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Differential salt-stress response during germination and vegetative growth in *in vitro* selected somaclonal mutants of *Cenchrus ciliaris* L.

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ABSTRACT

Four somaclonal mutants (S1, S4, S6 and M10) and their parental *Cenchrus ciliaris* L. cultivar Biloela were characterized under salinity conditions at germination and vegetative growth stages. Seeds of all somaclonal mutants had higher germination percentages than cv. Biloela seeds in the control and salt treatments. At 150 mM, germination was significantly higher in M10, S6 and S4 (72.3%, 66.3% and 61.8%, respectively) than in cv. Biloela (35.5%). Mutants grown under salinity along with cv. Biloela for 35 days had a different relative growth rate. S6 had the highest growth rate, indicating its potential tolerance to salt stress, whereas M10 was the most sensitive, with Bi, S4 and S1 being intermediate tolerant genotypes. Catalase enzyme activity (CAT) in M10 decreased in response to salt stress and was significantly associated with malondialdehide content, suggesting salt injury, whereas higher levels of CAT activity in S6 during salt stress were associated with increased salinity tolerance. The present results indicate that somaclonal variation and *in vitro* mutagenesis offer an effective tool for improvement of *C. ciliaris* because the somaclonal mutants showed differential tolerance to salt stress with respect to their parental and could be a better choice for use in a breeding program.

1. Introduction

In Argentina, crop expansion is forcing pastoral livestock production systems to relocate in drier lands, such as north-western Argentina (Guevara et al., 2009). *Cenchrus ciliaris* L. Syn. *Pennisetum ciliare* Link (Buffel grass), an apomictic, polyploid warm-season forage grass (Ozias-Akins, 2006; Snyder et al., 1955), is known worldwide for its tolerance to high temperatures (45 °C) and water stress, ease of establishment, productivity, and forage quality (Hacker and Waite, 2001; Kharrat-Souissi et al., 2011; Marshall et al., 2012). Hence, this species has been introduced as a forage resource to enhance the productivity of these marginal lands. In these environments, salinity, along with drought, is one of most important abiotic factors that contribute to severe declines of forage grass production and persistence (Griffa et al., 2010). Therefore, identifying and using plants adapted to saline soils is of increasing importance (Ashraf, 2009; Flowers and Flowers, 2005; Krishnamurthy et al., 2007; Nichols et al., 2009).

With the aim of obtaining new salt-tolerant *C. ciliaris* L. genotypes, a genetic improvement program is currently being conducted at the Institute of Physiology and Plant Genetic Resources (IFRGV-INTA), Córdoba, Argentina (López Colomba, 2009; López Colomba et al., 2011). Despite considerable efforts made to increase salt tolerance in *C. ciliaris* L. through conventional breeding, progress has been slow (López Colomba et al., 2011). *In vitro* mutation and selection techniques offer an alternative and effective tool for crop improvement programs through the generation of biotic and abiotic resistant plants (Jain, 2001, 2005; Maluszynski, 2001); nevertheless, forage grass species, including *C. ciliaris*, have been poorly studied. A protocol for somatic embryogenesis, plant regeneration and *in vitro* mutagenesis of *C. ciliaris* L. cultivar Biloela has been established by our group and several somaclonal mutants have been selected and already characterized using morphological and molecular markers (López Colomba, 2009; López Colomba et al., 2011). As a result of this work, four mutants, named M10, S1, S4 and S6, were identified for their differential behaviour in the field (López Colomba, 2009). However, these mutants have still not been characterized under salinity conditions.

Salt stress affects plants at various stages of development, including germination and establishment, vegetative growth, and finally reproduction and yield (Munns and Tester, 2008). Several authors reported that plant ability to germinate and establish seedlings on saline land is particularly important in perennial grasses (Abogadallah and Quick, 2009). Tolerance to salinity stress has been often associated with oxidative stress, since one of the consequences of exposure to salinity is the production of reactive oxygen species (ROS), such as superoxide radicals ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) (Apel and Hirt, 2004). Antioxidant defense system plays an important role in salt tolerance in various plant species (Abogadallah and Quick, 2009; Hasegawa et al., 2000). Increases in

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the activity of the enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) under salt stress conditions have been reported for leaves of tolerant genotypes of *Triticum aestivum*, *Chloris gayana*, *Oryza sativa*, *C. ciliaris* and *Setaria viridis* (Kim et al., 2004; Lanza Castelli et al., 2010; Luna et al., 2002; Sairam et al., 2002, 2005; Vaidyanathan et al., 2003). Most of these studies suggest a correlation between tolerance to salinity stress and the presence of an efficient antioxidant system (Ashraf, 2009; Gossett et al., 1994; Lanza Castelli et al., 2010; Luna et al., 2002; Mittova et al., 2004). In a previous work we measured oxidative stress characters, such as malondialdehide content (MDA), and found some antioxidant key enzymes to be promising indicators of *C. ciliaris* salt tolerant genotypes (Lanza Castelli et al., 2010).

