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Sequenced application of ascorbate-proline-glutathione improves salt tolerance in maize seedlings

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ABSTRACT

The role of antioxidants exogenously-applied individually or in sequences in the improvement of salt tolerance in maize seedlings, and their effects on changes in the activities of endogenous enzymatic and non-enzymatic antioxidants, and the concentrations of phytohormones in seedlings grown under 100 mM NaCl stress were assessed. The efficiency of maize seedlings to tolerate salt stress in terms of growth was noticed to varying degrees with antioxidants applied singly or in sequences. The healthy growth of salt-stressed seedlings was correlated with the improvements in the activities of enzymatic and non-enzymatic antioxidants, the concentrations of osmoprotectants and phytohormones, and tissue health in terms of relative water content and membrane stability index. Results show that, seed soaking in AsA, GSH and proline applied in sequences (i.e., $AsA_{0.50}$-$Pro_{0.50}$-$GSH_{0.50}$ or $GSH_{0.50}$-$Pro_{0.50}$-$AsA_{0.50}$) was better than their applications individually. In addition, the sequenced application of $AsA_{0.50}$-$Pro_{0.50}$-$GSH_{0.50}$ as integrated treatment was better, generating maize seedlings more tolerant to salinity than those generated from the sequenced application of $GSH_{0.50}$-$Pro_{0.50}$-$AsA_{0.50}$. Therefore, we recommend using the sequenced application of $AsA_{0.50}$-$Pro_{0.50}$-$GSH_{0.50}$ as integrated soaking treatment for maize to grow under salt stress.

1. Introduction

Nowadays, maize is considered as a direct staple food for millions of individuals and through indirect consumption as feed crop worldwide, making it as essential component of global food security (Campos et al., 2004).

Salinity is a widespread environmental stress that affects almost every aspect of the physiology and biochemistry of plants, significantly reducing the plant growth and productivity. A metabolic response to salt stress is the synthesis of compatible osmolytes. These compounds mediate osmotic adjustment and therefore protect sub-cellular structures and reduce oxidative damage caused by free radicals (Parida and Das, 2005; Younis and Tourky, 2014). Oxidative stress is initiated by reactive oxygen species (ROS), which are produced and fairly accumulated during normal aerobic metabolism. Failure to quench or inactivate the ROS may lead to the degradation of macromolecules in the plant cells such as membrane lipids, proteins and nucleic acids (Sharma et al., 2012). Of the main symptomatic parameters for oxidative stress developed in response to high salinity, we may refer to H_2O_2 production. Due to the imbalance in ROS production and antioxidant activity, H_2O_2 level increases with increasing salinity in different plants (Younis and Tourky, 2015). A rapid decline in H_2O_2 concentration in various plant tissues has been observed in response to exogenous application of various adaptive compounds (Hossain and Fujita, 2010; Azzedine et al., 2011; Rady et al., 2013; Rady and Hemida, 2015).

Plant possesses a battery of antioxidative mechanisms to scavenge the ROS. These include non-enzymatic antioxidants like ascorbic acid (AsA), reduced glutathione (GSH), α-tocopherol (TOC) and carotenoids, and enzymatic antioxidants such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) (Parida and Das, 2005; Younis et al., 2010). Counteraction of the adverse effects of salt stress can be managed by the exogenous application of compatible solutes, antioxidants and plant growth regulators (Gunes et al., 2007; Khattab, 2007; Younis et al., 2010; Rady, 2011; Rady and Hemida, 2015; Rady and Mohamed, 2015). Plants are found to synthesize low molecular weight antioxidants such as glutathione and ascorbate within the chloroplast stroma and cytosol using nicotinamide adenine dinucleotide phosphate (NADPH) as the ultimate electron donor (Kasote et al., 2015). These low molecular weight antioxidants are functioned to do as redox buffers that interact with numerous cellular components and influence plant growth

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and development by modulating processes from mitosis and cell elongation to senescence and death (Sharma et al., 2012). In addition, these antioxidants may influence gene expression associated with biotic and abiotic stress responses to maximize defense of stressed plants.

Vitamin C (ascorbic acid/ascorbate) is generated during aerobic metabolism, after which it reacts rapidly with superoxide ($O_2^{-\bullet}$), singlet oxygen ($O^{-\bullet}$) and ozone (chemically), and H_2O_2 (enzymatically) through ascorbate peroxidase to neutralize their toxic effects. Vitamin C also helps to regenerate antioxidant pigments, carotenoids (carotenes and xanthophylls) and vitamin E. Glutathione is a redox active molecule that can present in a reduced form (reduced glutathione; GSH) or an oxidized disulfide form (oxidized glutathione; GSSG). It plays important roles in biosynthetic pathways, detoxification, antioxidant biochemistry and redox homeostasis (Noctor et al., 2012). The GSSG is reduced to GSH by the enzyme glutathione reductase, which requires NADPH as the reducing power. The GSH acts as an antioxidant by quenching the ROS and is involved in the ascorbate–glutathione cycle, which eliminates damaging peroxides (Kasote et al., 2015). The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Islam et al., 2011; Abdelhamid et al., 2013). The increase in proline content is the most remarkable parameter in salt stressed plants (Rady et al., 2013; Fahramand et al., 2014; Rady and Mohamed, 2015). There are several techniques to enhance the endogenous proline accumulation for salt defense mechanism such as exogenous application (Rady and Mohamed, 2015), biosynthesis gene(s) over-expression (Hayat et al., 2012) and degradation gene(s) knock-out (Gupta and Huang, 2014). The endogenous proline accumulation in salt stressed plants has been utilized as effective indicator for salt tolerance. Moreover, multivariate biochemical and physiological parameters, growth performances and yields have been applied to classify salt tolerant cultivars in maize (Neto et al., 2006), wheat (El-Hendawy et al., 2005), rice (Zeng, 2005), cowpea (Murillo-Amador et al., 2006), tomato (Juan et al., 2005), seashore paspalum (Lee et al., 2008), and chickpea (Maliro et al., 2008).

