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Integrated application of salicylic acid and *Moringa oleifera* leaf extract alleviates the salt-induced adverse effects in common bean plants

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The effects of the integrated application of salicylic acid (SA; 1 mM) and *Moringa oleifera* leaf extract (MLE; 1 extract: 30 tap water) on the growth, yield and leaf anatomy of common bean (*Phaseolus vulgaris* L.) plants grown on a saline soil (EC = 6.23 – 6.28 dS m^{-1}) were investigated. The integrated application of SA and MLE, used as seed soaking or foliar spray, significantly improved growth characteristics (i.e., shoot length, number and area of leaves per plant, and plant dry weight), green pod and dry seed yields (pod weight per plant and per hectare, and seed weight per plant and per hectare) and leaf anatomy of common bean plants when compared with the controls (seed soaking and foliar spray with tap water). The integrated application of SA used as seed soaking and MLE used as foliar spray, which non-significantly exceeded the integrated application of SA used as foliar spray and MLE used as seed soaking, was found to be highly effective at improving the growth and yields of bean plants by alleviating the inhibitory effects of soil salinity stress.

Keywords: *Phaseolus vulgaris* L., salicylic acid, *Moringa oleifera* leaf extract, salt stress, anatomy, growth and productivity

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Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important vegetable Fabaceae crops, and is classified as a salt-sensitive plant (Maas and Hoffman, 1977). In developing countries, food legumes, including beans are an important component of the agricultural sectors due to their capacity to produce large quantities of protein-rich seed for human nutrition. Approximately, 20–30% of the produced bean in the Middle East and 50–10% in Latin America is affected by soil salinity (CIAT, 1992).

Moringa oleifera leaf extract (MLE; a plant biostimulant; Rady *et al.*, 2013) and salicylic acid (SA; an antioxidant; Semida and Rady, 2014a) are substances when applied singly as seed soaking and/or foliar spray modify plant growth and production with positive alterations in metabolic processes under salt stress conditions (Rady *et al.*, 2013; Semida and Rady, 2014a). *Moringa oleifera* Lam., a multipurpose tree from Moringaceae family is native to the sub-Hamaylian tract of India and Pakistan (Makkar and Becker, 1996; Shahzad *et al.*, 2013). The MLE is a rich source of antioxidants, some plant secondary metabolites and osmoprotectants. It is also a source of zeatin, a natural derivative of cytokinin, vitamins and several mineral elements, making it a potential natural growth stimulant (Rady *et al.*, 2013). The applications of MLE, used as seed soaking or foliar spray, have been shown to improve the plant tolerance to abiotic stresses, including salinity (Rady *et al.*, 2013; Yasmeen *et al.*, 2013; Howladar, 2014; Rady and Mohamed, 2015). These works have been shown that MLE applications maintained optimum tissue water status and membranes stabilities, enhanced antioxidant levels and activated plant defense system, increased levels of plant secondary metabolites, reduced uptake of undesirable Na^+ and/or Cl^-, and enhanced shoot or leaf K^+. These events lead to vigorous seedling growth, maximizing the crop performance (Yasmeen *et al.*, 2012; Rehman *et al.*, 2014).

Salicylic acid (SA) is classified as phenolic growth regulator, a non-enzymatic antioxidant, a signaling or messenger molecule in plants to induce responses of plants to environmental stressors. Providing plants with SA induces plant tolerance against various biotic and abiotic stresses by altering the activities of enzymatic antioxidants and reducing the generation of reactive oxygen species (ROS; Horvath *et al.*, 2007). SA plays an important role in the

regulation of some physiological processes in plants. It has been found that SA positively affects growth and development, ion uptake and transport, and membrane permeability (Simaei *et al.*, 2012). Depending on plant species, concentration, method and application time, SA has different effects on stress adaptation and damage development of plants (Metwally *et al.*, 2003). It has been shown that exogenous SA application has obtained a particular attention because it induces protective effects on plants under salinity (Simaei *et al.*, 2012).