In this work we evaluated the behaviour of somaclonal mutants of *C. ciliaris* L. under salt conditions at germination and vegetative stages to investigate if somaclonal variation and *in vitro* mutagenesis tools were effective to develop new salt-tolerant genotypes. Oxidative stress regulation, measured as MDA content and CAT activity, was evaluated during early vegetative stage to characterize tolerance of somaclonal mutants to salinity stress.

2. Materials and methods

2.1. Plant material

R3 seeds of somaclonal mutants of *C. ciliaris* L. (identified as M10, S1, S4 and S6) (López Colomba, 2009; López Colomba et al., 2011) and seeds of cv Biloela (Bi) were collected from plots established at IFRGV-INTA, Córdoba, Argentina (600 m a.s.l., 31° 24′S; 61° 11′W) in January–March 2011. Bi, which was used for the development of the mutant lines, was used here as the parental control. Seeds were stored in paper bags at 6 °C to prevent loss of viability.

2.2. Response to salt tolerance at the germination stage

Three independent experiments were conducted during September–December 2011. In germination tests five replicates of 20 seeds per treatment and per genotype were used. Seeds were disinfected for 15 min in 10% commercial bleach (NaClO 55 g L^{-1}) and placed on Whatman filter paper in 50-mm diameter Petri dishes moistened with 10 mL of test solution. Solutions included distilled water (0 mM), 50, 100, and 150 mM of NaCl, corresponding to 0, 0.2, 0.4 and 0.6 MPa osmotic potential, respectively. Seed incubation was performed in a growth chamber under the following conditions: 16 h light/8 h dark photoperiod (55 μmol m^{-2} s^{-1}) (4100 lux) at alternating temperatures of 25 °C (16 h) and 20 °C (8 h). Germinated seeds were counted and discarded every 2 days for 20 days. Seeds were considered to have germinated when radicals were at least 5 mm long. Rate of germination was estimated using a modified Timson's index of germination velocity: $GR = (\Sigma\ Gi)/t$, where Gi is the percentage of seed germination at 2-day intervals and t is the total germination period (Khan and Ungar, 1984). The maximum value possible using this index was 50.

In recovery experiments, seeds that did not germinate under high salt concentrations were transferred to distilled water to study germination recovery. Recovery percentage was calculated with the formula: $[(a - b)/(c - b)] \times 100$, where a is the number of seeds germinated in salt solutions plus those that recovered germination in distilled water, b is the number of seeds germinated in saline solution, and c is the total number of seeds tested (Khan and Ungar, 1984).

2.3. Measurement of relative growth rate (RGR)

Seeds of *C. ciliaris* L. somaclonal mutants and cv. Biloela (Bi) were sown in plastic trays (42 cm × 14 cm × 5 cm), with fine-mesh bottom filled with soil and vermiculite (1:1). The seeds were planted in rows approximately 2 cm apart and covered with a thin layer of soil. The trays were suspended in 10-L rectangular plastic trays (45 cm × 16 cm × 14 cm) filled with aerated Hoagland nutrient solution (Hoagland and Arnon, 1950). The salt treatment was started when seedlings had three folded leaves and was accomplished by weekly increments of 100 mM until reaching a final concentration of 500 mM. Nutrient solution without NaCl was used as control (0 mM). Evaporative conditions of nutrient solution were controlled regularly and the nutrient solution was renewed every 5 days. After 7 days of exposure to each concentration, nine plants were removed from the soil and vermiculite with their roots intact. Plants from both treatments were harvested and separated into aerial and radical part for fresh and dry weight determinations. Dry weight (DW) was determined after drying the plant parts in an oven at 65 °C for 72 h until constant weight was reached. RGR was determined as follows (Hilbert et al., 1981):

$$\mathrm{RGR} = (\ln\ \mathrm{DW}_2 - \ln\ \mathrm{DW}_1)/t_2 - t_1, \left(g\ g^{-1}\ d^{-1}\right)$$

where DW_1 is the initial total (aerial and radical part) dry weight, DW_2 is the final total dry weight and $(t_2 - t_1)$ is the difference in time interval between samplings (7 days).