Due to the considerable evidence of the adverse effects of salinity on plant growth, it was proved, from previous works, that exogenous application of low molecule antioxidants can alleviate the adverse salt stress effects (Aly-Salama and Al-Mutawa, 2009; Azzedine et al., 2011; Semida et al., 2014; Rady and Mohamed, 2015; Rady et al., 2016). Therefore, the aim of this study was to evaluate the potential increase of salt tolerance in maize seedlings by using the first time-applied technique of a sequenced application, used as seed soaking, of AsA, GSH and proline. Seedling growth, membrane stability and cell turgid, osmoprotectants, low molecular weight antioxidants, enzymatic antioxidants and phytohormones were assessed to see whether these parameters were improved, by using this new technique, to support the antioxidative defense system of maize seedlings to increase their salt tolerance.

2. Materials and methods

2.1. Plant material and growing conditions

Seeds of maize (*Zea mays* L., cv. Giza 129) were obtained from the Agricultural Research Center, Giza, Egypt. They (n=720) were surface-sterilized in 0.1% (v/v) mercuric chloride for 2 min, washed three-times in distilled water at 25 ± 2 °C, and then left to dry for 1 h. Sterilized seeds were divided into six groups (n=120 per group). Seeds of group 1 were soaked in distilled water for 24 h. Seeds of groups 2, 3 and 4 were soaked in proline (0.5 mM), glutathione (0.5 mM) and ascorbic acid (0.5 mM), respectively for 24 h. In addition, seeds of group 5 were sequentially soaked in glutathione (GSH; 0.5 mM) for 9 h, proline (Pro; 0.5 mM) for 8 h and ascorbic acid (AsA; 0.5 mM) for 7 h, and seeds of group 6 were sequentially soaked in AsA (0.5 mM) for 9 h, Pro (0.5 mM) for 8 h and GSH (0.5 mM) for 7 h. After finishing the soaking durations, seeds were allowed to air-dry overnight. The selection of AsA, Pro and GSH concentrations (0.5 mM for each) and the sequenced soaking periods were based on a preliminary study (data not shown). These concentrations and sequenced soaking periods resulted in the best growth in terms of seedling fresh and dry weights; therefore they were selected for this experiment which was designed in randomized complete blocks. Seeds of each group were divided into two sub-groups (n=60 per sub-group), one was germinated and grown using distilled water, while the second sub-group was germinated and grown in 100 mM NaCl. For each treatment, five seeds were placed on Whatman No. 1 filter paper in a 12 cm sterile Petri dish. Twelve replicate-Petri dishes, each with five seeds, were maintained for each treatment (sub-group). The treated seeds were allowed to germinate in the dark at 25 ± 2 °C. After germination, the Petri dishes were exposed to a 14 h photoperiod. The experiment was lasted after 15 d. The 15-d-old seedlings from each treatment (sub-group) were then collected for various measurements.

2.2. Seedling growth measurements

The 15-d-old seedlings (n=20) were selected at random from each of the different treatments and weighed (fresh weight; FW), then placed in an oven at 70 °C to reach a constant dry weight (DW) which was recorded.

2.3. Determination of the membrane stability index (MSI), electrolyte leakage (EL) and relative water content (RWC)

Excluding the midrib, twenty discs of fresh 2 cm-diameter fully expanded leaf were used to determine the RWC. Fresh weight (FW) of discs were taken and immediately floated on double distilled water in Petri dishes for 24 h, in dark, to saturate them with water. Any adhering water was blotted dry and the turgid weight (TW) was recorded. Dry weight (DW) was measured after dehydrating the discs at 70 °C for 48 h. The RWC was then calculated using the following formula (Hayat et al., 2007):

$$RWC\,(\%) = \left[(FW–DW)/(TW–DW)\right] \times 100$$

The MSI was estimated as described by Premchandra et al. (1990) and modified by Rady (2011). Duplicate 0.2 g samples of fully-expanded leaf tissue were used. 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 (EC_1) of the solution was recorded using a conductivity bridge. The second sample was boiled at 100 °C for 10 min, and the electrical conductivity was measured (EC_2). The MSI was then calculated using the formula:

$$MSI\,(\%) = \left[1–(EC_1/EC_2)\right] \times 100$$

The total inorganic ions leaked from fully expanded leaves were determined as per the method of Sullivan and Ross (1979). Twenty leaf discs (2 cm in diameter) were placed in a boiling tube containing 10 ml deionized water and the electrical conductivity (EC_1) was recorded. The contents were then heated to 45–55 °C in a water bath for 30 min 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. The EL was calculated using the formula:

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