In the arid and semiarid regions, including Egypt, salinity is considered one of the major factors affecting the agricultural productivity. Soil salinization in these regions is caused by some effective factors. These factors are low rainfall, poor drainage, poor irrigation water which contains considerable amounts of salts that accumulate in the soil surface layer, poor water management, high evaporation rate, proximity to the sea and/or the capillarity rise of salts from underground water into the root zone due to the excessive evaporation (Rady *et al.*, 2013; Gama *et al.*, 2007). Salinity reduces plant growth and yield due to an increase in water use efficiency, and changes the plant metabolic processes (Munns, 2002). Plants grown under saline conditions are stressed basically in three ways; water deficit caused by reduced water potential in the rhizosphere, Na^+ and Cl^- ions phytotoxicity and nutrient imbalance by the reduction in the uptake and/or shoot transport (Marschner, 1995).

The current investigation was designed to evaluate the potential effects of the integrated application of SA and MLE, used as seed soaking and foliar spray, on the growth, yields and leaf anatomy of *Phaseolus vulgaris* L. plants, exposed to moderate soil salinity stress (EC = 6.23–6.28 dS m^{-1}). The hypothesis tested is that integrated applications of SA and MLE, used as seed soaking and foliar spray, will protect the stress generated by salinity stress.

Materials and Methods

Soil analysis and preparation

In the two successive seasons of 2013 and 2014, two field experiments were conducted on a special Farm at Sherif Basha village, Beni Sueif Governorate; 29°06'20.4"N; 31°07'21.6"E, Egypt. In the 2013 season, daily temperatures ranged from 15.2º – 26.8ºC, with an average of 21.0º ± 2.5ºC. The daily relative

humidity averaged 58.0 ± 4.2%, and ranged from 31 – 85%. In addition, the daily temperatures ranged from 13.8º – 28.8ºC, with an average of 21.3º ± 2.8ºC, and the daily relative humidity averaged 56 ± 6.6% and ranged from 28 – 84% were for the 2014 season. Soil analysis of the experimental site in each season was carried out according to Black et al. (1965) and Jackson (1967). The results from physical and chemical analyses of the soils are shown in Table 1. Soil EC values were 6.23 and 6.28 dS m^{-1} for 2013 and 2014 seasons, respectively. These EC values classed the soils as being moderately saline (Dahnke and Whitney, 1988).

Plant material and experimental procedures

Healthy seeds of common bean (*Phaseolus vulgaris* L. cv. Bronco) were sown on 27 February 2013, and on 23 February 2014. Seeds were obtained from The Horticulture Research Institute, The Agricultural Research Centre, Giza, Egypt. They were sown at the equivalent of 95 kg ha^{-1} to achieve the recommended planting density. They were selected for uniformity by choosing those of equal size and of the same color. The selected seeds were washed with distilled water, sterilized in 1% (v/v) sodium hypochlorite for approx. 2 min, washed thoroughly again with distilled water, and left to dry at room temperature (20 ºC). Uniform, air-dried seeds were sown, after their soaking in water, salicylic acid (SA) or *Moringa oleifera* leaf extract (MLE), in hills in rows spaced 60 cm apart. The hills were spaced 10 – 15 cm apart in 3.0 m × 3.5 m plots. Thinning was done before the first irrigation to produce two plants per hill.

During soil preparation and plant growth, the soil was supplemented with the full dose of NPK fertilizer according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. These recommendations were for 450 kg ha^{-1} of calcium super-phosphate (15.5% P_2O_5), 120 kg ha^{-1} ammonium sulphate (20.5% N), and 60 kg ha^{-1} potassium sulphate (48% K_2O) during seed-bed preparation. An additional 120 kg ha^{-1} of ammonium sulphate and 60 kg ha^{-1} of potassium sulphate were added at the first irrigation, 2 weeks after each sowing. Irrigation water was added to 100% of the reference crop evapotranspiration (ETo), values from the Beni Sueif Governorate Meteo Station. All other recommended agricultural practices were followed according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. The experiment was arranged in a

randomized complete block design, with one level of each of water, SA and MLE with three replicate plots per treatment.