2.4. Response to salt tolerance at vegetative stage

For this experiment, seeds of *C. ciliaris* L. somaclonal mutants and cv. Biloela (Bi) were grown in pots (30 cm in diameter), in greenhouse under natural light and day/night temperature of 30/15 °C and 65–75% relative humidity. Twenty days after sowing, seedlings were placed individually in holes of a Styrofoam board (20 plants per board); the boards were set on rectangular plastic trays (30 cm × 20 cm × 60 cm) filled with aerated Hoagland nutrient solution (Hoagland and Arnon, 1950). The plants were maintained under these conditions during 10 days. Salinization was accomplished by gradually adding 50 mL of 1 M NaCl per L of nutrient solution (100 mM every 48 h). Nutrient solution without NaCl was used as control. Evaporative conditions of nutrient solution were controlled regularly and the nutrient solution was renewed every 5 days. When the treatment reached 300 mM NaCl, leaf samples (five plants per treatment) were collected at different times (24 and 48 h and 15 days) for biochemical determinations.

2.4.1. Lipid peroxidation assay

Lipid peroxidation in leaves was evaluated by measuring malondialdehyde content (MDA), as described by Heath and Packer (1968). About 100 mg of the frozen material was ground in 1.5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged. An aliquot of 0.5 mL of the supernatant was reacted with 0.5 mL 20% TCA containing 0.5% thiobarbituric acid (TBA) at 90 °C for 20 min, and cooled in an ice bath. The resulting mixture was centrifuged at 12,000 rpm for 10 min and the absorbance of the supernatant was measured at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. Each sample had a control without TBA (Hodges et al., 1999). MDA concentration was calculated by using extinction coefficient of 155 mM^{-1} cm^{-1}; results are expressed as mmol MDA mg fresh weight. The procedure was repeated five times using extracts from different samples. Data of MDA were expressed as percentage of control (100%).

2.4.2. Determination of antioxidant enzyme activity (CAT)

CAT activity was measured by following the consumption of H_2O_2 at 240 nm (Aebi, 1984). For antioxidant enzyme activities, 100 mg of frozen leaf samples were ground to a fine powder in liquid nitrogen and homogenized in 1.5 mL of 50 mM potassium phosphate buffer (pH 7.5), containing 1 mM EDTA and 1% (25 mg) PVPP (polyvinylpolypyrrolidone). Homogenates were centrifuged at

12,000 rpm at 4 °C for 30 min and the supernatant was used to determine protein concentration and antioxidant enzyme activity. One unit of CAT activity was defined as the amount of enzyme required for catalyzing the conversion of 1 μmol H_2O_2 into water per minute. Protein content in the enzyme extracts was determined according to the method of Bradford (1976). Data of CAT activity were expressed as percentage of control (100%).

2.5. Statistical Analysis

Data were subjected to an analysis of variance (ANOVA) and the means were compared by DGC test (Di Rienzo et al., 2002) at 5% level of significance ($p \leq 0.05$) using InfoStat statistics software (Di Rienzo et al., 2011). No data transformation was required because percentage of damage in morphological traits and data of biochemical parameters expressed as percentage of control were normally distributed. Standard error of mean was also calculated and is shown in figures.

3. Results

3.1. Response to salt tolerance at the germination stage

Seeds of all genotypes started to germinate on day 4 after sowing, regardless of salt concentration (Fig. 1A–D). Genotypes varied in their germination percentage with salinity level (Fig. 1A–D). For example, Bi had the highest germination percentage at 0 mM salinity on day 8, at 50 mM salinity on day 14, at 100 mM on day 10, and at 150 mM salinity on day 8, whereas S4 had the highest germination percentage at 0 mM on day 4, at 50 mM on day 8, at 100 mM on day 14 and at 150 mM on day 20.

Seed germination percentage after 20 days of 150 mM salt stress decreased in Bi with increasing salt concentrations. Seeds of S4, S6 and M10 had higher germination percentages (98.0%) than Bi (73.4%) in the control treatment (0 mM) (Table 1). Moreover, S1, S4 and S6 were not affected by exposure to low salinity (50 and 100 mM NaCl) as compared to the control. High salinity (150 mM NaCl) drastically decreased germination percentage of all genotypes evaluated, with the lowest values in S1 and Bi (32.9 and 35.5%, respectively) (Table 1).

A trend of linear decreasing germination percentage (expressed as percentage of control) with increasing NaCl concentrations was found in Bi; however, the linear relationship was not evident in somaclonal mutants (S1, S4, S6 and M10) (Fig. 2A). At 150 mM, germination was significantly high in M10, S4 and S6 (27%, 32% and 36% decrease, respectively), whereas the parental cv. Biloela (Bi) showed 52% of germination decrease (Fig. 2A). Germination rates, indicated by the index of germination velocity, decreased with increasing salt concentration (Fig. 2B). When exposed to high salinity stress (150 mM), S1 exhibited a significant decline in germination velocity with respect to the control. By contrast, a lower decrease in germination velocity was observed in M10 (Fig. 2B). In addition, at intermediate salinity

