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Impact of Presoaking and Foliar Spray Application by Maize Grain Extract in Alleviates Salinity Stress in Common Bean (Phaseolus Vulgaris l.) Plants Grown under Salt Stress

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Abstract

This investigation was carried out during the two successive seasons of 2014 and 2015 to investigate whether maize grains extract (MGE) could play a role in improving salt tolerance in bean plants. The MGE was exogenously applied as a seed soaking or foliar spraying to plants under salt stress (EC = 7.43–7.51 dS m–1). The impact of MGE on the growth and yield characteristics, physio-biochemical attributes, antioxidants and mineral nutrients of bean plants exposed to salt stress was assessed. The MGE-treated plants exposed to salt stress had higher growth and yield characteristics, leaf photosynthetic pigments, leaf tissue health in terms of relative water content and membrane stability index, concentrations of soluble sugars, free proline, ascorbic acid and mineral nutrients compared to MGE-untreated plants. Application of MGE as a mixture of aqueous extract: alcoholic extract at a rate of 1: 1 (v/v) was found to be more effective in alleviating salt stress damages in common bean plants compared to MGE as aqueous or alcoholic extract.

Key Words: common bean, salt stress, maize grains extracts, growth and productivity, antioxidants and osmoprotectants.

Introduction:

Common bean (Phaseolus vulgaris L.) is one of the most important Fabaceae vegetables produced for human nutrition due to its capacity to produce large quantities of protein-rich seed, particularly in the Middle Eastern developing countries. It is classified as a salt-sensitive plant (Maas & Hoffman 1977).

Salinity is one of the major limiting factors to crop performance (growth and productivity) in dry (arid and semi-arid) regions worldwide. The negative effect of salt stress on crop performance results in the disturbances in plant physiology through osmotic and/or ionic stress, causing physiological drought by affecting the water relations of the plant (Munns, 2002; Bargaz et al., 2016; Rady et al.,2020; Seif El-Yazal, 2020; Seif El-Yazal et al., 2020; Seif El-Yazal and Hussein, 2021), together with accumulation of the toxic amounts of salts in the leaf apoplasm that leads to dehydration and turgor loss, consequently death of cells and tissues (Megawer and Seif El-Yazal, 2008; Semida and Rady, 2014). Photosynthesis considers one of the most severely affected processes by salt stress. It is mediated by decrease of chlorophyll pigment (Sabra et al., 2012; Kchaou et al., 2013; Seif El-Yazal , 2020) and inhibition of rubisco (Soussi et al., 1998), herewith decreasing the leaf CO2 assimilation rate (Yiu et al., 2012). In addition, salt stress affects nitrogen metabolism by affecting various enzymes (Gong et al., 2013; Hemida et al.,2017; Seif El-Yazal, 2019a&b). However, plant antioxidative defense systems are reported to be stimulated by salt stress (Sairam et al., 2005; Seif El-Yazal, 2008; Rady, 2011; Semida and Rady, 2014; Rady and Hemida, 2016), and further stimulated by some exogenous applications to mitigate the adverse conditions of salt stress (Korkmaz et al., 2012; Yasmeen et al., 2013 Rady et al., 2013; Bargaz et al., 2016; Rady et al., 2018; Seif El-Yazal, 2020).

Nowadays, a growing interest has been observed with natural inexpensive biostimulants. Extracts of different plant parts such as natural phytohormones, osmoprotectants and antioxidants-containing leaves (i.e., Moringa oleifera – Rady et al., 2013; Yasmeen et al., 2013; Elzaawely et al., 2017), seeds (i.e., dry bean – Abd El-

Naem et al., 2007) or grains (i.e., maize – Rady and Seaf El-Yazal, 2009; Semida and Rady, 2014), in addition to seaweed extracts (Sabir et al., 2014; Battacharyya et al., 2015) have been reported to affect different physiological functions. The beneficial effects of these plant's natural extracts on growth, yield, chemical attributes and antioxidative defense systems in crop plants grown under normal or salt stress conditions have been reported.

Therefore, the current work was designed with objective to examine the changes in antioxidants and osmoprotectants under the effect of MGE, applied by seed soaking or plant foliar spray, on the Phaseolus vulgaris (L.) plants grown under salt stress (7.43–7.51 dS m–1) and to establish a relationship between the changes in antioxidants and osmoprotectants, and the degree of tolerance in terms of improvement in plant growth and yield, leaf tissue health and the concentrations of soluble sugars, free proline, ascorbic acid and mineral nutrients. The hypothesis tested, herein, is that MGE will positively modify the level of antioxidants and osmprotectants that will protect the stress generated by soil salinity stress. In addition, MGE as a natural extract will help to improve plant performance better than the expensive synthetic growth promoters.