Table 1: Physical and chemical properties of the experimental soil, and some chemical constituents of *Moringa oleifera* leaf extract (on dry weight basis) in two seasons

Parameter/component	2013 season	2014 season
The experimental soil		
Clay	50.0	49.8
Silt	30.5	30.2
Sand	19.5	20.0
Soil texture	Clay	
pH	7.72	7.76
EC (dS m^{-1})	6.23	6.28
Organic matter%	0.95	0.92
$CaCO_3$ (%)	5.79	5.66
CEC^* ($cmol_c$ kg^{-1})	33.8	34.2
Field capacity (%)	28.6	28.2
Available water (%)	12.8	12.4
Available N (mg kg^{-1} soil)	152.6	148.4
Available P (mg kg^{-1} soil)	12.2	11.4
Available K (mg kg^{-1} soil)	144.2	138.9
Available Fe (mg kg^{-1} soil)	21.2	19.3
Available Mn (mg kg^{-1} soil)	11.0	11.5
Available Zn (mg kg^{-1} soil)	4.1	3.9
Moringa oleifera leaf extract (values in mg g^{-1} DW)		
Amino acids	124.7	126.8
Proline	26.09	27.17
Total soluble sugars	151.4	160.3
Ash	111.3	114.9
Calcium	8.756	9.032
Magnesium	6.035	6.121
Potassium	27.68	29.02
Phosphorus	6.122	6.225
Sodium	0.674	0.662
Iron	1.873	1.920
Manganese	0.966	1.020
Zinc	0.453	0.510
Copper	0.208	0.225
Soluble phenols	2.252	2.164

Total carotenoids	2.243	2.462
Total chlorophyll	4.625	5.008
Ascorbic acid	3.247	3.448
Phytohormones (μg g^{-1} DW):		
Indole-3-acetic acid	0.873	0.904
Gibberellins	0.802	0.824
Zeatin	0.936	0.974
Abscisic acid	0.292	0.284

Preparation and analysis of moringa leaf extract

Fresh leaves harvested from fully matured *Moringa oleifera* trees were air-dried, grinded and extracted. For extraction, ethyl alcohol was added to leaf powder and the mixture was put for 4 hours on a Rotary Shaker. Extract was purified by filtering twice through Whatman No. 1 filter paper. After purification, the extract was subjected to a Rotary Evaporator to fully evaporate the alcohol. Centrifugation at 8,000 × *g* for 15 min was then conducted for supernant. Supernatant was diluted to 30 times and used to seed soaking and foliar spray applications. The extract was analyzed and its chemical constituents are presented in Table 1.

Applications of moringa leaf extract (MLE) and salicylic acid (SA)

For seed soaking, common bean seeds were soaked in distilled water, SA (1 mM) and MLE (1:30) using seed weight to solution volume ratio (1:5) for 30 min at room temperature. After soaking, seeds were given washings with distilled water and re-dried overnight at room temperature. At early morning, treated seeds were sown as mentioned before. Foliar spray of water, SA or MLE was done at early morning with a sprayer (Vol. 20 L) to run-off twice, at 25 and 40 days after sowing. The concentrations of SA and MLE, the number and timing of sprays, and the soaking duration were based on results from a preliminary pot trial (data not shown). To ensure optimal penetration into leaf tissues, 0.1% (v/v) Tween-20 was added to the foliar sprays as a surfactant.

Plant growth analysis and yield estimations

Fifty-day-old bean plants were removed from each of the nine treatments and the number of leaves plant^{-1} was recorded. Shoot lengths were measured using a meter scale, then the leaf areas were measured manually using a graph sheet, where the squares covered by the leaf were counted to note the leaf area. Plants were then placed in an oven at 70ºC until constant weight to record plant dry weight. At the marketable green pod stage of each experiment, all the green pods on plants of half-rows in each plot were collected and weighed. At the end of the experiments (dry seed stage), all dry pods on plants of the other half-rows in each plot were collected, and seeds were then extracted from the pods, air-dried and weighed.

Determination of leaf free proline, total soluble sugars and ascorbic acid

Leaf free proline concentrations (in μg g^{-1} DW) were determined using the rapid colourimetric method (Bates *et al.*, 1973). Proline was extracted from 0.5 g of each fresh leaf sample by grinding in 10 ml 3% (v/v) sulphosalicylic acid and the mixture was then centrifuged at 10,000 × *g* for 10 min. Two ml of the supernatant was placed in a test-tube, to which 2 ml of a freshly prepared acid ninhydrin solution was added. The tubes were incubated in a water bath at 90 ºC for 30 min and the reaction was terminated in an ice bath. Each reaction mixture was extracted with 5 ml toluene and vortex-mixed for 15 s. The tubes were allowed to stand for at least 20 min in the dark, at room temperature, to allow separation of the toluene and aqueous phases. Each toluene phase was then carefully collected into a clean test-tube and its absorbance was read at 520 nm. The free proline concentration in each sample was determined from a standard curve prepared using analytical grade proline, and expressed on a DW basis.