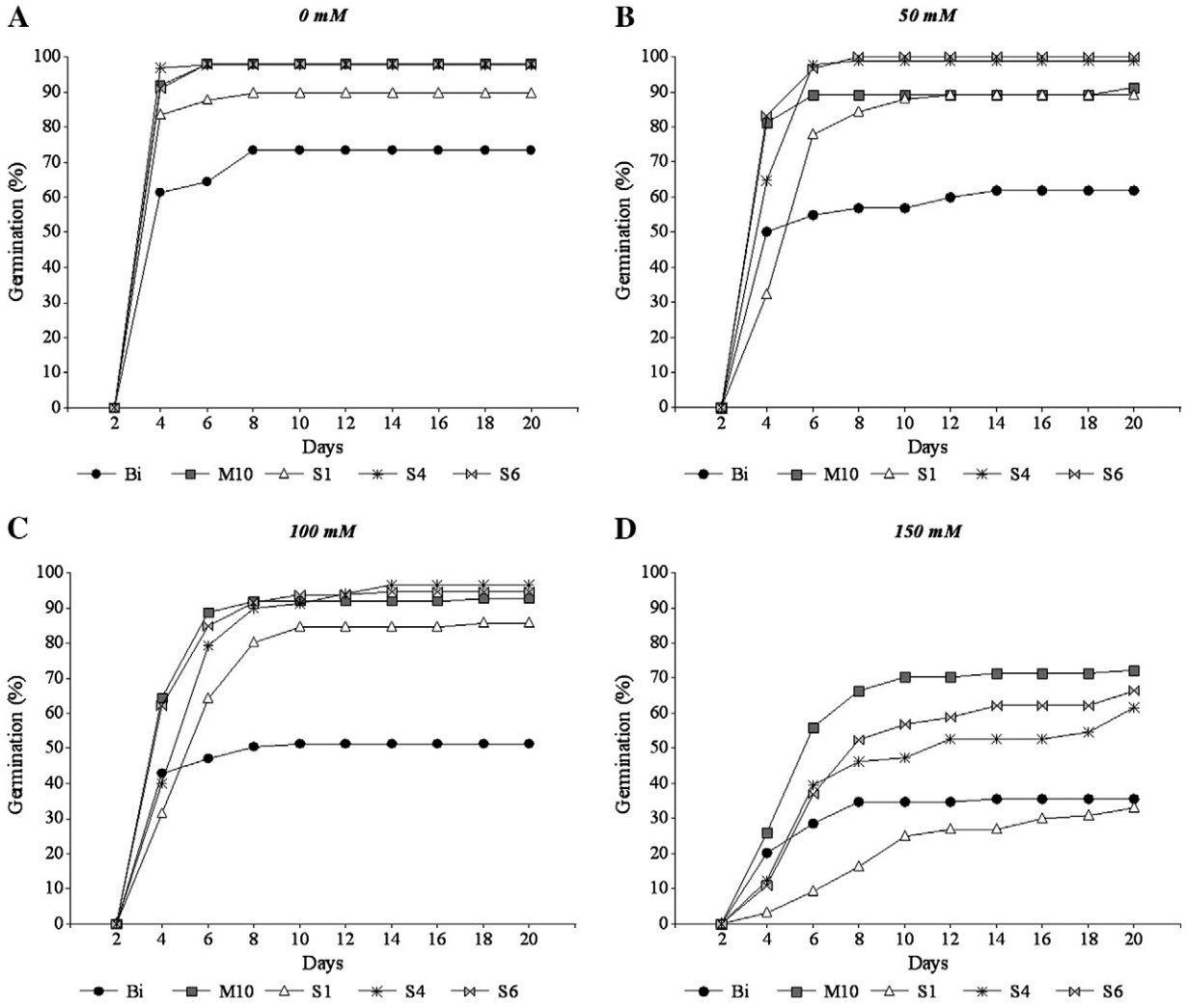

Fig. 1. Cumulative germination percentages of *Cenchrus ciliaris* cv. Biloela (Bi) and somaclonal mutant seeds (M10, S1, S4, S6) for each salinity level: 0 (A), 50 (B), 100 (C) and 150 (D) mM NaCl solutions over 20 days.

Table 1
Seed germination percentage of *Cenchrus ciliaris* cv. Biloela (Bi) and somaclonal mutants (M10, S1, S4, S6) after 20 days of exposure to different saline solutions (0, 50, 100 and 150 mM NaCl). Values are means ± SE ($n = 20$). Different letters within each column indicate significant differences among genotypes ($p < 0.05$), while different letters in each row indicate significant differences among concentrations of NaCl ($p < 0.05$).

