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Alleviation of drought-induced oxidative stress in maize (*Zea mays* L.) plants by dual application of 24-epibrassinolide and spermine

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ABSTRACT

Dual application [24-epibrassinolide (EBL) and spermine (Spm)] influence on the antioxidant machinery in water-stressed plants has received no attention. The present study, as a first investigation, was conducted with an aim to investigate the effects of EBL, Spm and their dual application on the ROS scavenging antioxidant defense machinery in plants subjected to drought conditions. This approach was assessed as possible mechanisms of drought tolerance and how these applications protect plants against oxidative stress. To achieve this goal, two maize hybrids (Giza 10 and Giza 129) were subjected to well-watered conditions and water-stressed conditions (75% and 50% of field capacity) with and without EBL and/or Spm foliar application. The grains were sown in plastic pots containing clay-loam (sand 37%, silt 28%, clay 35%) soil (Inceptisols; FAO), under greenhouse condition. Water deficiency significantly reduced growth, productivity, and membrane stability index, particularly in hybrid Giza 10. However, the follow-up treatment with the dual application (25 mg l^{-1} Spm + 0.1 mg l^{-1} EBL) detoxified the stress generated by drought and significantly improved the above parameters, particularly in hybrid Giza 129. Drought stress significantly increased H_2O_2 and $O_2^{\bullet -}$ contents and caused oxidative stress to lipids assessed by the increase in MDA content. However, they were significantly decreased in stressed plants treated with the dual application. Moreover, dual application alleviated the detrimental effects of drought on the electrolyte leakage. Activities of superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase and levels of ascorbate, glutathione, proline, and glycinebetaine were increased in response to drought treatments as well as foliar applications. Dual application significantly alleviated drought-induced inhibition in the activities of monodehydroascorbate reductase and dehydroascorbate reductase as well as in the ratios of AsA/DHA and GSH/GSSG. Overall, dual application improved the plant drought tolerance and decreased the accumulation of ROS by enhancing their scavenging through elevation of antioxidant enzymes activity and improving the redox state of ascorbate and glutathione.

1. Introduction

Drought is a major environmental stress factor that adversely affects the growth and productivity of plants (Ribaut et al., 2012). Plants can avoid the damage brought about by drought through several mechanisms such as compatible solutes accumulation (Marcińska et al., 2013). Organic solutes can accumulate to high level without disturbing intracellular biochemistry, protecting sub-cellular structures, mitigating oxidative damage caused by free radicals and maintaining the enzyme activities under different environmental stress conditions (Hanson et al., 1977; Hanson and Nelsen, 1978; Ashraf and Foolad, 2007; Marcińska et al., 2013; Talaat and Shawky, 2013, 2014a). Furthermore, during drought stress excessive generation of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$) occurs (Mittler, 2002). Generation of these ROS causes membrane deterioration, lipid peroxidation and DNA modifications, leading to irreparable metabolic and structural dysfunctions, ending in cell death (Miller et al., 2010). To cope with

Abbreviations: APX, ascorbate peroxidase; AsA, ascorbate; BRs, brassinosteroids; CAT, catalase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; DW, dry weight; ε, extinction coefficient; EBL, 24-epibrassinolide; EL, electrolyte leakage; FW, fresh weight; GB, glycinebetaine; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; MDHAR, monodehydroascorbate reductase; MSI, membrane stability index; $O_2^{\bullet -}$, superoxide radical; PAs, polyamines; ROS, reactive oxygen species; SOD, superoxide dismutase; Spm, spermine; TBARS, thiobarbituric acid reactive substance.

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ROS and maintain redox homeostasis, plants have developed a well-integrated antioxidant defense system, which is made up of antioxidant molecules and antioxidant enzymes such as superoxide dismutase, catalase, and enzymes involved in the ascorbate–glutathione cycle (Mittler, 2002). The high efficiency of these antioxidant enzymes is responsible for the alleviation of oxidative damage under abiotic stress (DaCosta and Huang, 2007; Zhang et al., 2008; Behnamnia et al., 2009; Talaat and Shawky, 2013, 2014b; Talaat, 2014).

Antioxidant molecules correlate positively with water stress and provide partial benefits against oxidative stress. Ascorbate (AsA) and glutathione (GSH) interact to form the central antioxidant system in plant cells, called the AsA–GSH cycle that helps prevent oxidative damage in plants (Noctor and Foyer, 1998). AsA plays an important role in the detoxification of ROS, including reducing H_2O_2 to water (Asada, 1999). This reaction is catalyzed by the ascorbate peroxidase (APX, EC 1.11.1.11) producing monodehydroascorbate radicals, which can be reduced by NADPH in a reaction catalyzed by monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) (Sano et al., 2005). Monodehydroascorbate radicals are able to spontaneously disproportionate to dehydroascorbate (DHA) and AsA (Asada, 2006). DHA reduction may occur via either a non-enzymatic reaction with GSH or enzymatically by dehydroascorbate reductase (DHAR, EC 1.8.5.1) leading to the production of AsA (Foyer and Halliwell, 1976). Recycling of GSH is catalyzed by glutathione reductase (GR, EC 1.6.4.2) that reduces glutathione disulphide (GSSG) by using NADPH (Noctor and Foyer, 2011). Maintaining high ratios of GSH/GSSG and AsA/DHA plays an important role in drought tolerance (Noctor and Foyer, 2011).

