

Research Note

Malondialdehyde content as a potential biochemical indicator of tolerant *Cenchrus ciliaris* L. genotypes under heat stress treatment

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Abstract

Buffel grass (*Cenchrus ciliaris* L.) is an important forage grass in arid and semiarid regions. As part of a genetic improvement programme, four genotypes [Biloela (Bl), Americana (Am), Texas (Tx) and Sexual (Sx)] were categorized by tolerance to heat stress in a greenhouse experiment. At 30 d after sowing, half of the plants (control plants) were transferred to growth chambers (28°C day per night), and the other half (pre-treated plants, Prt) were exposed to heat stress treatment (0, 24, 48 and 72 h; 45°C day per night). Malondialdehyde (MDA) content, an indicator of oxidative damage, was determined from foliar samples. During heat stress, Sx showed the earliest increase in MDA (at 24 h) followed by Tx (48 h) and Am and Bl (72 h). Results were compared with heat-stress tolerance evaluated as morphological traits at the end of recuperation (60 d after sowing). Fresh weight and aerial plant height were lowest in the Prt-Sx genotype and highest in Am and Bl genotypes; Tx showed intermediate tolerance. Results suggest that tolerance to heat stress in *C. ciliaris* genotypes could be related to the capacity for regulating the oxidative damage increase. Foliar MDA content might therefore be used in a genetic improvement programme of *C. ciliaris* as a potential biochemical indicator for a rapid, simple and low-cost identification of heat-stress tolerant genotypes.

Keywords: buffel grass, biochemical markers, breeding programme, heat stress tolerance, oxidative stress, Argentina

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Introduction

Buffel grass (*Cenchrus ciliaris* L.) is an important pasture grass used for cattle and sheep production in arid and semiarid regions worldwide (Saini *et al.*, 2007) and is particularly important in north-west Argentina (De Leon, 2004; Griffa, 2010). Four tetraploid genotypes of *C. ciliaris* included in a genetic improvement programme were previously characterized by molecular genetic discrimination (Griffa *et al.*, 2006) and by their tolerance to salt-stress conditions using morphological (Griffa *et al.*, 2010) and biochemical (Lanza Castelli *et al.*, 2010) characters. Because high-temperature stress is one of the major limiting factors affecting plant growth and crop productivity (Ahuja *et al.*, 2010; Sundaram and Rathinasabapathi, 2010), in this work they were categorized by their tolerance to heat stress. To identify tolerant genotypes, genetic improvement requires the use of sound selection criteria. As complex characters, such as yield, show multigenic inheritance and environmental influence, the additive use of biochemical and physiological characters as tolerance indicators has been proposed as a more reliable approach (Mittra, 2001; Ashraf, 2004, 2009). In that sense, oxidative stress has been mentioned as a common metabolic route of different stresses (Apel and Hirt, 2004), and it has been associated with genotypes tolerant to different abiotic stresses (Luna *et al.*, 2002; Ashraf, 2009). Oxidative stress is characterized by the production of reactive oxygen species (ROS), such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH). High-temperature stress can induce oxidative stress, which subsequently causes cell membrane injury, lipid peroxidation and pigment bleaching (Sairam *et al.*, 2000; Wahid and Close, 2007; Wang *et al.*, 2009). Under heat-stress conditions, malondialdehyde (MDA), a product of peroxidation of unsaturated fatty acids, has been used as a good indicator of free radical damage to cell membranes (Liu and Huang, 2000; Suzuki and Mittler, 2006).

Moreover, MDA content was correlated with high temperature stress tolerance (Huang *et al.*, 2001; Almeselmani *et al.*, 2006; Wang *et al.*, 2009). Because MDA content was found to be a good salinity-tolerance marker in *Chloris gayana* (Luna *et al.*, 2000, 2002) as well as in *C. ciliaris* (Lanza Castelli *et al.*, 2010), in the present study it was measured in *C. ciliaris* genotypes during 0, 24, 48 and 72 h of heat-stress treatment (45°C) and this information was compared with heat-stress tolerance, evaluated as morphological traits.