Genotype	NaCl concentration			
	0 mM	50 mM	100 mM	150 mM
Bi	73.4 ± 4.2 d	62.0 ± 9.0 c	51.2 ± 3.9 b	35.5 ± 2.8 a
M10	98.0 ± 2.7 e	91.4 ± 8.1 e	92.8 ± 2.8 e	72.3 ± 8.7 c
S1	89.7 ± 9.6 e	89.0 ± 3.7 e	85.7 ± 5.7 e	32.9 ± 12.2 a
S4	98.0 ± 2.9 e	98.9 ± 2.4 e	96.7 ± 4.7 e	61.8 ± 12.4 c
S6	98.0 ± 4.4 e	100.0 ± 0.0 e	94.7 ± 3.5 e	66.3 ± 4.9 c

concentrations (50 and 100 mM), Bi was the most affected genotype in terms of germination percentage.

Recovery treatments of ungerminated seeds (seeds transferred to distilled water after 20 days of salt treatment) showed significant differences ($p < 0.05$). At 150 mM, recovery ranged from 18.6% for M10 to 87.8% for S6 (Fig. 3).

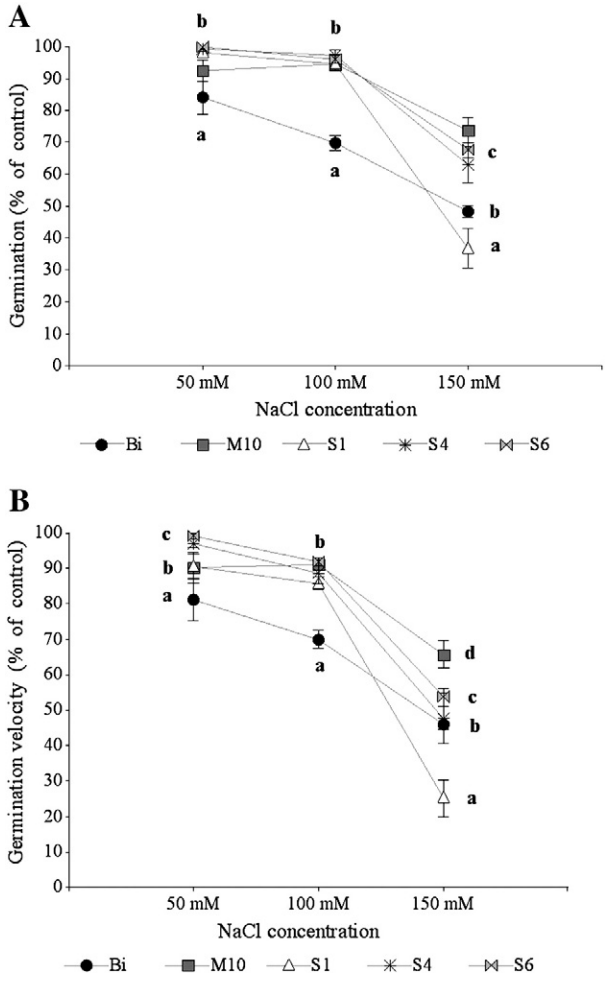

Fig. 2. Germination (A) and germination velocity (B) in *Cenchrus ciliaris* cv. Biloela (Bi) and somaclonal mutants (M10, S1, S4, S6) under different salt concentrations (50, 100 and 150 mM NaCl) for 20 days. Results are expressed as percentage of the control. The data represent the means ± standard error. Different letters indicate significant differences ($p < 0.05$).

3.2. Measurement of growth rate

After 35 days of growth under control conditions, significant differences in relative growth rate (RGR) were found (Table 2). RGR was higher in Bi than in all the somaclonal mutants (S1, S4, S6 and M10), with growth differences among the mutants. RGR of all genotypes studied was markedly affected by salt stress (Fig. 4A–B). For example, when subjected to 200 mM NaCl stress (on day 14), M10 showed the largest RGR reduction, whereas S6, Bi and S1 showed an increase. However, after this initial enhancement, plant growth of cv. Bi along with M10, S4 and S1 declined at 500 mM NaCl, whereas the smallest reduction was observed for S6 (Fig. 4B).

3.3. Response to salt tolerance at the vegetative stage

3.3.1. Lipid peroxidation assay

Lipid peroxidation in terms of foliar MDA content was similar in all genotypes under control conditions and remained constant during the experiment (data not shown). By contrast, MDA content was significantly ($p \leq 0.05$) higher under salinity stress in all genotypes. Moreover, it increased significantly in M10, as compared with Bi, S1, S4, and S6 at 24 h. At 48 h, the highest MDA content was recorded in S1, whereas the other mutants, S6 and S4, had similar MDA content to that of Bi genotype. After 15 days of exposure to salt stress (300 mM NaCl), all genotypes showed a decrease in MDA content (Fig. 5A), S6 being the mutant that showed the most significant decrease.