To extract and determine the total soluble sugars, 0.2 g sample of fresh leaves was homogenized in 10 ml of 96% (v/v) ethanol and washed with 5 ml 70% (v/v) ethanol (Irigoyen *et al.*, 1992). The extract was centrifuged at 3,500 × g for 10 min and the supernatant was stored at 4 ºC for measurement. Total soluble sugar concentrations were determined by reacting 0.1 ml of the ethanolic extract with 3 ml of freshly prepared anthrone reagent [150 mg anthrone plus 100 ml of 72% (v/v) sulphuric acid] and placed in a boiling water

bath for 10 min. After cooling, the absorbance of the mixture was recorded at 625 nm using a Bausch and Lomb-2000 Spectronic Spectrophotometer.

To extract and determine the ascorbic acid (AsA) from bean leaf samples, leaf material (1.0 g) was obtained from each replicate-plot of each treatment, homogenized immediately in liquid N_2 and extracted with 10 ml 5% (w/v) trichloroacetic acid (TCA) (Kampfenkel *et al.*, 1995). The homogenate was centrifuged at 4°C for 5 min at 15,600 × *g*. The supernatant was transferred to a clean reaction vessel and immediately assayed for AsA content in a 1.0 ml reaction mixture containing 50 µl 10 mM DTT, 100 µl 0.2 M phosphate buffer (pH 7.4), 0.5% (v/v) Nethylmaleimide, 10% (w/v) TCA, 42% (v/v) H_3PO_4, 4% (v/v) 2,2'-dipyridyl, and 3% (w/v) $FeCl_3$.

Leaf anatomy

For anatomical study, leaf samples were taken at 50 DAS. Samples were taken from the middle of the fifth leaf from apex. They were killed and fixed in FAA solution (50 ml 95% ethyl alcohol + 10 ml formalin + 5 ml glacial acetic acid + 35 ml distilled water) for 48 h. Thereafter, samples were washed in 50% ethyl alcohol, dehydrated and cleared in tertiary butyl alcohol series, embedded in paraffin wax of 54–56 °C m.p. Cross sections, 20 µ thick, were cut by a rotary microtome, adhesived by Haupt's adhesive and stained with the crystal violet–erythrosine combination (Sass, 1961), cleared in carbol xylene and mounted in Canada balsam. The sections observed and documented using an upright light microscope (AxioPlan, Zeiss, Jena, Germany). Measurements were done, using a micrometer eyepiece and an average of five readings were calculated.

Statistical analysis

The values for all parameters were subjected to statistical analysis (Gomez and Gomez, 1984). The 'F' test was applied to assess the significance of each treatment at the 5% level of probability ($P \leq 0.05$).

Results

Growth characteristics

Growth characteristics (i.e., shoot length, number and area of leaves per plant, and plant dry weight) of salt-stressed common bean plants were positively affected by salicylic acid (SA) and *Moringa oleifera* leaf extract (MLE) over two seasons as shown in Table 2. Integrated treatment applications of SA and MLE (i.e., seed soaking in SA + foliar spray with MLE and seed soaking in MLE + foliar spray with SA) significantly increased all growth characteristics compared to the control (seed soaking in tap water + foliar spray with tap water). The integrated treatment of seed soaking in SA + foliar spray with MLE was found to be the best, increasing shoot length, number of leaves per plant, leaf area per plant and plant dry weight by 14.6%, 13.0%, 20.6% and 34.6%, respectively in 2013 season and by 10.6%, 15.1%, 24.2% and 34.2%, respectively in 2014 season compared to the controls.