Materials and methods

Seeds of four tetraploid genotypes of *C. ciliaris* [Americana (Am), Biloela (B), Texas 4464 (Tx) and Sexual line (Sx)] were grown in pots (30 cm in diameter × 25 cm in depth) containing sterilized soil, sand and vermiculite (1:1:1), in a greenhouse under natural light and day/night temperatures of 30/15°C. Thirty days after sowing, half of the plants, here referred to as pre-treated plants (PTr), were transferred to a growth chamber set at 16-h day length, 45% RH and ~550 mmol m⁻² s⁻¹ light intensity and with constant 45 ± 1°C day/night temperature. To choose experimental conditions, sampling time and different temperatures (30, 35, 40 and 45°C) were previously assessed. Genotypes showed diminution in fresh weight of aerial part (FWA) and in height of aerial part (HA) at 45°C and after 3 d duration of treatment (data not shown); therefore, this was the experimental condition selected. The other half of the plants were kept as control plants (Ct) under normal conditions in another chamber, set at constant 28 ± 1°C temperature, 16 h day length, 45% RH and ~550 mmol m⁻² s⁻¹ light intensity. All treatments were watered regularly to avoid drought stress. Leaf samples were collected during periods (0, 24, 48 and 72 h) of heat-stress treatment (45°C) to evaluate MDA content according to Heath and Packer (1968) and Hoodges *et al.* (1999). MDA content was calculated by using an extinction coefficient of 155 mm⁻¹ cm⁻¹; results are expressed as mmol MDA mg FW.

After heat-stress treatment, Ct and PTr plants were transferred to a greenhouse to allow recovery. At the end of this period, about 60 d from sowing, the morphological traits FWA and HA were measured following Griffa (2010) and Griffa *et al.* (2010). Results were expressed as percentage of high temperature stress-induced damage in PTr plants as compared to Ct plants and were calculated as follows:

$$\text{Percentage of damage} = [(X_c - x_i) / X_c] \times 100,$$

where X_c represents the mean value (HA or FWA) of control plants and x_i represents the value of each treated plant.

Statistical analysis

The analysis included two replicates with 10 foliar samples per genotype. Data were submitted to an analysis of variance (ANOVA), and the means were compared using the method DGC (Di Rienzo *et al.*, 2002) ($P < 0.05$), using InfoStat statistics software (Di Rienzo *et al.*, 2010).

Results and discussion

Among the different indices used to measure heat-stress tolerance in crop plants, growth reduction has been indicated as a potential criterion for genotype selection (Huang *et al.*, 2001; Wang *et al.*, 2009). Consequently, morphological traits were evaluated after recovery, at about 60 d after sowing. The percentage of morphological damage of FWA and HA was lower in PTr-Am and Bl than in PTr-Tx and Sx genotypes (Figure 1a and b). In turn, PTr-Tx showed a lower percentage of morphological damage than Sx (Figure 1a and b). As a result, the genotypes Am and Bl were categorized as tolerant, whereas Tx showed an intermediate tolerance with respect to Sx, which was the most sensitive to heat stress pre-treatment. A similar trend was observed under salinity stress (Griffa *et al.*, 2010; Lanza Castelli *et al.*, 2010).

Data on morphological traits were compared with results of MDA behaviour evaluated during the 72 h of heat-stress treatment. In Ct treatment (28°C), all genotypes showed a similar foliar MDA level, which remained constant during the experiment (initial value: 10 mmol mg⁻¹ FW, data not shown). By contrast, under heat-stress (45°C) treatment, foliar MDA level was different among the genotypes. The earliest MDA increase in the Sx genotype was correlated with the highest damage in morphological traits in the same genotype after recovery (Figures 2, 1a and b). In agreement with this finding, Wang *et al.* (2009) observed that high-temperature stress reduced growth and caused cell membrane injuries in two wheat cultivars, with more pronounced changes in the more sensitive one. On the other hand, MDA increase in the middle of the heat-stress treatment, at 48 h in Tx genotype, was consistent with the intermediate damage level exhibited by this genotype, as observed in the evaluation of morphological traits (Figures 1a,b and 2). By contrast, the last MDA increase (72 h) in both Bl and Am was correlated with the least severe morphological damage after recovery (Figures 1a,b and 2). Several investigations have demonstrated the relationships between increase in growth characters, antioxidant defence induction and increased tolerance to heat stress (Huang *et al.*, 2001; Almeselmani *et al.*, 2006; Wang *et al.*, 2009; Sundaram and Rathinasabapathi, 2010). In addition, under salinity stress, decreasing MDA level

