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Physiological and Ascorbate -Glutathione pathway-related genes responses under drought and heat stress in crested wheatgrass

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ABSTRACT

Drought and heat stress are two factors that limit the growth of cool-season plant species in many regions of the world. The objective of this experiment was to study the expression patterns of genes involved in ascorbate and glutathione pathways, while assessing the physiological responses of plants and their tolerance to drought stress and heat stress. The results of this study indicated that there were variations in the plants' tolerance to drought and heat among the crested wheatgrass genotypes. Based on the real time-PCR results genes involved in the biosynthesis (GalLDH and γ-ECS) and recycling (APX, GR, DHAR, MDHAR) of ascorbate and glutathione, also DREB2 were significantly affected by stress. The expression of the *DREB2* gene increased substantially in all genotypes under drought and heat stress, which was also associated with high levels of expression genes involved in the Asc-Glu pathway. Based on our results, it seems that all of the genes involved in the Asc-Glu pathway probably had the DRE/CRT element in their promoter region for the *DREB2* gene. Sequencing the *DREB2* gene showed that leaky mutations occurred in two genotypes collected from cold and wet regions. As a result, the *DREB2* probably cannot bind with the dehydration-responsive elements (DRE/CRT, as a cis-acting element) and, although the expression of the *DREB2* gene was increased, there was no significant change in the level of expression genes involved in the Asc-Glu pathway. Based on physiological analyses, the ranking of the genotypes' tolerance to drought would appear as 'AC3' > 'AC5' > 'AC6' > 'AC1' > 'AC2' > 'AC4' and the ranking of tolerance to heat stress would be 'AC5' > 'AC1' > 'AC6' > 'AC4' > 'AC2' > 'AC3'. Finally, our results indicated that tolerance to drought and heat associated positively with the expression of genes involved in the Asc-Glu pathway and natural habitat of genotypes. It was also found that the *DREB2* plays a key role in regulating the expression of genes involved in the Asc-Glu pathway.

1. Introduction

Heat stress and drought stress are two major environmental factors that can limit the management of turfgrass in semi-arid and arid regions during summer months (Du et al., 2013; Li et al., 2008). Models pertaining to the prediction of weather demonstrate that some regions in the world may be facing reduced amounts of rainfall due to the increase in average global temperatures, which could probably be associated with an increase in the incidence and persistence of drought periods in the coming years (Parrotta et al., 2016; Xu and Zhou, 2006). Heat stress and drought stress cause oxidative damage to plant cells through increased production of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hydroxyl radical (OH*) (Du et al., 2013). The balance between the production and removal of ROS may be disturbed by environmental stresses (Koca et al., 2007). To cope with reactive oxygen species during stress, the turfgrass has evolved an effective antioxidant defense system that includes both enzymatic and non-enzymatic antioxidants (Etemadi et al., 2015).

Abbreviations: APX, Ascorbate peroxidase; Asc-Glu pathway, ascorbate-glutathione cycle; EL, electrolyte leakage; CAT, catalase activit; DHAR, dehydroascorbate reductase; DREBs, dehydration responsive element binding proteins; DRE/CRT, dehydration responsive element; GalLDH, galactono-1,4-lactone dehydrogenase; γ-ECS, gamma-glutamylcysteine synthetase; GR, glutathione reductase; H_2O_2, hydrogen peroxide; OH*, hydroxyl radical; MDA, malondialdehyde; MDHAR, monodehydroascorbate reductase; POD, peroxidase; $^1O_2$, singlet oxygen; ROS, reactive oxygen species; RWC, relative water content; SOD, superoxide dismutase; TNC, total nonstructural carbohydrates; TFs, transcription factors

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The ascorbate-glutathione cycle (Asc-Glu pathway) is involved extensively in the defense against oxidative stress (Neto et al., 2005). In the Asc-Glu pathway, ascorbate and glutathione are two crucial non-enzymatic compounds that can be recycled while H_2O_2 is scavenged. It is known that ascorbate and glutathione are constantly regenerated and biosynthesized in plants in order to scavenge reactive oxygen species (ROS) (Shan and Liang, 2010). L-galactono-1, 4-lactone dehydrogenase (GalLDH) and Gamma-glutamylcysteine synthetase (γ-ECS) are key enzymes for ascorbate and glutathione biosynthesis, respectively (Dringen, 2000; Wheeler et al., 1998). The enzymes of the Asc-Glu pathway comprise monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) as well as ascorbate peroxidase (APX) (Noctor and Foyer, 1998).

Transcription factors (TFs) are commonly known among regulators in all organisms. They play key roles in the regulation of gene expression, especially under stress conditions (Hassan et al., 2015; Nakashima et al., 2009). Dehydration-responsive element binding proteins (DREBs) constitute a large family of TFs that are involved in the transduction pathway of plant stress signaling. They can exclusively bind to dehydration responsive elements (DRE/CRT, as a cis-acting element) and activate the expression of many stress-inducible genes (Rehman and Mahmood, 2015). Also, various studies have shown that the over-expression of the *DREB* gene in different plants caused the increase in tolerance to abiotic stress such as drought, heat, and salt (Liu et al., 2015).