Table 2: Effect of integrated application of salicylic acid (SA) and moringa leaf extract (MLE) on some growth traits of common bean (*Phaseolus vulgaris* L. cv. "Bronco") plants grown under moderate soil salinity in 2013 and 2014 seasons

Treatments		Parameters			
Seed soaking	Foliar spray	Shoot length (cm)	Number of leaves plant^{-1}	Leaf area plant^{-1} (dm^2)	Plant dry weight (g)
2013 season					
Tap water	Tap water	26.7b[†]	7.67b	9.44b	6.02b
MLE	SA	29.6a	8.19a	11.10a	7.82a
SA	MLE	30.6a	8.67a	11.38a	8.10a
2014 season					
Tap water	Tap water	28.4b	7.63b	9.52b	6.14b
MLE	SA	30.5a	8.15a	11.47a	7.84a
SA	MLE	31.4a	8.78a	11.82a	8.24a

[†]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.

Total soluble sugars, free proline and ascorbic acid (AsA) concentrations

Over two seasons, the concentrations of total soluble sugars, free proline and AsA of salt-stressed common bean plants were positively affected by salicylic acid (SA) and *Moringa oleifera* leaf extract (MLE) as shown in Table 3. Integrated treatment applications of SA and MLE (i.e., seed soaking in SA + foliar spray with MLE and seed soaking in MLE + foliar spray with SA) significantly increased the concentrations of all mentioned parameters compared to the control (seed soaking in tap water + foliar spray with tap water). The integrated treatment of seed soaking in SA + foliar spray with MLE was found to be the best, increasing the concentrations of soluble sugars, free proline and AsA by 28.0%, 75.8% and 170.2%, respectively in 2013 season and by 35.5%, 59.2% and 159.5%, respectively in 2014 season compared to the controls.

Table 3: Effect of integrated application of salicylic acid (SA) and moringa leaf extract (MLE) on leaf concentrations of photosynthetic pigments, total soluble sugars, free praline and ascorbic acid (AsA) of common bean (*Phaseolus vulgaris* L. cv. "Bronco") plants grown under moderate soil salinity in 2013 and 2014 seasons

Treatments		Parameters		
Seed soaking	Foliar spray	Total soluble sugars (mg g^{-1} DW)	Free proline (µg g^{-1} DW)	AsA (µg g^{-1} FW)
2013 season				
Tap water	Tap water	3.22c[†]	240.2c	11.4c
MLE	SA	3.90b	381.2b	26.0b
SA	MLE	4.32a	422.2a	30.8a
2014 season				
Tap water	Tap water	3.41c	259.6c	12.1c
MLE	SA	4.32b	362.8b	27.1b
SA	MLE	4.82a	413.4a	31.4a

[†]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.

Green pod and dry seed yields

Pod and seed yields of salt-stressed common bean plants shown in Table 4 were positively affected by salicylic acid (SA) and *Moringa oleifera* leaf extract (MLE) over two seasons. Integrated treatment applications of SA and MLE (i.e., seed soaking in SA + foliar spray with MLE and seed soaking in MLE + foliar spray with SA) significantly increased pod and seed yields compared to the control (seed soaking in tap water + foliar spray with tap water). The integrated treatment of seed soaking in SA + foliar spray with MLE was found to be the best, increasing pod weight plant^{-1}, pod weight ha^{-1}, seed weight plant^{-1} and seed weight ha^{-1} by 76.5%, 74.1%, 20.7% and 20.4%, respectively in the first season and by 81.7%, 82.1%. 22.1% and 22.6%, respectively in the second season compared to the controls.

Table 4: Effect of integrated application of salicylic acid (SA) and moringa leaf extract (MLE) on green pod and dry seed yields of common bean (*Phaseolus vulgaris* L. cv. "Bronco") plants grown under moderate soil salinity in 2013 and 2014 seasons

Treatments		Parameters			
Seed soaking	Foliar spray	Pods weight plant^{-1} (g)	Pods weight ha^{-1} (ton)	Seed weight plant^{-1} (g)	Seed weight ha^{-1} (ton)
2013 season					
Tap water	Tap water	39.2c†	5.60c	11.1b	1.57b
MLE	SA	62.2b	8.77b	13.0a	1.83a
SA	MLE	69.2a	9.75a	13.4a	1.89a
2014 season					
Tap water	Tap water	40.4c	5.69c	11.3b	1.59b
MLE	SA	65.3b	9.21b	13.4a	1.89a
SA	MLE	73.4a	10.36a	13.8a	1.95a

†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.