Materials and Methods:

Experimental Procedures:

Two field experiments were conducted on both 2014 and 2015 summer seasons at a Special Farm, a newly-reclaimed saline soil (EC = 7.43–7.51 dS m–1) located in Demo, Egypt (30°54055″E 29°17006″N). Daily temperatures ranged from 14.5 to 27.1 °C with an average of 20.8 ± 2.6 °C, and daily relative humidity averaged 55 ± 4.5%, in a range between 25 and 85%. The Paulista cultivar of common bean (Phaseolus vulgaris L.) was selected for this study as an exportation crop. Seeds were selected for uniformity by the selection of those equal in size and like in color. The selected seeds were washed with distilled water, sterilized with a 1% sodium hypochlorite solution for 2 min and thoroughly washed again with distilled water. Commercial rhizobia inoculants were applied as peat slurry containing 107 Rhizobium g–1. Seeds were field sown on two different locations in the same Farm, one location (EC = 7.51 dS m–1) for 2014 season (28 February) and the other location (EC = 7.43 dS m–1) for 2015 season (25 February), each with 21 experimental units for 7 treatments (3 replicates each–1) including the control. The recommended seed rate of 95 kg ha–1 for common beans was used. Each experimental unit consisted of nine rows, 5 m long and 0.7 m wide, within row spacing was of approximately 7.5 cm. Thinning of plants (two hill 1) was performed prior to the first irrigation. During preparation and plant growth, the soil was supplemented in total with ammonium sulphate (20.5% N), calcium superphosphate (15.5% P2O5) and potassium sulphate (48% K2O) at rates of 200 kg ha–1, 200 kg ha–1 and 100 kg ha–1, respectively as recommended. Prior to sowing, physical and chemical soil characteristics of the two locations of the two seasons were determined as described by Black et al. (1965) and Jackson (1973), as shown in Table 1. Electrical conductivity (ECe) was measured using a soil paste extract. The ECe values were 7.51 and 7.43 dS m–1 at the two locations of 2014 and 2015 seasons, respectively. These ECe values classed the soil as being saline at the two locations according to Dahnke and Whitney (1988). The treatments were as follows:

Treatments	Seed soaking	Soaking time	Foliar spray	No. of sprays	Dates of sprays
T1 (Control)	Tap water	2 h	Tap water	2 times	At 25 and 40 days after sowing
T2	MGE_1		Tap water		
T3	MGE_2		Tap water		
T4	MGE_{1+2}		Tap water		
T5	Tap water		MGE_1		
T6	Tap water		MGE_2		
T7	Tap water		MGE_{1+2}		

Note: MGE1 = Aqueous extract of maize grains, MGE2 = Alcoholic extract of maize grains, and MGE1+2 = Mixture of aqueous and alcoholic extracts of maize grains.

The experimental design was complete randomized blocks. The experimental units were irrigated to that of reference crop evapotranspiration (ET0) values. Seven irrigations were supplied totaling approximately 2830 m3 ha 1. All other recommended agricultural practices for common bean were carried out as recommended (Abdelhamid et al., 2013). Seed soaking treatments were for 2 h at 25 ± 2 °C, and soaked seeds were allowed to air-dry overnight at room temperature. Foliar sprays were conducted for plants to run off, using 0.1% (v/v) Tween-20 that added to sprays as a surfactant to ensure optimal penetration into leaf tissues.

Preparation of Maize Grains Extracts (MGE):

To prepare the MGE, a weight of 0.5 kg of maize grains of a genotype Balady (a local type frequently handled by many farmers) was stored in water-wetted cotton or clean cloth until the grains were mushy. Then, mushy grains were ground well with distilled water and filtered under vacuum through Whatman No. 1 paper. The obtained aqueous extract was condensated to obtain an extract of 2% active ingredients. The aqueous extract (MGE1) was stored in a refrigerator at –20 °C until use. Another weight of maize grains was soaked in ethanol (95%) until the grains were mushy. Then, mushy grains were ground well with distilled water and filtered under vacuum through Whatman No. 1 paper. The alcoholic extract (MGE2) was evaporated using a big fan for quite excluding the alcohol and condensate the extract up to 2% active ingredients. The alcoholic extract was stored in a refrigerator at –20 °C until use. Each extract (aqueous or alcoholic) was used singly for seed soaking or plant foliar spraying or in a mixture (MGE1+2) of 1 aqueous extract: 1 alcoholic extract (v/v). Chemical characteristics of MGE1+2, which were determined and identified by GC/MS in a specialized laboratory in the National Research Center, are presented in Table 2.

Plant Sampling:

At 50 days after sowing (DAS), 9 plants were randomly selected from each replication and phenotyped; shoot length, number of leaves plant–1, leaf area plant–1, shoot fresh weight (FW) and shoot dry weight (DW) plant–1 were recorded. The harvest for marketable green pods was performed several times 2-day

intervals beginning from 60 DAS in both seasons. Average pod weight, number of pods plant–1, pods weight plant–1 and ha–1. Pods yield was recorded in kg for each experimental unit and has been converted to t ha 1.

