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Modulation of salt stress effects on the growth, physio-chemical attributes and yields of *Phaseolus vulgaris* L. plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract

Mostafa M. Rady*, Gamal F. Mohamed

Botany Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt

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ABSTRACT

Two field experiments were aimed to study the effects of salicylic acid (SA; 1 mM) and *Moringa oleifera* leaf extract (MLE; 1 extract: 30 tap water), used singly or in combination on the growth, physio-chemical attributes and yields of common bean (*Phaseolus vulgaris* L.) plants grown on a saline soil (EC = 6.23 – 6.28 dS m^{-1}). The SA or MLE application, used as seed soaking or foliar spray, improved growth characteristics (i.e., shoot length, number and area of leaves per plant, and plant dry weight) and physio-chemical attributes (i.e., RWC% and MSI%, concentrations of total chlorophylls, total carotenoids, total soluble sugars, free proline and ascorbic acid, contents of N, P, K and Ca, and ratios of K/Na and Ca/Na) in bean plants. In addition, green pod and dry seed yields were improved when compared with the controls (tap water seed soaking or foliar spray). Combined treatments of SA and MLE (i.e., seed soaking in SA + foliar spray with SA, seed soaking in SA + foliar spray with MLE, seed soaking in MLE + foliar spray with SA, and seed soaking in MLE + foliar spray with MLE) significantly increased all abovementioned parameters compared to the control (seed soaking in tap water + foliar spray with tap water). In contrast, there were significant reductions in leaf EL% and Na% under these combined treatments. The combined seed soaking in SA + foliar spray with MLE treatment was found to be highly effective at improving the growth and yields of bean plants by alleviating the inhibitory effects of soil salinity stress.

1. 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). Food legumes, including beans, are an important component of the agricultural sectors of developing countries 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).

Salinity considers one of the major factors affecting the agricultural productivity worldwide, particularly in the arid and semiarid regions. In these regions, soil salinization caused by some effective factors such as 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 (Gama et al., 2007; Rady et al., 2013). Salinity reduces plant growth and yield due to a reduction in utilize water, and changes the plant metabolic processes (Munns, 1993, 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 (Munns and Termaat, 1986; Marschner, 1995).

Plant biostimulants (i.e., *Moringa oleifera* leaf extract; MLE) and antioxidants (i.e., salicylic acid; SA) are substances when applied as seed soaking and/or foliar spray positively modify plant growth and production with alterations in metabolic processes (European Biostimulants Industry Council, 2012; Rady et al., 2013; Semida and Rady, 2014a) under normal or stress conditions.

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 obtained from fresh moringa leaves posses' high antioxidant activity are rich in some plant secondary metabolites and osmoprotectants (Rady et al., 2013). They also found that MLE is also a source of zeatin, a

* Corresponding author. Fax +2 0846343970.
E-mail addresses: mrady2050@gmail.com, mmr02@fayoum.edu.eg (M.M. Rady).

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natural derivative of cytokinin, vitamins and several mineral elements, making it a potential natural growth stimulant. Like other biostimulants, the potential of MLE when applied through seed or plant foliage have been shown to improve the plant tolerance to abiotic stresses, including salinity (Yasmeen et al., 2013; Rady et al., 2013; Howladar, 2014). These reports and others have been shown that MLE application improved crop performance, resulting from vigorous seedling growth, maintained optimum tissue water status, improved membranes stability, 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^+ (Yasmeen et al., 2012; Rehman et al., 2014). The MLE act at low or even diluted concentration of 1:30, and the chemical composition of this extract may vary with species, season of collection and extraction procedure used.

Salicylic acid (SA) is classified as phenolic growth regulator, a non-enzymatic antioxidant, a signaling or messenger molecule in plants to modify plant responses to environmental stressors. It 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 time application, SA has different effects on stress adaptation and damage development of plants (Metwally et al., 2003). Some earlier reports have shown that exogenous SA has obtained particular attention because of inducing protective effects on plants under NaCl salinity, and the effects of cytotoxicity induced by salt stress can be ameliorated by the exogenous application of SA (Simaei et al., 2011, 2012).

The present work was designed with objective to evaluate the potential effects of the exogenous application of MLE and/or SA on the changes in growth, yields and endogenous physio-chemical constituents of *Phaseolus vulgaris* L. plants, exposed to moderate soil salinity stress (EC = 6.23 – 6.28 dS m^{-1}) and to establish a relationship between the changes in physio-chemical constituents and the degree of tolerance, in terms of improvement in growth and yields. The hypothesis tested is that exogenous applications of MLE and/or SA used as seed soaking and/or foliar spray will elevate the level of some antioxidants and osmoprotectants that will protect the stress generated by salinity stress.

2. Materials and methods

2.1. Soil analysis and preparation, plant material and experimental procedures

Two field experiments were conducted in two successive seasons (2013 and 2014) 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 15.2°– 26.8 °C, with an average of 21.0° ± 2.5°C. The daily relative humidity averaged 58.0 ± 4.2%, and ranged from 31 to 85%. In addition, the daily temperatures ranged from 13.8° – 28.8 °C, with an average of 21.3° ± 2.8°C, and the daily relative humidity averaged 56 ± 6.6% and ranged from 28 to 84% were for the 2014 season.

Healthy common bean (*Phaseolus vulgaris* L.) cv. Bronco seeds 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, and were sown at the equivalent of 95 kg ha^{-1} to achieve the recommended planting density. Seeds 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 °C). 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.

Table 1
Physical and chemical properties of the experimental soil in two seasons.

Parameter	2013 season	2014 season
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

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.

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 according to Dahnke and Whitney (1988). 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.

2.2. 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 h 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 8000 × *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 2.