3.3.2. Determination of antioxidant enzyme activity (CAT)

Relatively low CAT activity was detected in leaves of all genotypes under control conditions, with no significant differences (data not shown). After 24 h of exposure to 300 mM NaCl, salt stress induced CAT activity in mutants and Bi genotype. However, this increase differed among mutants with respect to Bi. The lowest CAT activity was observed in M10, whereas CAT activity was induced in S1, as in Bi genotype. The mutants S4 and S6 had lower CAT activity than Bi and S1 (Fig. 5B). At 48 h, while CAT activity remained constant in Bi genotype, in S1 it decreased until the end of the experiment. By contrast, M10, S4, and S6 increased, with S6 showing the highest CAT activity. Significantly higher levels of CAT activity were observed in S6 than in Bi, M10, S4 and S1 at day 15 of exposure to salt stress, S1 being the mutant that showed the most significant decrease.

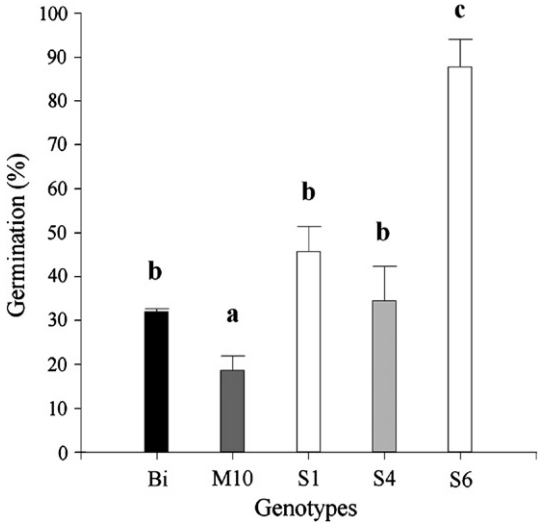

Fig. 3. Recovery germination percentage of *Cenchrus ciliaris* cv. Biloela and somaclonal mutants (M10, S1, S4, S6) exposed to 150 mM NaCl. Vertical bars are means of recovery percentage ± SE. Different letters indicate significant differences ($p < 0.05$).

Table 2
Relative growth rate (RGR) of *Cenchrus ciliaris* cv. Biloela and somaclonal mutants (M10, S1, S4, S6) grown under control (C) and salt stress (S) conditions for 35 days (500 mM). Different letters indicate significant differences ($p < 0.05$).

Genotype	Treatment	RGR (aerial)	RGR (root)
Bi	C	0.02930 c	0.00676 e
M10		0.01803 b	0.00375 c
S1		0.00541 a	0.00060 a
S4		0.02512 c	0.00531 d
S6		0.01855 b	0.00199 b
Bi	S	0.00691 b	0.00112 b
M10		0.00758 b	0.00148 c
S1		0.00391 a	0.00054 a
S4		0.00396 a	0.00072 a
S6		0.00926 c	0.00169 c

4. Discussion

Somaclonal variation and random mutagenesis followed by selection have been used to develop tolerant mutants, which have been released as cultivars in several crops such as rice, wheat, cotton, rapeseed, sunflower, sesame, grapefruit and banana (Ahloowalia et al., 2004; Jain, 2001, 2005). In our previous works, several putative mutants were isolated in the primary screening. These *C. ciliaris* L. somaclonal mutants were already characterized using morphological and molecular markers (López Colomba, 2009; López Colomba et al., 2011); in addition, four mutants (named S1, S4, S6 and M10) were selected based on testing of their progenies in the field.