Parameter	2014 season	2015 season
Clay	48.2	48.6
Silt	30.4	30.2
Sand	21.4	21.2
Soil texture	Clay	
pH	7.84	7.80
EC (dS m-1)	7.51	7.43
Organic matter %	0.88	0.90
CEC* (cmolc kg-1)	33.5	34.9
Field capacity (%)	27.4	28.2
Available water (%)	13.2	13.5
Available N (mg kg-1 soil)	146.8	150.6
Available P (mg kg-1 soil)	12.4	13.4
Available K (mg kg-1 soil)	142.2	148.8
Available Fe (mg kg-1 soil)	21.4	22.3
Available Mn (mg kg-1 soil)	12.1	13.0
Available Zn (mg kg-1 soil)	4.1	4.4

Table 1: Physical and chemical properties of the experimental soil during soil preparation for sowing in 2012 and 2013 seasons. *CEC; cation exchange capacity.

Physio-Biochemical Attributes:

Fresh and dried leaves of common bean plants harvested at 50 DAS were evaluated. Fresh leaves were assessed for concentrations of total chlorophylls and total carotenoids (mg g–1 FW) using a colorimetric method according to Arnon (1949), following an extraction by homogenization of fresh leaves in 80 % acetone. Relative water content (RWC%; Hayat et al., 2007) and membrane stability index (MSI%; Premchandra et al., 1990; Rady, 2011) were also assessed in full expanded fresh leaves. Total soluble sugars (mg g–1 DW) were determined using dried leaves according to Irigoyen et al. (1992), following an extraction by homogenization of dried leaves in 5 ml of 96% (v/v) ethanol and washed with 5 ml 70% (v/v) ethanol, afterwards freshly-prepared anthrone reagent [150 mg anthrone plus 100 ml of 72% (v/v) sulphuric acid] was used to record the values at 625 nm using a Bausch and Lomb-2000 Spectronic Spectrophotometer. Free proline (µg g–1 DW) was extracted by sulphosalicylic acid (3 %) and determined colorimetrically using the acid ninhydrin reagent as described by Bates et al. (1973). Ascorbic acid (AsA) concentration in leaves was determined using the method of Mukherjee and Choudhuri (1983), following an extraction of fresh fully-expanded leaf sample (0.5 g) in 10 ml of 6% (w/v) TCA, and then the extract was mixed with 2 ml of 2% (w/v) dinitrophenylhydrazine, followed by the addition of one drop of 10% (w/v) thiourea in 70% (v/v) ethanol and the absorbance was recorded at 530 nm after boiling for 15 min and adding 5 ml of 80% (v/v) H2SO4. Fresh samples of leaves were dried at 70 °C to constant weights before they were ground to a fine powder for analyses of macronutrients and sodium concentrations. Total nitrogen (N; mg g–1 DW) concentration was determined using the micro-Kjeldahl method. Phosphorus (P; mg g–1 DW) concentration was colorimetrically determined using stannous chloride-ammonium molybdate reagent as described by King (1951) after its extraction by sodium bicarbonate according to Olsen et al. (1954). Potassium (K+) and sodium (Na+) were determined using a flame photometer (Gallenkamp Co., London, UK) as described by Brown and Lilliand (1966).

Statistical Analysis:

All data were subjected to an analysis of variance for a complete randomized blocks design. Significant differences between means were compared at $P \leq 0.05$ using Duncan's multiple range test. The statistical analysis was carried out using COSTAT computer software (CoHort Software version 6.303, Berkeley, CA, USA).

Results:

Table 2 show that, maize grains extract (MGE) is rich in osmoprotectants (i.e., free proline, soluble sugars and K+), mineral nutrients (i.e., N, P, K, Ca, Mg, Fe, Mn, Zn, Cu and I), and antioxidants and vitamins [i.e., ascorbic acid (vitamin C; AsA), glutathione (GSH) and B-group vitamins]. The MGE is also rich in phytohormones [indoles, indole-3-acetic acid (IAA), gibberellic acid (GA3) and zeatin-like cytokinins]. In addition, it has antioxidant activity (DPPH-radical scavenging activity) of approximately 82.5%.