Leaf anatomy

Leaf anatomy of salt-stressed common bean plants were positively affected by salicylic acid (SA) and *Moringa oleifera* leaf extract (MLE) in 2014 seasons as shown in Table 5 and Fig. 1. Integrated treatment applications of SA and MLE (i.e., seed soaking in SA + foliar spray with MLE and seed soaking in MLE +

foliar spray with SA) increased midvein length, midvein width, median vascular bundle width, median vascular bundle length, blade thickness, palisade tissue thickness and spongy tissue thickness compared to the control (seed soaking in tap water + foliar spray with tap water). The integrated treatment of seed soaking in SA + foliar spray with MLE was found to be the best, increasing the abovementioned anatomy parametrs by 34.2%, 31.8%, 23.5%, 21.7%, 38.9%, 66.7% and 50.0%, respectively compared to the controls.

Table 5: Effect of integrated application of salicylic acid (SA) and moringa leaf extract (MLE) on leaf anatomy of common bean (*Phaseolus vulgaris* L. cv. "Bronco") plants grown under moderate soil salinity in 2014 season

Treatments		Parameters						
Seed soaking	Foliar spray	midvein length (µ)	midvein width (µ)	median vascular bundle width (µ)	median vascular bundle length (µ)	blade thickness (µ)	palisade tissue thickness (µ)	spongy tissue thickness (µ)
Tap water	Tap water	1825	1100	425	575	180	60	80
MLE	SA	2325	1450	450	700	170	60	80
SA	MLE	2450	1450	525	700	250	100	120

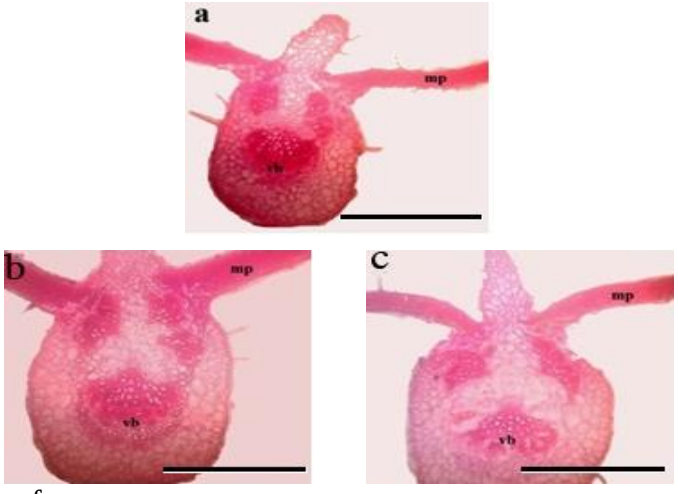

Fig. 1. Leaf anatomy

a. Control (seed soaking and foliar spray with tap water), b. SA seed soaking + MLE foliar spray, and c. MLE seed soaking + SA foliar spray. (mp = mesophyll and vb= vascular bundle). Scale bar = 200µ

4. Discussion

Soil salinity causes inhibitory effects on growth and productivity of plants such as reductions of water availability to plant roots that disturbs plant tissue water status and metabolic processes, leading to decreases in meristematic activity and cell enlargement. It also causes an increase in respiration rate due to the higher energy requirements (Kaydan and Okut, 2007; Abdul Qados, 2015). Salinity stress causes over-production of reactive oxygen species (ROS) in plant tissues. A balance between the generation and degradation of ROS is required to avoid oxidative injury and to maintain metabolic functions under stress conditions. In plant tissues, the level of ROS is controlled by an antioxidant system that consists of antioxidant enzymes and non-enzymatic low molecular weight antioxidant molecules, including proline and ascorbic acid (Semida and Rady, 2014a and 2014b). In the present study, the reduction in plant growth and productivity under the adverse conditions of soil salinity (controls of Tables 2 and 4) could be attributed to the osmotic effect resulting from salt stress that causes increase of growth inhibitors (i.e., abscisic acid), decrease of growth promoters (i.e., indole-3-acetic acid gibberellins) and disturbance of the water balance of saline-stressed plants. These inhibitory effects of salinity lead to stomatal closure, ionic imbalance, reduction in photosynthesis, disturbance in ionic homeostasis, accumulation of toxic ions and consequently inhibition of growth (Rady *et al.*, 2013; Semida and Rady, 2014b; Rady, 2011).