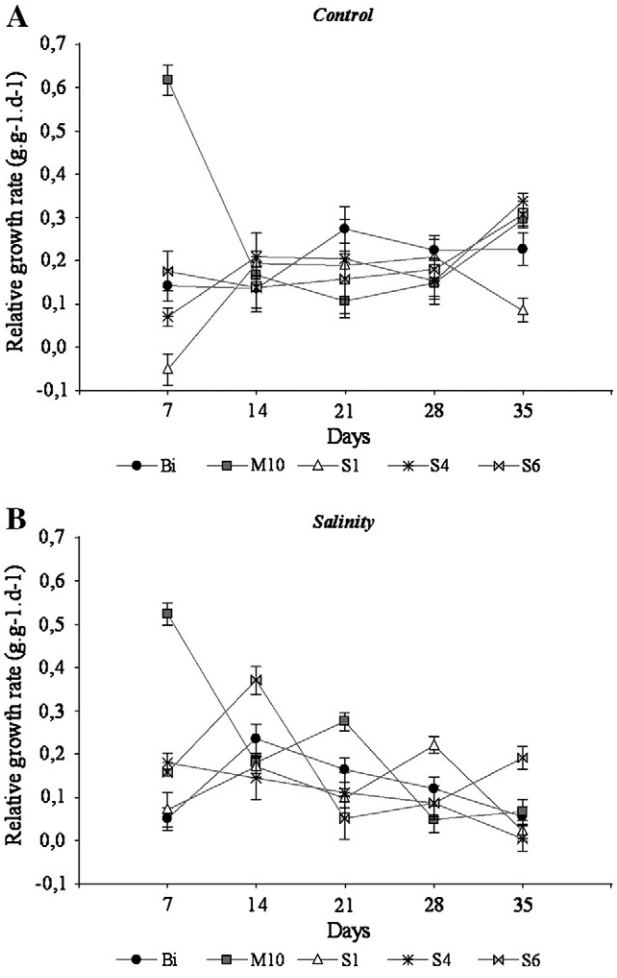

Fig. 4. Relative growth rate of *Cenchrus ciliaris* cv. Biloela and somaclonal mutants (M10, S1, S4, S6) grown under control (A) (0 mM) and salt stress (B) conditions (days 7, 14, 21, 28, and 35 correspond to 100, 200, 300, 400, and 500 mM NaCl). The data represent the means ± standard error.

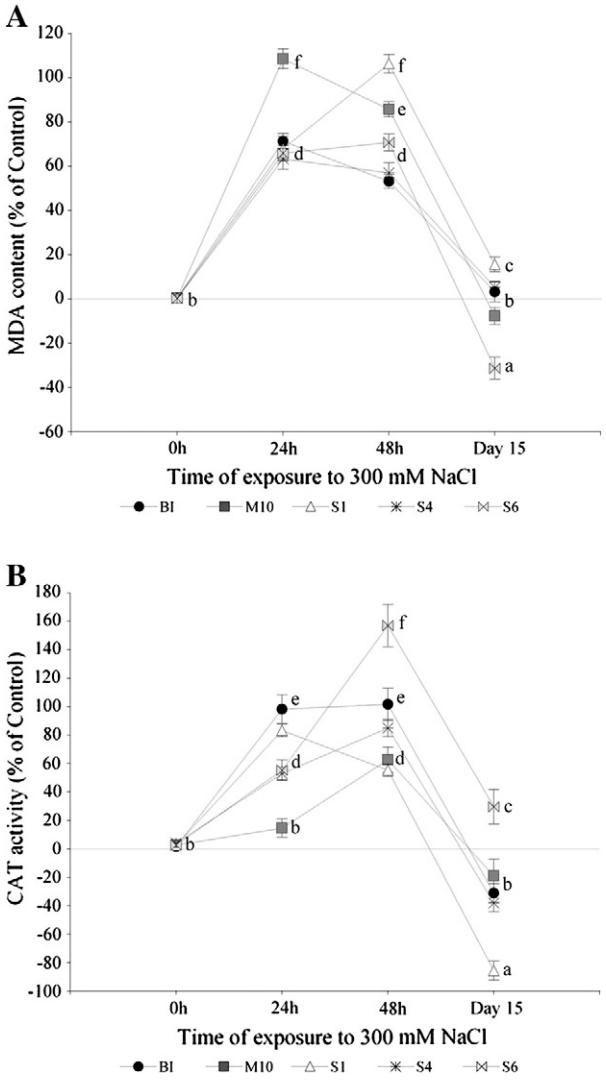

Fig. 5. Effects of salt stress on MDA content (A) and CAT activity (B) of *Cenchrus ciliaris* L cv. Biloela and somaclonal mutants (M10, S1, S4, S6) after 24 h, 48 h and 15 days of exposure to 300 mM NaCl. Values were expressed as percentage of control plants (0 mM NaCl). Bars represent mean ± standard error. Different letters indicate significant differences ($p < 0.05$).

Salt tolerance during germination and early seedling stages is critical for the establishment of plants that can grow in saline soil (Al-khateeb, 2006). In *C. ciliaris*, seed germination is inherently poor and becomes unpredictable, especially in semi-arid regions characterized by low rainfall, due to its extended period of dormancy (Bhattarai et al., 2008; Sharif-Zadeh and Murdoch, 2001). In this study, seeds of S1, S4, S6 and M10 somaclonal mutants had higher germination percentages than *C. ciliaris* cv. Biloela in the control and salt treatments. The relationship between salinity and germination rate was linear, or close to being linear in cv. Biloela, which suggests a simple physical cause, and may be indicative of the importance of osmotic limitation in determining germination rate. Similar findings were reported for other grasses (Easton and Kleindorfer, 2009; Joshi et al., 2005). Despite the reduction in germination rate in S4, S6 and M10 at high salt concentrations, germination percentages remained uniformly high, suggesting that the osmotic stress imposed was enough to slow water uptake, but not to prevent the seeds from achieving the required water content for germination.