Under saline soil conditions (EC = 7.43–7.51 dS m–1), growth characteristics (i.e., shoot length, number of leaves plant–1, leaves area plant–1, and shoot fresh and dry weights; Table 3) and green pods yield traits (i.e., average pod weight, number of pods plant–1, and pods weight plant–1 and ha–1; Table 4) of common bean plants treated with MGE, which used as seed soaking or foliar spraying, were significantly increased compared to the controls (i.e., plants treated with tap water) in both growing seasons (2014 and 2015). In general, MGE treatment as seed soaking was more effective than MGE treatment as foliar spraying. Treatment of seed soaking in MGE1+2 (mixture of aqueous extract: alcoholic extract at 1: 1 v/v) exceeded the all other treatments including the control. This treatment exceeded the control by 47.5 and 46.8% for shoot length, 30.8 and 28.5% for number of leaves plant–1, 171.4 and 162.5% for leaves area plant–1, 134.1 and 96.6% for shoot fresh weight, 87.8 and 72.0% for shoot dry weight, 55.9 and 56.5% for average pod weight, 84.6 and 76.5% for number of pods plant–1, 188.3 and 176.1% for pods weight plant–1, and 188.7 and 176.3 for pods weight ha–1 in both 2014 and 2015 growing seasons, respectively.

The same trends were exhibited for leaf concentrations of photosynthetic pigments (i.e., total chlorophylls and total carotenoids; Table 5), leaf tissue health [i.e., relative water content (RWC) and membrane stability index (MSI); Table 5], leaf concentrations of osmoprotectants and antioxidants (i.e., soluble sugars, free proline and AsA; Table 6), and mineral nutrients (i.e., N, P and K) and the ratio of K/Na (Table 7). These results are true in both growing seasons. The most significant increases recorded by the treatment of seed soaking in MGE1+2 in both seasons were as follows: 90.9 and 96.3% for concentration of total chlorophylls, 42.9 and 44.1% for MSI, 64.4 and 77.9% for concentration of soluble sugars, 91.4 and 98.2% for concentration

of free proline, 47.8 and 40.6% for concentration of AsA, 36.3 and 33.0% for concentration of K, and 295.0 and 292.6% for K/Na ratio, respectively compared to the controls. On the other hand, the concentration of Na was significantly decreased by the superior treatment (seed soaking in MGE1+2) compared to the all other treatment including the control (Table 7). This treatment reduced the concentration of Na by 65.5 and 66.1% in both seasons, respectively compared to the controls.

Parameter	Unit	Value	
		2014	2015
Osmoprotectants:			
Soluble sugars	mg g^{-1} DW	69.7	71.2
Proline		5.32	4.97
Mineral nutrients:			
Nitrogen (N)	mg g^{-1} DW	24.8	25.1
Phosphorus (P)		3.12	3.08
Potassium (K)		27.3	27.0
Magnesium (Mg)		2.51	2.64
Calcium (Ca)		3.26	3.18
Iron (Fe)		1.21	1.24
Manganese (Mn)		0.84	0.79
Zinc (Zn)		0.51	0.55
Iodine (I)		1.28	1.14
Copper (Cu)		0.23	0.25
Antioxidants and vitamins:			
Total B-group vitamins	mmol g^{-1} DW	129	133
Ascorbic acid (vitamin C)		1.62	1.59
Glutathione		0.92	0.88
DPPH-radical scavenging activity	%	82.4	82.7
Phytohormones:			
Total indoles	µg g^{-1} DW	3.24	3.32
Indole-3-acetic acid		1.72	1.84
Gibberellic acid		1.96	1.92
Zeatin		2.69	2.78

Table 2: Chemical components of the tested maize grains extract (MGE1+2; on dry weight basis) identified by GC/MS.

Treatments		Parameters				
Seed soaking	Foliar spray	Shoot length (cm)	Leaves No. plant^{-1}	Leaves area plant^{-1} (m^2)	Shoot FW (g)	Shoot DW (g)
2014 season						
Tap water		40.2d	12.0c	0.07d	35.5d	7.4d
MGE_1	Tap water	59.1a	15.3a	0.19a	80.1a	13.3a
MGE_2	Tap water	58.0a	14.0b	0.15b	62.9b	12.3b
MGE_{1+2}	Tap water	59.3a	15.7a	0.19a	83.1a	13.9a
Tap water	MGE_1	54.0b	13.7b	0.13c	49.3c	11.6c
Tap water	MGE_2	49.0c	13.3b	0.13c	47.0c	11.3c
Tap water	MGE_{1+2}	53.3b	13.7b	0.15b	49.6c	12.3b
2015 season						
Tap water		44.0c	13.0b	0.08d	41.7d	8.2d
MGE_1	Tap water	64.2a	16.3a	0.21a	80.3a	13.1ab
MGE_2	Tap water	58.3ab	16.0a	0.18b	67.3b	12.9b
MGE_{1+2}	Tap water	64.6a	16.7a	0.21a	82.0a	14.1a
Tap water	MGE_1	63.0a	15.7a	0.16c	59.0c	12.3b
Tap water	MGE_2	54.3b	15.7a	0.15c	55.0c	11.1c
Tap water	MGE_{1+2}	59.0ab	16.0a	0.18b	59.3c	12.8b

Table 3: Effect of seed soaking or foliar spray with maize grains extract (MGE) on some growth traits of common bean (Phaseolus vulgaris L., cv. "Paulista") plants grown under salt stress conditions in two seasons.

Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $p \leq 0.05$ by Duncan's multiple range test.
Note: MGE1 = Aqueous extract of maize grains, MGE2 = Alcoholic extract of maize grains, and MGE1+2 = Mixture of aqueous and alcoholic extracts of maize grains.

Treatments		Parameters			
Seed soaking	Foliar spray	Average pod weight (g)	No. of pods plant^{-1}	Pods weight plant^{-1} (g)	Pods weight ha^{-1} (ton)
2014 season					
Tap water		2.02c	12.3d	24.8e	5.3e
MGE_1	Tap water	3.12a	21.3ab	66.5b	14.3ab
MGE_2	Tap water	3.00a	20.3bc	60.9c	13.1c
MGE_{1+2}	Tap water	3.15a	22.7a	71.5a	15.3a
Tap water	MGE_1	2.92ab	20.3bc	59.3cd	12.7cd
Tap water	MGE_2	2.85b	19.5c	55.6d	11.9d
Tap water	MGE_{1+2}	3.03a	20.7b	62.7bc	13.4bc
2015 season					
Tap water		2.09b	13.2c	27.6d	5.9d
MGE_1	Tap water	3.21a	22.6a	72.5ab	15.5ab
MGE_2	Tap water	3.13a	21.7b	67.9b	14.6b
MGE_{1+2}	Tap water	3.27a	23.3a	76.2a	16.3a
Tap water	MGE_1	3.07a	22.0ab	67.5b	14.5bc
Tap water	MGE_2	3.00a	20.7b	62.1c	13.3c
Tap water	MGE_{1+2}	3.15a	22.3a	70.2b	15.0b

Table 4: Effect of seed soaking or foliar spray with maize grains extract (MGE) on green pods yield and its components of common bean (Phaseolus vulgaris L., cv. "Paulista") plants grown under salt stress conditions in two seasons.

Mean values in each column for each year followed by a different lower-case letter are significantly different at $p \leq 0.05$ by Duncan's multiple range test.
Note: MGE1 = Aqueous extract of maize grains, MGE2 = Alcoholic extract of maize grains, and MGE1+2 = Mixture of aqueous and alcoholic extracts of maize grains

Treatments		Parameters			
Seed soaking	**Foliar spray**	**Total chlorophylls**	**Total carotenoids**	**RWC (%)**	**MSI (%)**
2014 season					
Tap water		0.88d	0.35d	57.2c	44.3b
MGE_1	Tap water	1.48b	0.48a	84.3a	61.3a
MGE_2	Tap water	1.20c	0.43bc	79.6b	59.8a
MGE_{1+2}	Tap water	1.68a	0.49a	86.1a	63.3a
Tap water	MGE_1	1.27c	0.44b	80.2ab	58.9a
Tap water	MGE_2	1.19c	0.42c	79.9b	58.7a
Tap water	MGE_{1+2}	1.34bc	0.45b	81.5a	59.7a
2015 season					
Tap water		0.82c	0.40c	54.2c	45.1c
MGE_1	Tap water	1.56a	0.52a	84.5a	62.9ab
MGE_2	Tap water	1.36b	0.46b	79.1b	59.1b
MGE_{1+2}	Tap water	1.61a	0.54a	85.9a	65.0a
Tap water	MGE_1	1.41b	0.47b	79.4ab	60.1ab
Tap water	MGE_2	1.31b	0.45b	79.0b	58.2b
Tap water	MGE_{1+2}	1.48ab	0.48b	80.1a	60.3ab

Table 5: Effect of seed soaking or foliar spray with maize grains extract (MGE) on leaf concentration of photosynthetic pigments (mg g-1 fresh weight) and leaf tissue health (relative water content; RWC and membrane stability index; MSI) of common bean (Phaseolus vulgaris L., cv. "Paulista") plants grown under salt stress conditions in two seasons.

Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $p \leq 0.05$ by Duncan's multiple range test.
Note: MGE1 = Aqueous extract of maize grains, MGE2 = Alcoholic extract of maize grains, and MGE1+2 = Mixture of aqueous and alcoholic extracts of maize grains.