Studies in recent years have shown that there are several methods of improving turfgrass' tolerance to heat and drought. The use of species and genotypes that exhibit a stronger tolerance to heat and drought can be an important way to facilitate turfgrass management (Du et al., 2013; Wang et al., 2008). Grass species and genotypes vary in their responses to heat and drought stress, which involve changes in the physiological, biochemical and molecular aspects of the plant (Abraham et al., 2008). Understanding the relative involvement of each physiological, biochemical and molecular characteristic in heat and drought tolerance is important in selecting grass genotypes to facilitate the breeding of genotypes that are more tolerant to heat and drought (Liu et al., 2008; Soliman et al., 2012).

Crested wheatgrass (*Agropyron cristatum* L.) is a cool-season perennial grass characterized by high resistance to abiotic stress and diseases (Han et al., 2014). This perennial grass has a good potential to be used as a low-input turfgrass due to its tolerance to stress, its slow vertical growth rate, adaptation to dry and sandy soils and its ability to grow in arid and semi-arid regions (Bayat et al., 2016). There are currently no information about Iranian crested wheatgrass genotypes with respect to their drought and heat tolerance. Undoubtedly, the development of Iranian crested wheatgrass genotypes that are more tolerant to drought and heat can increase the adaptability of this wheatgrass to warm and dry environments. Such developments would favorably benefit the management of turfgrass.

The objective of this study was to evaluate the physiological, biochemical and molecular responses of the Iranian crested wheatgrass genotypes, along with the expression of genes that encode the Asc-Glu pathway under drought stress and heat stress. The ultimate aim was to select genotypes that are more resistant to drought and heat. The results of this study may provide ways for the selection and breeding of turfgrass. Such programs could make turfgrass more tolerant to drought and heat in hot and dry climates.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of six genotypes of Iranian crested wheatgrass (*Agropyron cristatum* L.) ('AC1', 'AC2', 'AC3', 'AC4', 'AC5' and 'AC6') were gathered in January 2016 from six regions in Iran (Table 1). All seed collections were kept at a constant temperature of 5 °C at the Turfgrass Seed Testing Centre, Department of Horticulture at the Isfahan University of Technology, Isfahan, Iran. Seeds of the Iranian desert wheatgrass genotypes were planted in polyvinyl chloride (PVC) tubes (50 cm deep and 15 cm in diameter) filled with sterilized sandy loam soil (50% fine-loamy, mixed mesic Typic Hapludult; 50% sand) which had been sterilized in an oven at 180 °C for 8 h. The bottom of the pots were covered with coarse nylon sheets before planting so as to preserve the soil and allow for drainage of water from the tubes. Grass genotypes were first grown in a greenhouse for 3 months. Pots were watered daily to maintain soil moisture at field capacity. One hundred milliliters of soluble fertilizer 15–15–15 ($N\text{-}P_2O_5\text{-}K_2O$) was applied weekly at a concentration of 4 g L^{-1} to each container to facilitate plant establishment before the treatments were implemented. Grasses were cut every 2 weeks with scissors, and the clippings were removed. The grass was frequently cut to maintain a height of 4 cm. The pots were then transferred to growth chambers that provided 10 to 12 hours of light (13-h photoperiod, relative humidity of 50% to 60%, photosynthetic photon flux density of 500 μmol.m^{-2}.s^{-1} at the canopy level) and temperatures of 22 °C day / 18 °C night. Plants were allowed to adapt to the conditions of the growth chamber for two weeks before the start of the experiment.

2.2. Treatments and experimental design

Genotypes of the Iranian crested wheatgrass were exposed to three treatments for a duration of 24 days. The first treatment was the control in which the plants were kept at optimal temperature conditions (22 °C day / 18 °C night) and were watered daily. These temperatures are within the optimum temperatures for the growth of cool-season grasses. The control plants were situated in pots and the moisture of the soil was kept within the 60–80% range of water holding capacity throughout the experiment. The second treatment was to withhold irrigation, thereby causing drought stress. Accordingly, the plants were maintained at optimal temperature conditions (22 °C day / 18 °C night) and were not watered. Water stress was initiated by withholding water from the soil for 24 days. The third treatment was to apply heat stress. The plants were subjected to 35 / 30 °C (day / night) and were watered daily. Plants were situated in soils with a moisture of 60–80% of water holding capacity throughout the experiment (Abraham et al., 2008; Larkindale and Huang, 2004).

2.3. Measurements

Physiological and biochemical properties of the six Iranian crested wheatgrass genotypes were evaluated in response to drought stress and heat stress after 0, 8, 16, 24 days by measuring hydrogen peroxide (H_2O_2) content, malondialdehyde (MDA) content, electrolyte leakage (EL), relative water content (RWC), proline content, total nonstructural carbohydrates (TNC) content, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD) and turf quality.

2.3.1. Hydrogen peroxide contents (H_2O_2) and Malondialdehyde content (MDA)

Samples of Agropyron genotypes were ground finely by using liquid N_2 in ice-chilled mortar and pestle and were homogenized in 4% ice-cold trichloro acetic acid. The homogenate was then centrifuged at 15,000*g* for 15 min at 5 °C. The supernatant was used for the calculation of hydrogen peroxide content (H_2O_2; Guo et al., 2006) and malondialdehyde content (MDA; Heath and Parker, 1968).

2.3.2. Relative Water Content (RWC) and Electrolyte Leakage (EL)

The amount of water in leaves was determined by measuring the relative water content (RWC) calculated by the following formula. RWC (%) = (FW-DW)/(TW-DW) × 100, where FW is the leaf fresh weight, DW is leaf dry weight for tissues dried at 80 °C for 4 d, and TW is the turgid weight of leaves after being soaked in water for 4 h at 20 °C