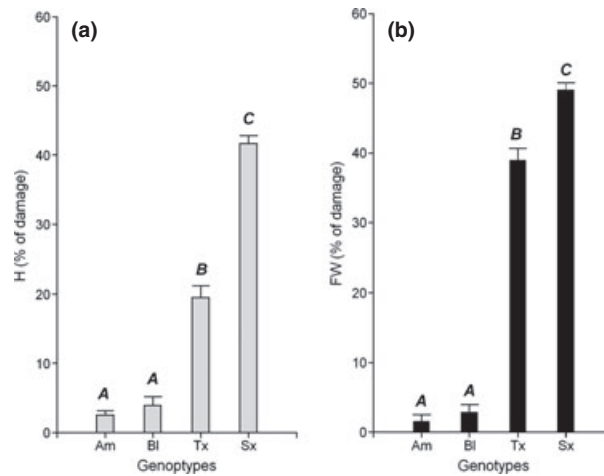


Figure 1 Morphological damage caused by high-temperature stress in (a) fresh weight of aerial part (DFWA) and (b) height of aerial part (DAH) of *Cenchrus ciliaris* L. genotypes. (DFWA and DAH were measured at the end of recovery, 60 d after sowing, in control (Ct) and pre-treated (Ptr) plants; and 30 d after sowing Ct and Ptr plants were exposed to heat stress treatment during 72 h at 28 and 45°C temperature stress respectively). Values were expressed as percentage of Ct and represent percentage of DFWA and DAH, as compared to Ct. DGC test Alpha = 0.05%. Different letters indicate significant differences ($P < 0.05$). Error bars indicate standard error.

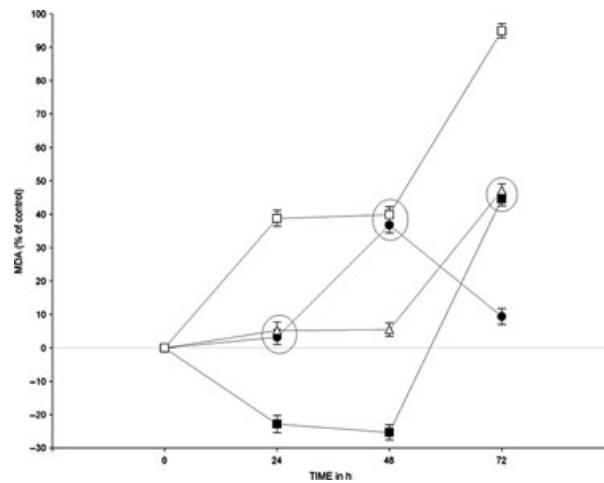


Figure 2 Effect of high temperature stress on malondialdehyde (MDA) content in *Cenchrus ciliaris* L. genotypes: Americana (△), Biloela (■), Texas (●) and Sexual (□). Foliar MDA content evaluated in control (28°C) and pre-treated plants during heat stress (at 0, 24, 48 and 72 h, at 45°C). Values were expressed as percentage of control plants (MDA initial values: 10 mmol mg⁻¹ FW). DGC test Alpha = 0.05%. Two points in the same circle represent non-significant differences. Error bars indicate standard error.

was correlated with increasing antioxidant defences in the most tolerant genotype of *C. ciliaris* (Lanza Castelli *et al.*, 2010). On the other hand, Chandra and Dubey (2010) reported lower tolerance to drought conditions in diploid than tetraploid and hexaploid *Cenchrus* species, and this was related to higher MDA accumulation in diploids. It is important to know that categorization by MDA content under heat stress showed a similar trend under salinity stress: Am and Bl were the

most tolerant, whereas Tx and Sx were the least tolerant genotypes to oxidative stress (Lanza Castelli *et al.*, 2010). As both Bl and Am genotypes showed good performance under both abiotic stresses, and as these cultivars also presented the greatest divergence from the Sx line (Griffa *et al.*, 2006), Am and Bl can be suggested as potential parents for future crossings.

In conclusion, the capacity to regulate lipid peroxidation and increased oxidative damage could be one of

the mechanisms included in the tolerance to heat stress in *Cenchrus ciliaris* L. genotypes. Therefore, within the framework of a *C. ciliaris* genetic improvement programme, the foliar MDA level could be used as potential biochemical indicator for a rapid, simple and low-cost identification of heat-stress-tolerant genotypes.

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