Treatments		Parameters		
Seed soaking	Foliar spray	Soluble sugars (mg g^{-1} DW)	Free proline (µg g^{-1} DW)	AsA (mmol ascorbate g^{-1} DW)
2014 season				
Tap water		17.4c	105d	2.01c
MGE_1	Tap water	27.7a	189ab	2.89a
MGE_2	Tap water	24.2b	175bc	2.72b
MGE_{1+2}	Tap water	28.6a	201a	2.97a
Tap water	MGE_1	24.1b	175bc	2.70b
Tap water	MGE_2	23.8b	170c	2.64b
Tap water	MGE_{1+2}	24.3b	178bc	2.78ab
2015 season				
Tap water		19.5d	110c	2.24c
MGE_1	Tap water	33.0a	211a	3.01a
MGE_2	Tap water	27.9bc	201ab	2.88b
MGE_{1+2}	Tap water	34.7a	218a	3.15a
Tap water	MGE_1	28.8b	198b	2.85b
Tap water	MGE_2	26.3c	189b	2.74b
Tap water	MGE_{1+2}	29.5b	201ab	2.91ab

Table 6: Effect of seed soaking or foliar spray with maize grains extract (MGE) on the leaf concentrations of total soluble sugars, free proline, ascorbic acid (AsA) and glutathione (GSH) of common bean (Phaseolus vulgaris L., cv. "Paulista") plants grown under salt stress conditions in two seasons

Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $p \leq 0.05$ by Duncan's multiple range test.
Note: MGE1 = Aqueous extract of maize grains, MGE2 = Alcoholic extract of maize grains, and MGE1+2 = Mixture of aqueous and alcoholic extracts of maize grains.

Treatments		Parameters				
Seed soaking	Foliar spray	N	P	K	Na	K/Na ratio
		(mg g^{-1} DW)				
2014 season						
Tap water		17.8b	1.58c	18.2b	6.23a	2.92e
MGE_1	Tap water	22.8a	2.60ab	24.5a	2.24b	10.94ab
MGE_2	Tap water	22.2a	2.48b	23.8a	2.36b	10.08bc
MGE_{1+2}	Tap water	23.0a	2.68a	24.8a	2.15b	11.53a
Tap water	MGE_1	22.0a	2.46b	23.6a	2.38b	9.92cd
Tap water	MGE_2	21.8a	2.44b	23.3a	2.50b	9.32d
Tap water	MGE_{1+2}	22.3a	2.55ab	23.8a	2.34b	10.17bc
2015 season						
Tap water		18.4b	1.62c	19.1b	6.14a	3.11e
MGE_1	Tap water	23.0a	2.76a	25.0a	2.12b	11.79ab
MGE_2	Tap water	22.2a	2.65ab	24.1a	2.18b	11.06bc
MGE_{1+2}	Tap water	23.2a	2.84a	25.4a	2.08b	12.21a
Tap	MGE_1	22.2	2.64a	24.0	2.20	10.91d

water		a	b	a	b	
Tap water	MGE_2	21.9 a	2.52b	23.7 a	2.18 b	10.87d
Tap water	MGE_{1+2}	22.4 a	2.72a	24.2 a	2.16 b	11.20b c

Table 7: Effect of seed soaking or foliar spray with maize grains extract (MGE) on leaf concentrations of some macro-nutrients (N, P and K) and Na, and ratio of K/Na of common bean (Phaseolus vulgaris L., cv. "Paulista") plants grown under salt stress conditions in two seasons

Mean values (n = 9) in each column for each year followed by a different lower-case letter are significantly different at $p \leq 0.05$ by Duncan's multiple range test.
Note: MGE1 = Aqueous extract of maize grains, MGE2 = Alcoholic extract of maize grains, and MGE1+2 = Mixture of aqueous and alcoholic extracts of maize grains.

Discussion:

