South African Journal of Botany 106 (2016) 221–231

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Exogenous applications of moringa leaf extract effect on retrotransposon, ultrastructural and biochemical contents of common bean plants under environmental stresses

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ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form 1 June 2016
Accepted 14 July 2016
Available online 3 August 2016

Edited by AR Ndhlala

Keywords:
Phaseolus vulgaris
Salinity
High temperature
Gamma rays
Retroelement
Phytohormones
Hydrogen peroxide
Glutathione
Ultrastructure

ABSTRACT

Moringa oleifera leaf extract (MLE) is rich in amino acids, ascorbate, zeatin, minerals, and many other compounds known for their growth-promoting potential. Therefore, a pot experiment was aimed to study the effect of foliar spray with aqueous extracts of *Moringa oleifera* leaves on some physiological, biochemical, and molecular markers of common bean plants (*Phaseolus vulgaris*) grown under salt stress (200 mM), high temperature (45 °C), and gamma rays (200 Gy). The results showed that the shoot and root length, fresh and dry weights of shoots and roots, photosynthetic pigments, and phytohormone contents were decreased significantly in stressed plants as compared with untreated plants under all environmental stresses. On the other hand, total soluble sugars content, glutathione, malondialdehyde, $O_2^{\bullet-}$, and H_2O_2 contents were significantly increased in stressed plants as compared to untreated plants. The exposure to different environmental stresses caused damage in leaf ultrastructure, which led to both vesiculation and swelling in the chloroplast stroma. In addition, many retrotransposons were showed to be activated in response to environmental stresses. Retrotransponson detecting the differences between control and stress samples in terms of different fragment length which reflects the effect of MLE and both stresses. The foliar application of moringa leaf extract caused significantly increased in all the above parameters. Results of this study suggested that MLE as antioxidant could activate the antioxidants in plants to enable them to alleviate the oxidative damage leading to improvements in physiological and molecular attributes in plants grown under the adverse conditions of environmental stresses.

1. Introduction

The world population is increasing at an alarming rate and is expected to reach about six billion by the end of 2050, but food productivity is decreasing due to the effect of various abiotic stresses and climatic change; minimizing these losses is a major area of concern for all nations to cope with increasing food requirements. Also, plants are subjected to various environmental stresses and affect on their growth. The adverse effect of environmental stresses is the enhanced accumulation of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) (Ashraf, 2009). Excess of ROS caused phytotoxic reactions such as lipid peroxidation, DNA mutation, and protein degradation.

Salinity is one of the most important abiotic stress factors limiting plant growth, photosynthesis, and productivity (Mohamed and Gomaa, 2012). Approximately 22% of the world agricultural land is saline (FAO, 2004). Egypt is one of the countries that suffer from severe salinity problems. For example, 33% of the cultivated land, which comprises only 3% of total land area in Egypt, is already salinized due to low precipitation (25 mM annual rainfall) and irrigation with saline water (El-Hendawy et al., 2004).

High temperature is considered one of the most important environmental factors that affect growth and development of plant (Roy and Ghosh, 1996). High temperature stress can create a water deficit in plants tissues which in turn lead to injury of cell membranes and to reduction in rates of transpiration, protein synthesis, and ion uptake and transport. Also, high temperature can inhibit photosynthetic enzymes as well as loss of permeability of cellular membranes (Levitt, 1980).

Gamma rays can damage or modify important components of plant cells and have been reported to affect the morphology, anatomy, biochemistry, and physiology of plants depending on the radiation dose (Ashraf et al., 2003).

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http://dx.doi.org/10.1016/j.sajb.2016.07.010
0254-6299/

Exogenous application of plant growth regulators, antioxidants, certain nutrients, organic and inorganic chemicals have been used to promote plant growth and development for inducing abiotic and biotic stress tolerance that results in higher economic return (Mohamed and Gomaa, 2012; Abd El-Rahman and Mohamed, 2014). The continuous use of synthetic chemicals to quench ROS is usually not cost-effective and environmentally friendly. The search for safe and effective naturally occurring antioxidants is now focused on edible plants, especially spices and herbs (Nakatani, 1997). Among naturally occurring plant growth enhancers, *Moringa oleifera* has attained enormous attention because of having cytokinin like zeatin, antioxidants such as ascorbic acid, flavonoid, phenolics, carotenoids, amino acids, macro and micro nutrients in its leaves (Foidl et al., 2001; Yasmeen, 2011). Treatment of many crops with moringa leaf extract (MLE) promoted seed germination, growth and productivity under normal (Nouman et al., 2012) and stress conditions (Yasmeen et al., 2012, 2013).

Retrotransposons (RTs) are the main groups of TEs. The latter move ('or jump') by copy-and-paste way of life cycles through molecular steps of transcription, reverse transcription, and integration of the cDNA copies back into host genome (Geuking et al., 2009). Many retrotransposons were shown to be activated in response to environmental stresses and activation is under the control of *cis*-regulatory sequences strikingly similar to those of plant defense genes (Grandbastien, 1998).

