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Individual and combined effects of drought and heat on antioxidant parameters and growth performance in Buffel grass (*Cenchrus ciliaris* L.) genotypes

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ABSTRACT

Drought and heat stress are two critical threats to crop growth and sustainable agriculture worldwide. In the last decade, many studies focused on the response of crops to a single stress, nevertheless studying the response of plants to a combination stress may be critical to our understanding of stress tolerance in plants and the development of tolerant genotypes. Buffel grass (*Cenchrus ciliaris* L.) is a warm-season grass known in arid and semiarid regions for its tolerance to heat and drought stress, productivity, and forage quality. However, in our previous works, several accessions have exhibited different responses to abiotic stresses. Therefore, the objective of this work was to evaluate the effects of combination of drought and heat stresses on biochemical parameters and plant growth and to compare the impacts of the stresses separately and when combined. We found that sensitive genotype exhibited higher lipid peroxidation content, lower total reducing power values and reduced catalase and superoxide dismutase activities than tolerant under drought or heat stress or combination stress. In this study, heat stress had a predominant effect on buffel grass genotypes over drought stress, which explained why simultaneous application of heat and drought revealed similar biochemical and growth responses to the heat stress. Antioxidant metabolism seems to be critical for tolerate abiotic stress. This study may provide useful information to perform a rapid and low-cost characterization in new buffel grass germplasm and to identify genotypes with better growth performance under drought and heat conditions.

1. Introduction

Drought and heat stress are known as major threats to growth and development of agricultural crops. Abiotic stresses frequency, duration and severity are anticipated to be increased in the future, which might have severe effects for crop and forage productivity and subsequently livestock production (Zhou et al., 2017). In the last decade, many investigations have been performed to figure out the response of plant species to a unique abiotic stress, whilst in ecosystems, plants might simultaneously be exposed to multiple abiotic stresses (Mittler, 2006; Pandey et al., 2015).

Plants have evolved various morphological, cellular, physiological, biochemical and molecular adaptations to preserve themselves in abiotic stress situations (Pandey et al., 2015). Reactive oxygen species (ROS) including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH), are one of the earliest known biochemical responses of eukaryotic cells to abiotic stresses. ROS are produced mostly in chloroplasts, mitochondria, and peroxisomes (Apel and Hirt, 2004). Drought and heat stresses dramatically increase ROS levels which lead in oxidative damage of proteins, DNA and lipids (Apel and Hirt, 2004; Farooq et al., 2009; Mittler, 2002; Gill and Tuteja, 2010). Particularly, when ROS directly attack membrane lipids, the malondialdehyde (MDA), a product of peroxidation of unsaturated fatty acids, increases their content (Gill and Tuteja, 2010). MDA has been considered as an indicator of oxidative damage in various crops including forage grass species (Luna et al., 2002; Moller et al., 2007; Bi et al., 2016) and it has been used as a suitable indicator for tolerant genotype selection (Luna et al., 2002; Lanza Castelli et al., 2010; Tommasino et al., 2012). ROS also acts as signaling molecules in many biological

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processes such as stomatal closure, growth, development, and stress signaling (Suzuki et al., 2012).

Due to the dual roles of ROS, plants are able to fine-tune ROS concentrations between certain thresholds by means of production and scavenging mechanisms (Sekmen et al., 2014). Since ROS homeostasis is disrupted under stress, induced enzymatic antioxidant defenses are considered as an important factor of plant stress tolerance (Mittler et al., 2011; Suzuki et al., 2011, 2012; Sekmen et al., 2014; You and Chan, 2015). Higher plant species generally apply a defense system, which is involved with antioxidative enzymes and non-enzymatic compounds to protect plants against ROS. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) among others (Ashraf, 2009; Gill and Tuteja, 2010; Sharma et al., 2012; You and Chan, 2015).

Knowledge regarding the response of plants to multiple abiotic stresses is limited and also its required be improved for a better understanding of mechanisms underlying stress tolerance in plants species (Rizhsky et al., 2002). When plants deal with multiple abiotic stresses-induced factors concurrently, their adaptation strategy will be governed by the interaction of abiotic stress factors, which means a new level of stress (Mittler, 2006). In general, plant responses to multiple stresses are majorly determined by the more severe stress (dominant stressor) (Pandey et al., 2015). However, it depends largely on the age of plant, the genotype, the stress-susceptibility or tolerance behaviour of plant, and severity of multiple factors involved with abiotic stress (Silva et al., 2010). Several authors underline the need to develop crops with better performance and resilience to abiotic stress combination and indicate the complex interaction between drought and heat stress (Mittler, 2006; Feller and Vaseva, 2014; Pandey et al., 2015).