Salinity, as one of the major abiotic stresses limiting crop performance, is proved to cause overproduction of reactive oxygen species (ROS). To maintain the metabolic functions under salt stress conditions, a balance between generation and degradation of ROS is required to avoid the oxidative injuries. Under stress conditions such as salt stress, plants utilize most of their resources to improve defense mechanisms rather than growth and development (Kolbert et al., 2012;). Salt stress is proved to inhibit plant performance (i.e., growth and productivity) (Shoresh et al., 2011; Abouelsaad et al., 2016). Salt stressed-plants suffer from physiological drought which causes physiological disruptions in different metabolic process (Soussi et al., 1998; Ghallab and Seif El-Yazal, 2006;2007; Garriga et al., 2015; Rady and Mohamed, 2015), negatively affecting plant growth and productivity. It has been found that soaking different crop seeds in and/or foliar spraying different crop plants with some biostimulating substances cause improvements in plant growth and productivity (Rady and Seaf El-Yazal, 2009; Rady et al., 2013; Yasmeen et al., 2013; Semida and Rady, 2014; Rady and Mohamed, 2015; Elzaawely et al., 2017). Among the antioxidant system, non-ezymatic low molecular weight antioxidants (i.e. proline and ascorbic acid, etc.) are reported to control the level of ROS in plant tissues (Schutzendubel and Polle, 2002; Rady and Hemida, 2016). It is, therefore, expected that the level of antioxidants tends to increase with the exposure of common bean plants to salt stress. However, the interesting finding found out in the current study is that maize grain extract (MGE) applied by seed soaking or plant foliar spraying for common bean grown on a saline soil (EC = 7.43–7.51 dS m–1) significantly improved the concentrations of ascorbic acid (AsA), free proline and soluble sugars (Table 6). This result may be due to that MGE as a plant biostimulant is rich in some growth stimulants. It contains abundant concentrations of soluble sugars, free proline, various mineral nutrients, phytohormones; GA3, indoles and zeatin, as well as GME contain significant concentrations of ascorbic acid (AsA), glutathione (GSH) and B-group vitamins (Table 2). In addition, it has an antioxidant activity (assessed in term of DPPH-radical scavenging activity) at approximately 82.5%. These growth stimulants, together with the high antioxidant activity, have been found to play important roles in many physio-biochemical activities in salt-stressed common bean plants when treated with MGE that help them to alleviate the deleterious effects of salt stress. Thus, using MGE as a soaking or foliar spray solution for bean seeds or plants alleviated the inhibitory effects of saline soil conditions on all studied parameters, showing improvements in plant growth and yield (Tables 3 and 4). Phytohormones and antioxidants found in MGE could be considered as key tools of the mechanisms by which the MGE applications alleviated the deleterious effects of salt stress. Alleviation of salt stress effects occurred by seed soaking in or plant foliar spray with MGE may be attributed to the stimulative materials found in MGE. In general, seed soaking treatments are found to more effective than plant foliar spray treatments. This finding may be attributed to that seeds absorbed various stimulant substances from MGE that enabled seed to strongly germinate under salt stress conditions (data not shown) and seedlings obtained from these MGE-soaked seeds showed a vigorous growth in terms of fresh and dry weights, and also exhibited a significant improvement in leaf tissue health in terms of increased relative water content (RWC) and membrane stability index (MSI) (Table 5). In addition, leaf photosynthetic pigments showed significant increased concentrations with MGE application under salt stress and this preferred result may be attributed to increase of chlorophyll biosynthesis and/or decrease of chlorophyll degradation by chlorophyllase enzyme. Leaf chlorophyll is among the most important physiological indicators reflecting the stress of the plant, in part, due to its reliance on water and nutritional availability (Rady et al., 2015; Bargaz et al., 2016). In the current study, plants pretreated (soaking seeds) with MGE had greater leaf chlorophyll and carotenoids concentrations than those foliar sprayed with MGE. The reduction in chlorophylls in the salt-stressed plants (controls) might be due to disorganization of thylakoid membranes, more degradation than synthesis of chlorophyll via the formation of proteolytic enzymes such as chlorophyllase that is responsible for the chlorophyll degradation and damaging to the photosynthetic apparatus (Rong-hua et al., 2006), and this led to reducing accumulated ions in plants (Abdelhamid et al., 2010; Bargaz et al., 2016). However, MGE application restored and significantly increased the mineral nutrients in common bean plants (Table 7), which may attribute to that MGE is rich in mineral nutrients and increased absorption by the increase occurred in osmoprotectants (soluble sugars and proline; Table 6). Soluble sugars play a central role in osmotic adjustment in almost all plants under salt stress conditions. In this study, soluble sugars concentration found to significantly increase in response to MGE application under salt stress compared to the control. Bargaz et al. (2016) reported that soluble sugar accumulation together with free proline and ascorbic acid improved common bean plant tolerance to salinity and consequently enhanced plant performance (growth and yield). It is a recent phenomenon that the application of MGE, as seed soaking or plant foliar application, caused an increase in the concentrations of antioxidants such as free proline and ascorbic acid. The increase in these antioxidants, on the basis of molecular, physiological and genetic approaches, is the consequence of enhanced expression of DET2 gene, which enhanced the tolerance to oxidative stress in Arabidopsis thaliana (Cao et al., 2005). Ascorbate is considered as a most powerful ROS scavenger due to its ability to donate electrons in a number of enzymatic and non-enzymatic reactions. It can provide a protection to membranes by directly scavenge the $O2^-$ and OH^- and by regenerate α-

tocopherol from tocopheroxyl radical. In chloroplast, ascorbate acts as a cofactor of violaxanthin de-epoxidase, thus sustaining dissipation of excess excitation energy (Smirnoff, 2000). In addition to the importance of ascorbate in the ascorbate-glutathione cycle, it also plays an important role in preserving the activities of enzymes that contain prosthetic transition metal ions (Noctor and Foyer, 1998). The ascorbate redox system consists of l-AsA, mono-dehydroascorbate and dehydroascorbate. Both oxidized forms of can be chemically reduced by glutathione to ascorbate (Foyer and Halliwell, 1976).