Common bean (*Phaseolus vulgaris* L) is the most important grainlegume cultivated for human consumption (Ogbonnaya et al., 2003). In Egypt, bean is one of the most popular vegetable crops and hence an export reached about 24,704 tons in 2006 (FAO, 2009).

Therefore, this study aimed to measure the potential effects of the exogenous application of moringa leaf extract on some physiological, biochemical, molecular, and ultrasturcture parameters of common bean plants grown under different environmental stresses and help the plants to overcome these stresses and increased yield productivity.

2. Materials and methods

2.1. Plant material and growth conditions

A pot experiment was conducted in a wire-house at the Faculty of Education, Ain Shams University, Cairo, Egypt (30°20′ N; 31°53′ E) from 25 October 2014 to 5 January 2015. During this period, daily temperatures ranged from 15.3 to 26.6 °C, with an average of 20.2 ± 3.4 °C. Daily relative humidity averaged 55.8 ± 8.4%, and ranged from 41.5 to 70.6%.

Seeds of common bean (*Phaseolus vulgaris* cv. Karnac) plants were obtained from Agricultural Research Station at AL Kanater area near Cairo, Ministry of Agriculture, Egypt. Homogeneous seeds were surface sterilized by 0.01 M $HgCl_2$ solution for 3 min and then washed thoroughly with distilled water. Common bean seeds were sown during 25 October 2014 and plants were harvested on 5 January 2015.

Ten, uniform, air-dried common bean seeds were sown in each pot (25 cm in diameter and 25 cm in depth) containing approximately 3.5 kg of homogeneous loamy clay soil. Each treatment contains ten pots. A granular commercial *Rhizobium leguminosarum* (obtained from the Biofertilizer Inoculum Production Unit, Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Centre, Giza, Egypt) was incorporated into the top 30 mm of soil in each pot with the seeds at the time of sowing.

2.2. Treatment and sample preparation

The pots were divided into four groups. Each group contained fives pots as replications. The first group was irrigated with the water holding capacity of the soil (80%) and served as control. The second group, dry seeds were irradiated at National Centre for Radiation Research and Technology, Nasr City. Gamma irradiation source was by using Cesium-137 with 200 Gy and then the seeds planting in the pots. The third group, the seedlings after 15 days from sowing were irrigated with 200 mM sodium chloride. The fourth group, the seedlings after 15 days from sowing were transferred to an incubator and were exposed to high temperature (45 °C) for 5 h for 2 days and then transferred to the normal temperature.

2.3. Applications of moringa leaf extract (MLE)

The treated plants were foliar sprayed with water or 1:30 solution of MLE at early morning with a sprayer (Vol. 20 L) to run-off twice, at 30 and 37 days after sowing (vegetative stage). The concentrations of MLE, and the number and timing of sprays, 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.

2.4. Plant sampling

After 40 days of sowing, the plants (vegetative stage) were collected to determine the following parameters (shoot and root lengths, fresh and dry weights of shoots and roots), photosynthetic pigments, pytohormones, total soluble sugars, MDA, H_2O_2, O_2, and glutathione. In addition, ratro marker and ultrastructure examination were done in leaves of untreated and treated plants. After 70 days of sowing (harvested stage), yield was collected and seed yield was analyzed.

2.5. Source of moringa leaves

Young leaves/branches of moringa were harvested from young full grown trees located in botanical garden at Faculty of Education, Ain Shams University.

2.6. Preparation of moringa leaf extract

Fresh mature moringa leaves were collated from mature moringa trees at the Faculty of Education Ain Shams University. After collection, 200 g of leaves was washed and stored overnight at freezing temperatures which yielded 125 ml of MLE. The extracted MLE was sieved through cheesecloth and diluted 30 times with distilled water to prepare 1:30 solution of MLE (Nouman et al., 2012).

2.7. Biochemical analyses in moringa leaves

2.7.1. Determination of total phenols

Levels of soluble phenols in moringa leaves were determined in accordance with Dihazi et al., 2003). A known weight of fresh samples was selected and extracted with 80% cold methanol (v/v) for three times at 90 °C. The combined extract was collected and filtered through Whatman No. 1 filter paper. After filtration, the filtrate was made up to a known volume with cold methanol. A known volume of the extract (0.5 ml) was added to 0.5 ml folin–Cicalteu reagent and shacked well. The mixture was allowed to stand for 3 min. One milliliter of saturated sodium carbonate solution (25 g Na_2CO_3 were dissolved in 1000 ml distilled water at 70–80 °C and cooled down and filtered) was added to the mixture and shaken well. The mixture was allowed to stand for 60 min. The optical density was measured at 725 nm UV–Vis spectrophotometer. The quantity of total phenolic compounds was calculated according to the standard curve of Tannic acid (99.5%).

2.7.2. Determination of total flavonoids

The total flavonoids were measured by the method of Bushra et al. (2009). Briefly, extracts of each plant material (1 ml) were diluted with 4 ml water in a 10 ml volumetric flask. 5% $NaNO_2$ solution (0.3 ml) was added to each volumetric flask; after 5 min, 10% $AlCl_3$ (w/w) was added, and at 6 min, 1.0% M NaOH (2 ml) was added.