

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:

Common bean (*Phaseolus vulgaris* L.) is an important legume plant for local consumption and exportation, which strongly affected by salt stress. The objective of this study was to assess 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 ($EC = 7.43\text{--}7.51\text{ dS m}^{-1}$) in two field experiments during 2014 and 2015 seasons. The effect 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.

Introduction:

As one of the most important vegetable crops grown in Egypt, common bean (*Phaseolus vulgaris* L.) has a great interest for local consumption and export. It is, nowadays, widely cultivated on salt-affected newly-reclaimed soils in Egypt. 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), together with accumulation of the toxic amounts of salts in the leaf apoplast that leads to dehydration and turgor loss, consequently death of cells and tissues (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) and inhibition of rubisco (Soussi et al., 1998), herewith decreasing the leaf CO_2 assimilation rate (Yiu et al., 2012). In addition, salt stress affects nitrogen metabolism by affecting various enzymes (Gong et al., 2013). However, plant antioxidative defense systems are reported to be stimulated by salt stress (Sairam et al., 2005; 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).

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 ($7.43\text{--}7.51\text{ dS m}^{-1}$) 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 osmoprotectants 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°54'055"E 29°17'006"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 (EC_e) was measured using a soil paste extract. The EC_e values were 7.51 and 7.43 dS m⁻¹ at the two locations of 2014 and 2015 seasons, respectively. These EC_e 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		

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 condensed 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