Brassinosteroids (BRs) are plant steroid hormones that control cell division and elongation, xylem differentiation, pollen tube growth, reproductive and vascular development, membrane polarization, proton pump activation, source/sink relationships, enzyme activation, gene expression, nucleic acid and protein synthesis, photosynthesis, and stress responses (Krishna, 2003; Talaat and Shawky, 2012). Although many efforts have been made to recommend the BRs as plant growth regulators for wide spread utilization in agriculture, the underlying mechanisms for BR-mediated plant growth and development and stress tolerance are still poorly understood. Exogenous application of 24-epibrassinolide (EBL) modified antioxidant enzymes activity, antioxidant molecules content, osmoprotectants accumulation, lipid peroxidation, H_2O_2 content, electrolyte leakage, and membrane stability index in plants under water stress conditions (Zhang et al., 2008; Behnamnia et al., 2009; Yuan et al., 2010; Anjum et al., 2011; Li et al., 2012; Mahesh et al., 2013). Recently, Morales et al. (2014) reported that EBL stimulates the AsA synthesis pathway by increasing the AsA precursor formation.

Polyamines (PAs), namely putrescine (Put), spermidine (Spd) and spermine (Spm), are ubiquitous, polycationic, aliphatic amines that modulate several biological processes in plants, including cell division, cell proliferation, embryogenesis, reproductive organ development, root growth, and gene expression. PAs also play major roles in plant stress tolerance by scavenging free radicals, involving in activation of expression of genes encoding antioxidant enzymes, and acting directly as stress-protecting compounds due to their acid-neutralizing and antioxidant properties, as well as to their membrane-stabilizing abilities (Shevyakova, 1981; Takahashi and Kakehi, 2009). Spm was the most effective PA in improving plant drought tolerance. It has a longer chain and more positive charges, and it could provide more effective neutralizing and membrane stabilizing (Farooq et al., 2009). Previous reports have demonstrated that exogenous application of Spm ameliorated water stress by enhancing the antioxidant enzymes activity, increasing the amount of antioxidants, decreasing MDA, H_2O_2, and $O_2^{\bullet-}$ contents and electrolyte leakage, and thus significantly promoted plant growth and its productivity (Farooq et al., 2009; Yiu et al., 2009; Radhakrishnan and Lee, 2013).

Maize is one of the most important crops; however, it is highly sensitive to drought stress (Ribaut et al., 2012). EBL or Spm play crucial roles in plant development and also promote tolerance to a range of abiotic stresses. Although much has been learned about their roles in plant development, the mechanisms by which EBL or Spm control plant stress responses are not fully known. To the best of our knowledge, this is the first study to investigate the effect of the dual application of EBL and Spm on the plant drought-tolerance. The present study was conducted to test the hypothesis that the single foliar application of EBL or Spm as well as their dual foliar application may moderate drought effects in plants by monitoring the changes in the scavenging capacity of the antioxidant defense system, which represents the executive system of the protective response. To verify this hypothesis, two maize genotypes were treated with or without EBL and/or Spm under well-irrigated and drought-stressed conditions. Results were quantified by measuring several physiological parameters, including the activities of the antioxidant enzymes (SOD, CAT, APX, MDHAR, DHAR, and GR), contents of the antioxidant molecules (AsA and GSH) and their redox ratios, concentrations of the osmoprotectants (proline and glycinebetaine), contents of MDA, H_2O_2, and $O_2^{\bullet-}$, electrolyte leakage, and membrane stability index. Further, this investigation was directed to establish a relationship between the changes in these parameters and the degree of tolerance, in terms of improvement in plant growth and productivity. The objective of this approach was to define the effect of EBL and/or Spm applications on some physiological processes in order to understand the mechanisms regarding the alleviation of drought injuries in treated plants.

2. Materials and methods

2.1. Experimental design and plant growth conditions

A pot experiment was conducted during the two successive seasons of 2013 and 2014 in the greenhouse of the Department of Plant Physiology, Faculty of Agriculture, Cairo University, Giza, Egypt. The experiment was performed in a completely randomized design with three factors: two maize hybrids (Giza 10 and Giza 129), three soil water conditions (well-watered condition and water-stressed conditions at 75% and 50% of field capacity), and four spraying treatments [0.00 (double distilled water; DDW), 25 mg l^{-1} Spm, 0.1 mg l^{-1} EBL, and 25 mg l^{-1} Spm + 0.1 mg l^{-1} EBL]. The experiment included twenty four treatments and each treatment had nine replicates.

These two genotypes were selected based on their high yield productivity and we tried to increase their drought tolerance by using EBL and/or Spm foliar application.

The clay-loam (sand 37%, silt 28%, clay 35%) soil (Inceptisols; FAO), collected from the Faculty of Agriculture, Cairo University Experimental Station, was sieved (pore size, 2 mm) and diluted with quartz sand (particle diameter <1 mm; 2:1, soil:sand, v/v). Chemical soil properties are presented in Table 1 and were determined before planting according to Cottenie et al. (1982). Fertilization was carried out by adding ammonium nitrate (33.5% N), calcium superphosphate (15.5% P_2O_5), and potassium sulfate (48% K_2O) at the rate of 2.0, 2.0, and 0.5 g pot^{-1}, respectively, before planting, as well as 2.0 g pot^{-1} ammonium nitrate 30 days after planting. For each plastic pot (30 cm diameter, 35 cm depth, filled with 15 kg of the soil mixture), four grains thinned to two after germination were planted on June 3 in both seasons. Grains of two maize hybrids (Giza 10 and Giza 129) were obtained from the Agriculture Research Center, Ministry of Agriculture, Giza. All pots were irrigated to soil saturation before planting. After planting,