Common bean seed and plant treating with salicylic acid (SA; 1 mM) and *Moringa oleifera* leaf extract (MLE; 1 extract: 30 water by volume), used as seed soaking and foliar spray, respectively, significantly improved plant growth characteristics, plant productivity and anatomy, as well as osmoprotectants and ascorbic acid (AsA) under the adverse conditions of the studied soil salinity. Analysis of MLE revealed presence of essential macro- and micro-nutrients such as Ca, Mg, K, P, Fe, Mn, Cu and Zn. MLE also contains antioxidants including proline and AsA coupled with proline and total soluble sugars as osmoprotectants. It is also rich in phytohormones such as indole-3-acetic acid (IAA), gibberellins (GAs) and zeatin as a cytokinin (Table 2). This diverse composition of MLE indicates that this extract can be used as a plant biostimulant. Many researches highlighted MLE role to improve plant growth

and development in different crops (Rady *et al.*, 2013; Yasmeen *et al.*, 2012; Rehman *et al.*, 2014), which is also evident from results of the present study. Improved seedling growth traits i.e. shoot length, number and area of leaves per plant, and plant dry weight by MLE application might be due to the enhanced mobilization of germination related metabolites/inorganic solutes such as zeatin, ascorbic acid, Ca and K presented in MLE (Table 1) to the growing plumule and/or the increase in amylase activity and reducing sugars, contributing to early vigor and increased plant growth (Foidl *et al.*, 2001; Afzal *et al.*, 2012). In addition, the increased MLE content of IAA, GAs and zeatin encouraged plant growth and productivity under salt stress conditions. Seed soaking and/or seedling foliar application with MLE might provide strong and energetic start for earlier emergence and completed other phenological events well in time (Rehman *et al.*, 2014). Coupled with MLE, exogenous SA treatments ameliorated the negative effects of salt stress on growth characteristics of plants, which could be attributed to that SA causes an increase of CO_2 assimilation and photosynthetic rate and also causes an increase in mineral uptake by the salt-stressed plants (Fariduddin *et al.*, 2003; Szepesi *et al.*, 2005). In addition, the increase in growth parameters of salt-affected plants in response to SA might be attributed to the protective role of SA on membranes that might increase the tolerance of plants to salt stress (Aftab *et al.*, 2010). The SA-mediated increase in growth parameters of common been plants under salt stress could be also due to the SA-induced antioxidant function and metabolic activity in plants (Gunes *et al.*, 2007). However, combined treatments of SA + MLE showed the best results under saline condition, particularly the integrated treatment of seed soaking in SA + foliar spray with MLE, which ameliorated the deleterious effects of salt stress through preventing decreases in growth characteristics and plant productivity (Tables 2 and 4).

Maintenance of green leaf area and number of leaves per plant (Table 2) maximized photosynthesizing leaves, increasing sink capacity fulfilled through supply of photo-assimilates from stayed green leaves (Thomas and Howarth, 2000), and/or re-translocation of stem reserves of present study as a result of the application of cytokinin-rich MLE with SA that induces cytokinin biosynthesis. Presence of zeatin-like cytokinin in MLE prevents premature leaf senescence and maintains higher leaf area for photosynthetic activity. During

late stage of growth, endogenous levels of cytokinin are usually decreased and exogenously-applied cytokinin (found in MLE) can delay this process (Tetley and Thimann. 1974), possibly through activation of cytokinin dependant isopentenyl transferase (*ipt*) biosynthesis, increasing chlorophyll concentrations. Collectively with MLE, SA may also ameliorate the adverse effects of salt stress by increased levels of antioxidants. SA is a part of an extremely complex signal-transduction network, and its mode of action may differ between the different systems used. SA may influence the activity of certain enzymes directly, or may (directly or indirectly) induce expression of the genes responsible for these stress-protective mechanisms (Horvath *et al.*, 2007). The increased growth vigor of bean plants under salt stress were positively reflected in green pod and dry seed yields that might be attributed to more assimilate partitioning to developing edible parts and have been correlated with cytokinin levels (Zeatin) found in MLE (Dietrich *et al.*, 1995; Rady *et al.*, 2013), which was supported also by the exogenous SA in the combined MLE + SA treatments.

