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Presoaking application of propolis and maize grain extracts alleviates salinity stress in common bean (*Phaseolus vulgaris* L.)

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ABSTRACT

Presoaking application of propolis extract (PE) or maize grain extract (MGE) for bean seeds to improve their germination and growth under 100 mM NaCl stress was investigated. The potential effects of these extracts on seed germination, seedling growth, dehydration tolerance, antioxidant system and the concentrations of some osmoprotectant molecules and phytohormones were evaluated. PE or MGE alleviated the adverse effects of NaCl-stress to varying degrees. Despite the preference for MGE, soaking seeds in either extract, prior to germination and growth under NaCl-salinity, increased the seed germination percentage, seedling growth, the cell membrane stability index, the relative water content, the concentrations of free proline, total free amino acids, total soluble sugars, indole-3-acetic acid (IAA) and gibberellic acid (GA_3), and the activity of the antioxidant system, while it reduced lipid peroxidation, electrolyte leakage and abscisic acid (ABA) compared to soaking seeds in distilled water. These results are important as the potential of MGE or PE to alleviate the harmful effects of NaCl stress offers an opportunity to increase the resistance of common bean plants to growth under saline conditions.

1. Introduction

Common been (*Phaseolus vulgaris* L.) considers one of the most important vegetable crops grown in Egypt, and occupies a great figure in local consumption and export. Beans are widely cultivated on newly-reclaimed soils in Egypt. However, most of these soils are affected by different levels of salinity. Salinity is one of the major limiting factors to plant growth and crop productivity in many arid and semi-arid areas in the world. Salt stress affects plant physiology, both at whole plant and cellular levels, through osmotic and ionic stress. It generates a physiological drought or osmotic stress by affecting the water relations of the plant (Munns, 2002). Accumulation of the toxic amounts of salts in the leaf apoplasm leads to dehydration and turgor loss, consequently death of cells and tissues. Photosynthesis considers one of the most severely affected processes during salinity stress, which is mediated by decreased chlorophyll pigment (Sabra et al., 2012; Kchaou et al., 2013), inhibition of rubisco (Soussi et al., 1998) and closure of stomata, thereby, decreasing the leaf CO_2 assimilation rate (Yiu et al., 2012). It has also been demonstrated that salinity stress affects the nitrogen metabolism by affecting various enzymes (Soussi et al., 1998; Gong et al., 2013). All these and other alter processes lead to poor plant growth and subsequently the productivity. However, lipid peroxidation and the antioxidant system are reported to be stimulated by salt stress (Sairam et al., 2005; Rady, 2011), and further stimulated by some applications to alleviate the adverse conditions of salinity (Yasmeen et al., 2012; Korkmaz et al., 2012; Rady et al., 2013).

In recent years, a growing interest has been observed with natural bio-stimulating substances. Propolis (bee glue) is the generic name for the resinous substance collected by honeybees (*Apis mellifera* L.) and considered to be effective against a variety of bacteria, viruses, fungi and molds. There are numerous literature data identifying the chemical composition of propolis (Crane, 1990; Bankova et al., 2000; Abd El-Hady and Hegazi, 2002). They reported that propolis contains several important compounds which observed to affect the activity of many physiological processes in plants. Amino acids, sugars, certain vitamins (particularly, B-group, C and E), several minerals, terpenes and sesquiterpenes are considered to be some of these important compounds. We are aware the importance of these compounds for plants, particularly terpenes and sesquiterpenes. Terpenoids are considered to be the precursors of many phytohormones (particularly, gibberellins), which are necessary for growing plants under various stresses.

Extracts from plant parts such as phytohormone and antioxidant-containing leaves, seeds and/or grains has been reported to affect different physiological functions. The beneficial effects of plant's natural extracts on growth, yield and some chemical constituents have been reported on seaweed extracts (Zhang and Ervin, 2004; Sultana et al., 2005; Bai et al., 2007), dry

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bean extracts (Abd El-Naem et al., 2007), *Moringa oleifera* extracts (Yasmeen et al., 2012, 2013; Rady et al., 2013), and also on maize grain extract (Rady and Seaf El-Yazal, 2009).

The present work was designed with objective to examine the changes in antioxidant system under the effect of PE or MGE, applied by seed soaking, on the *P. vulgaris* L. seedlings, exposed to 100 mM NaCl and to establish a relationship between the changes in antioxidant system and the degree of tolerance, in terms of improvement in seedling growth, cell membrane stability, drought tolerance and the concentrations of phytohormones, free proline, total free amino acids and total soluble sugars. The hypothesis tested is that PE or MGE will positively modify the level of antioxidant system that will protect the stress generated by NaCl-salinity. In addition, these extracts will help in enhancing bean performance better than synthetic growth promoters.