Buffel grass (*Cenchrus ciliaris* L. syn. *Pennisetum ciliare* (L.) Link) is an apomictic, polyploid warm-season grass (Ozias-Akins, 2006) used for cattle and sheep grazing in arid and semiarid regions worldwide (Saini et al., 2007). Buffel grass is known to be tolerant to heat and drought stress and easy to establish with high productivity and quality (Hacker and Waite, 2001; Kharrat-Souissi et al., 2010; Marshall et al., 2012). However, genetic variability was found when several accessions were exposed to abiotic stresses (Mansoor et al., 2002; Kharrat-Souissi et al., 2012; Al-Dakheel et al., 2015; Al-Dakheel and Iftikhar Hussain, 2016). In Argentina, the species is cultivated mainly in the Northwestern region (De León, 2003; Griffa et al., 2010), in areas with a considerable dry season during a long part of the year, and strong sunshine in the summer (Guevara et al., 2009). In our previous work, the effect of heat stress on biochemical parameters was investigated in buffel grass genotypes (Tommasino et al., 2012). However, understanding of combined effect of heat and drought on biochemical parameters, biomass production and the relationship between the biochemical responses to single and combined stress remained unclear. Therefore, the objective of this work was to evaluate the effects of combination of drought and heat stresses on biochemical parameters and plant growth in different genotypes of *Cenchrus ciliaris* and to compare the impact of the stresses separately and when combined.

2. Materials and methods

2.1. Plant material

In this study, two genotypes (Register Number: RN51 and RN1) of buffel grass (*Cenchrus ciliaris* L.) were used in all experiments because they showed different responses (tolerant and sensitive genotypes, respectively) to salt and heat stress as observed in our previous studies (Lanza Castelli et al., 2010; Tommasino et al., 2012). In addition, a widespread genotype (RN49) and two somaclonal mutants, named as J20 and S6 were used in a combined stress assay under controlled conditions. J20 was obtained through mutation and in vitro selection assay for drought tolerance (López Colomba et al., 2011). While S6 is a somaclonal variant that is already determined as salt tolerant genotype (López Colomba et al., 2013).

2.2. General growth conditions and treatments application

For all assays, including treatments and control, 0.2 g seeds of individual genotypes were sown in pots (25 cm in diameter × 15 cm in depth) containing 2.76 kg sand and soil substrate (1:1) previously dried in stove at 105 °C for 48 h. After sowing the seeds, the surface of all pots were covered with 200 g of sieved soil substrate. The pots were watered and the soil water content (SWC) was calculated after the complete drainage. This value was considered as the maximum amount of water capable to be retained by the substrate (100% SWC). The SWC was determined via gravimetric method. Afterwards, the pots were transferred to a growth chamber, under following conditions: temperature (28 °C ± 2 °C), photoperiod (16/8 h light/dark), humidity (40%) and photosynthetic photon flux density (PAR) (250 μmol m^{-2} s^{-1}). Pots were watered daily until SWC reached to 80%. Seedlings emerged after 15 days past sown and we kept 35 small seedlings in each pot.

2.2.1. Drought stress evaluation

Water stress assays were performed using the RN1 and RN51 genotypes following the protocol described by Tommasino (2013). Briefly, 30 days after sowing the seeds, drought treatment was carried out by interrupting irrigation until 30% SWC was obtained. This 30% SWC value was used for this study because buffel grass genotypes have been previously reported to showing water stress symptoms under drought conditions (Tommasino, 2013). Pots with 80% of SWC were considered as control treatment. A completely randomized design was performed with six repetitions (pots) per genotype and treatment. Five plants of control and treatment were collected to evaluate biochemical parameters after 24, 48 and 72 h when pots obtained 30% of SWC. Whilst, growth performance was measured after 54 days after sowing (DAS).

2.2.2. Heat stress evaluation

RN1 and RN51 seeds were grown in pots as previously described. Thirty DAS, half of the pots, were exposed to heat-treatment (H), in a growth chamber already set for 16 h day length, 45% RH, 250 μmol m^{-2} s^{1} light intensity and with constant 45 ± 1 °C day/night temperature during 72 h constantly. The other half pots were considered as control (C) and were kept in a growth chamber under normal conditions (28 °C, at 16 h day length, 45% RH and 250 μmol m^{-2} s^{-1} light intensity). All treatments were watered regularly (80% SWC) to avoid drought stress. Leaf samples from five plants of heat treated and control pots were collected at 24, 48 and 72 h of 45 °C to evaluate biochemical parameters. At the end of 72 h of heat treatment, C and H pots were kept under normal growth conditions (28 °C, at 16 h day length, 45% RH and 250 μmol m^{-2} s^{-1} light intensity). The growth performance was measured after 54 DAS, through the same characters as mentioned above.

2.2.3. Combined drought and heat stress evaluation

RN1 and RN51 seeds were grown as previously described. Thirty DAS, the drought treatment was carried out by interrupting irrigation until 30% SWC was obtained. Then, the temperature of the chamber was raised to 45 ± 1 °C day/night temperature during 72 h to provoke heat stress treatment and the pots were watered regularly to keep 30% SWC. A completely randomized design was performed with six repetitions (pots) per genotype and per treatment. Leaf samples from five plants of each pot were harvested at 24, 48 and 72 h of combined drought and heat stress treatment for evaluation of biochemical parameters. Then, pots were transferred to growth chamber at 28 °C, at 16 h day length, 45% RH under 250 μmol m^{-2} s^{-1} light intensity, via 30%