Soluble sugars are significantly increased in salt-stressed bean plants by the exogenous applications of MLE and/or SA (Table 3). They contribute to osmotic adjustment (Hayashi *et al.*, 1997), and can directly or indirectly modulate the expression of genes involved in metabolic processes, storage functions, and defence (Hebers and Sonnewald, 1998). It has been indicated that the oxidative damage generated during salinity stress is due to the imbalance in production of ROS and antioxidant activity alterations (Hernández *et al.*, 1993). To avoid the damage caused by oxidative stress, plants have developed many antioxidant systems; among non-enzymatic ones, the accumulation of proline is one of the most frequent changes induced by salinity or drought, although there is controversy concerning whether its accumulation is a stress resistance mechanism or a mere indicator of the existence of stress (Thakur and Sharma, 2005). One of the substrates of the Halliwell–Asada cycle is ascorbic acid (AsA) that also act as a non-enzymatic antioxidant in an isolated way on being involved in the direct reduction of ROS during different types of stress (Del Río *et al.*, 2006), taking part in the control of the H_2O_2 levels. This situation is reflected in the total concentration of AsA in our study, which are increased either with the treatments of MLE or SA, and its maximum concentration was noted with the combined application of SA seed soaking +

MLE foliar application, perhaps to overcome $O_2^{\bullet-}$ accumulation, since the AsA can directly eliminate $O_2^{\bullet-}$ and H_2O_2 in a non-enzymatic way (Foyer *et al.*, 1991). The healthy metabolic state of the stressed bean plants pretreated or treated with MLE resulted in the healthy plant growth (Table 2). This may be attributed to that MLE is excellent source in minerals, amino acids, soluble sugars and some antioxidants (Table 1). These increased proline and AsA antioxidants supported the antioxidant system in bean plants to enable them to tolerate salt stress. Collectively with MLE, SA causes increases in the concentrations of proline and AsA which, in turn, protect plants against the ROS generation and membrane injury, or may result in the synthesis of other substances having a protective effect on plants grown under salt stress (Xu *et al.*, 2008).

All enhanced parameters (i.e., growth traits, total soluble sugars, free proline, AsA and final yields) by the integrated application of SA and MLE were accompanied with the improved leaf anatomy (Table 5), which gave an opportunity to a good translocation of the absorbed nutrients into healthy cells to be used in different metabolic processes positively reflecting in vigorous growth and satisfactory yield under the adverse conditions of the tested saline soil (Semida *et al.*, 2014).

Salt stress tolerance in bean plants, in this study, was improved with the elevated antioxidant system, including non-enzymatic antioxidants (i.e., free proline and AsA) by the application of minerals, AsA, cytokinins, GAs and IAA-containing MLE (Table 1) singly or in combination with SA. *Moringa oleifera* leaves is a rich source in zeatin (Foidl *et al.*, 2001), minerals and other phytohormones, so the effectiveness of MLE in mitigating salinity stress. Coupled with MLE, SA supported the bean antioxidant defence system through increase of free proline and AsA concentrations, maintaining tissue water balance and ionic homeostasis (Rady and Mohamed, 2015).

Conclusion

This study revealed that the inhibitory effects of salt stress on the growth and productivity of common bean plants could be alleviated by the exogenous application of SA and *Moringa oleifera* leaf extract (MLE) that could protect the plants against injuries by salt stress. Soaking bean seeds in SA (1 mM)

applied in integration with foliar spray with MLE (1 extract: 30 water by volume) was the most effective integrated treatment in providing bean plants with salt tolerance when grown under moderate soil salinity. The increased concentrations of free proline and AsA as antioxidants and soluble sugars with free proline as osmoprotectants under salt stress by the abovementioned effective integrated treatment supported bean plants to improve their growth and productivity. *Moringa oleifera* trees are began to spread worldwide, so MLE became to easily obtain, and SA is readily available. Therefore, they could be used in combination (SA seed soaking + MLE foliar spray) to prevent losses in plant growth and productivity under salt stress and may have significant practical applications.

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