The increased proline concentration observed in common bean plants due to seed soaking in or plant foliar spray with MGE may be attributed to that MGE are rich in free proline (Table 2). Cellular proline accumulates from about 5% of the amino acid pool under normal conditions up to 20–80% under stress due to increased synthesis and decreased degradation in many plant species (Kavi Kishor et al., 2005) to enhance plant tolerance by reducing ROS damage. The mechanism by which free proline reduces ROS damage and enhancing plant tolerance is that proline reduces salt stress effects by detoxification of ROS produced as a result of salt poisoning. Free proline may physically quench singlet oxygen or react directly with hydroxyl radicals (Siripornadulsil et al., 2002). These reactions result in reduced ROS damage and a more reducing cellular environment (higher AsA and proline levels; Table 6). Free proline is a compatible osmolyte, is not charged at neutral pH and is highly soluble in water. It can drive influx of water or reduce the efflux. This provides cell turgor (higher RWC; Table 5) that is necessary for cell expansion. Free proline seems to have diverse roles under osmotic stress conditions, such as stabilization of proteins, maintenance of membrane integrity and subcellular structures, and protecting cellular functions by scavenging ROS (Kavi Kishor et al., 2005). In the present study, the increased concentrations of antioxidants and proline pool resulted in an increase in the capacity of tolerance to salt stress may be attributed to antioxidants enriching-MGE and the higher antioxidant activity of MGE (Table 2). The increased tolerance to the stress was emerged in terms of improved common bean plant growth (fresh and dry weights; Table 3). Based on these findings, we suggest that plants supplied with MGE, as a seed soaking or a plant foliar spray, could optimally stimulate free proline and soluble sugars acting as osmoprotectants for the overall osmotic adjustment, and also stimulate AsA acting as an effective antioxidant under salt stress conditions (Abdelhamid et al., 2013; An and Liang, 2013; Semida and Rady, 2014). Biosynthesis of osmoprotectants, such as sugars and free proline, together with antioxidants, such as AsA, has been reported as an adaptive strategy to mediate salt stress (Bargaz et al., 2016). In addition to acting as osmosolutes, they also act as N storage compounds and/or hydrophobic protectants for enzymes and cellular structures (Abdelhamid et al., 2013; Taie et al., 2013). The osmo-tolerance responses observed of plant growth and nitrogen fixation in salt-stressed M. sativa, P. vulgaris and P. acutifolius are thought to be associated with high proline and carbohydrate accumulation (Özge and Atak, 2012).

Previous researches have shown that soil salinity significantly increased Na+ concentration in faba bean (Abdelhamid et al., 2010) and Phaseolus vulgaris (Bargaz et al., 2016). The increase in leaf Na+ concentration may be due to increased concentrations of Na+ in the growing medium ultimately resulting in the increased uptake of Na+ by plant (Abdelhamid et al., 2010). Findings herein exhibit a decrease in Na+ concentration by the application of MGE. This may be attributed to the positive role of MGE in improved plant growth and yield (Tables 3 and 4), increased concentration of photosynthetic pigments (Table 5), increased total soluble sugar, free proline (Table 6) and increased nutrient concentrations such as N, P and K(Table 7), consequently increasing the plant adaptive capacity to salinity by exclusion of Na+ (Munns and Tester, 2008). Moreover, Lenis et al. (2011) reported that salinity-tolerant genotypes have less leaf scorch and a greater capacity to prevent Na+ and Cl–-transport from soil solution to stems and leaves than that of sensitive genotypes. Application of MGE significantly increased K+ concentration in common bean leaves under soil salinity conditions (Table 7). The increase in K+ concentration by MGE under salt stress could be related to a gradient competition and resulting in selective uptake between K+ and Na+ which causes an increase in uptake of K+ together with the amount of K+ absorbed from MGE by seed or by plant leaf. Results of this study confirmed an increase of N, P and K+ concentrations, while exhibited a reduction of Na+ concentration, and consequently an increase of K+/Na+ ratio, indicating a salt tolerance of common bean plants is associated with an enhanced K+/Na+ ratio with the application of MGE.

According to the fact that MGE is rich sources of zeatin, GA3 and indoles (Table 2), soaking common bean seeds in or foliar spraying plants with this biostimulant (MGE) strengthens plant defense system against salt stress. A possible involvement of genes in stress responses is often inferred from changes in the transcript abundance in response to a given stress trigger. Where MGE is rich source in antioxidants, mineral nutrients and phytohormones, so the effectiveness of these extracts in alleviating the salt stress by better plant growth and productivity, endogenous antioxidants and osmoprotectants might be due to cytokinin mediated stay green effect. Further work in this regard is necessary to identify, exactly, the mode of action of MGE that explain exactly how seed and plant tolerate salt stress.

Conclusion:

Application of MGE, as a soaking solution for seeds or a foliar spray solution for plants, improved the level of antioxidants and osmoprotectants such as ascorbic acid, free proline and soluble sugars in common bean plants grown under salt stress conditions. The effects of MGE were more pronounced under salt stress when used as a soaking solution for seeds, thereby increasing the tolerance of plants to salt stress and improving plant performance (growth and productivity). The MGE was found to be an effective strategy as a plant biostimulant for salt-stressed common bean plants.

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