2. Materials and methods

2.1. Plant material and growing conditions

Seeds of common bean (*P. vulgaris* L., cv. Nebraska) were obtained from the Agricultural Research Center, Giza, Egypt. They ($n = 240$) were surface-sterilized in 0.1% (v/v) mercuric chloride for 2 min and washed three-times in distilled water at 25 ± 2 °C. The seeds were divided into three groups ($n = 80$ per group) and soaked for 4 h in distilled water (group 1), PE (1% active ingredients; group 2), or MGE (2% active ingredients; group 3), then allowed to air-dry overnight. The selection of these concentrations (1% and 2% active ingredients of PE and MGE, respectively) was based on a preliminary study (data not shown). These concentrations resulted in the best growth, therefore they were selected for this experiment which was designed in randomized blocks. Seeds of each group were divided into two sub-groups ($n = 40$ per sub-group). One sub-group of each was germinated using distilled water, while the second sub-Group of each was germinated in 100 mM NaCl. For each treatment, five seeds were placed on Whatman No. 1 filter paper in a 12 cm sterile Petri dish. Forty replicate Petri dishes, each with five seeds, were maintained for each treatment. The seeds were allowed to germinate in the dark at 25 ± 2 °C. The number of germinated seeds was recorded each day until it remained constant. After germination, the Petri dishes were exposed to a 13 h photoperiod. The experiment was terminated after 12 d. The 12-d-old seedlings from each treatment were then collected for various measurements.

2.2. Preparation of propolis (PE) and maize grain (MGE) extracts

For the extraction of propolis, local raw material of propolis was collected from honeybee colonies of the apiary of Faculty of Agriculture, Fayoum University. The collected samples were mixed together and active ingredients were extracted by ethanol (95%) as described by Vechet (1978). The ethanol extraction was filtered and the alcohol was evaporated under vacuum (30 °C) using rotary evaporator, Buchi model-011. The extract was diluted by distilled water to the final concentration required (1% active ingredients) and kept cool at 4 °C until use or used immediately for seed soaking.

MGE was prepared as described by Rady and Seaf El-Yazal (2009). A weight of 0.5 kg of maize grains of a genotype Balady (a local type frequently handled by many farmers) were soaked in 1 l water for 48 h then, well ground and filtered under vacuum through Whatman No. 1 paper. The obtained extract was condensated to obtain an extract of 2% active ingredients, and then used immediately for seed soaking.

Table 1
Some of the chemical characteristics of the propolis (PE) and maize grain (MGE) extracts identified by GC/MS.

Parameter (units)	PE	MGE
Total terpenoids (%)	2.28	–
Total flavenoids (%)	0.26	–
Phenolic acids (%)	0.31	–
Total gibberellins (ppm)	–	2.83
Total indoles (ppm)	–	3.24
Indole-3-acetic acid (ppm)	–	1.82
Cytokinins (ppm)	–	1.96
Total sugars (%)	1.37	2.97
Total amino acids (%)	0.21	0.11
Ascorbic acid; vitamin C (ppm)	97	30
Total B-group vitamins (ppm)	158	–
Vitamin E (ppm)	64	–
Nitrogen (ppm)	–	115
Phosphorus (ppm)	–	17
Potassium (ppm)	164	127
Magnesium (ppm)	52	–
Calcium (ppm)	67	–
Iron (ppm)	23	12
Mn (ppm)	13	6
Iodine (ppm)	11	–
Zn (ppm)	10	5
Cu (ppm)	7	3

Chemical characteristics of PE and MGE, which were determined and identified by GC/MS in a specialized laboratory in National Research Center, are presented in Table 1.

2.3. Plant growth analysis

Twelve-d-old seedlings taken from each treatment were weighed for fresh weight, and then placed in an oven at 70 °C to reach a constant dry weight, which was recorded.

2.4. Determination of membrane stability index, electrolyte leakage and relative water content

The membrane stability index (MSI) was estimated as described by Rady (2011) using duplicate 0.2 g samples of fully-expanded leaf tissue. Each sample was placed in a test-tube containing 10 ml of double-distilled water and heated at 40 °C in a water bath for 30 min. The electrical conductivity (C_1) of the solution was recorded using a conductivity bridge. A second sample was boiled at 100 °C for 10 min, and the conductivity was measured (C_2). The MSI was calculated using the formula:

$$\text{MSI}(\%) = \left[1 - \left(\frac{C_1}{C_2}\right)\right] \times 100$$

The total leakage of inorganic ions from fully-expanded leaves was determined using the method of Sullivan and Ross (1979). Twenty leaf discs were placed in a boiling tube containing 10 ml deionised water and the electrical conductivity (EC_1) was recorded. The contents were then heated to 45–55 °C for 30 min in a water bath and the electrical conductivity (EC_2) was recorded. The sample was then boiled at 100 °C for 10 min and the electrical conductivity (EC_3) was recorded. Electrolyte leakage was calculated using the formula:

$$\text{Electrolyte leakage}(\%) = \left[\frac{EC_2 - EC_1}{EC_3}\right] \times 100$$

Excluding the midrib, fresh 2 cm-diameter fully-expanded leaf discs ($n = 3$ per sample) were used to determine the relative water content (RWC). The discs were weighed (fresh mass; FM) and immediately floated on double-distilled water in Petri dishes for 24 h, in the dark, to saturate them with water. Any adhering water was blotted dry and the turgid mass (TM) was measured. The dry