The ability to recover germination has implications for seedling establishment in saline environments, particularly when initial rainfall is insufficient to flush salts from the soil surface. In such situations, imbibed seeds may be able to survive until subsequent rains permit completion of germination. A large number of the ungerminated seeds of S6 remained viable and germinated when transferred to distilled water after exposure to 150 mM for 20 days. Accordingly, salinity is considered to affect seed germination through osmotic effects, ion toxicity or their combination (Zhang et al., 2012). High germination recovery ability in S6 appears to be due to osmotic effect, whereas negative impact of 150 mM salt concentration on germination in M10 appears more related to ion toxicity than to osmotic effect.

Levels of salt tolerance during germination and at the later stages of development in some species are markedly different (Abogadallah and Quick, 2009; Abogadallah et al., 2010). Usually, however, species that are highly tolerant to salt stress during germination are also highly tolerant at growth and reproduction stages. In this work, response of *C. ciliaris* L. somaclonal mutants to the application of salt concentrations varied at germination and early growth stages. For instance, under salt stress, M10 was characterized by consistently higher germination percentages than those of the cv. Biloela and S1 somaclonal mutant. However, this trend was not observed in early growth assays, which, agrees with previous findings in apomictic and sexual genotypes of *C. ciliaris* (Griffa et al., 2010).

When the mutants were grown under salt conditions along with cv. Biloela for 35 days, their performance in terms of growth rate was different. S6 had the highest growth rate of all genotypes, indicating its potential tolerance to salt stress, whereas M10 was the most sensitive, with Bi, S4 and S1 being intermediate tolerant genotypes. This morphological characterization under salt stress was compared with the capacity of mutants to regulate oxidative damage.

Environmental stresses, including high salinity, cause oxidative stress via the production and accumulation of ROS. A risk for serious cellular damage may arise when ROS are overproduced under stress conditions. In addition, free radical-induced peroxidation in lipid membrane is both a reflection and a measure of stress-induced damage at the cellular level (Lanza Castelli et al., 2010; Tommasino et al., 2012). Content of lipid peroxidation at 24–48 h in M10 was significantly higher than in the control and the other mutants, suggesting its inefficiency in using the incident light when exposed to salt (Chandran and Puthur, 2009).

Based on our results, it was of further interest to analyze the effectiveness of free radical scavenging mechanisms operating in control cv. Biloela and somaclonal mutants of *C. ciliaris* L. Antioxidant defense system is positively associated with salt tolerance in plants. SOD dismutes oxygen radicals into H_2O_2 (Mittler, 2002). H_2O_2 is a ROS metabolized by catalase or ascorbate-peroxidase in plants (Noctor and Foyer, 1998). Some investigations have demonstrated higher activities of antioxidant enzymes in drought-tolerant cultivars of rice and wheat (Guo et al., 2006; Lascano et al., 2001). In agreement with these observations, Bi and S6 showed higher CAT activity than the other mutants throughout the experiment; this behavior was associated with salt tolerance stress evaluated as increases in growth rate and decreases in MDA levels. By contrast, the present results show that in M10, CAT activity decreased in response to salt stress and it was significantly associated with the lowest growth rate and increase in MDA content, suggesting that salt injury on somaclonal mutants is related to a decline in antioxidant CAT enzyme activity. The association of salt injury with the decrease of antioxidant enzyme activities has been observed in several forage grasses (Abogadallah and Quick, 2009; Abogadallah et al., 2010; Luna et al., 2000, 2002), including *C. ciliaris* (Lanza Castelli et al., 2010). Since oxidative stress has been considered a common metabolic route of different stresses (Apel and Hirt, 2004; Mittler, 2002), some parameters of oxidative stress could be proposed as a tool to identify tolerance to different abiotic stress factors. MDA content was a useful tool to characterize salt tolerance in four *C. ciliaris* L. genotypes (Lanza Castelli et al., 2010) and also in *C. gayana* diploid cultivars (Luna et al., 2000, 2002). In our study, oxidative damage evaluated as foliar MDA content was related to tolerance of Bi and mutants; consequently it could be used as biochemical indicator for a rapid, simple and low-cost identification of tolerant *C. ciliaris* L. genotypes.

In summary, in the present study four somaclonal mutants with differential response to salt tolerance in germination and early growth were identified. The analysis of these somaclonal variants in comparison to *C. ciliaris* cv. Biloela indicated that they exhibited a differential response to oxidative stress and it was in accordance with their tolerance to salt stress. All these results indicate that somaclonal variation and *in vitro* mutagenesis offer an effective tool for improvement in *C. ciliaris* because the somaclonal mutants obtained showed differential tolerance to salt stress with respect to their parental, cv. Biloela, and could be a better choice for use in a breeding program.

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