Plant Physiology and Biochemistry 102 (2016) 53–61

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

# Genotypic variation in response to salinity in a new sexual germplasm of *Cenchrus ciliaris* L.

Mariana Quiroga [a, *], Exequiel Tommasino [a], Sabrina Griffa [a], Andrea Ribotta [a], Eliana López Colomba [a], Edgardo Carloni [a], Karina Grunberg [a, b]

[a] Instituto de Fisiología y Recursos Genéticos Vegetales, Centro de Investigaciones Agropecuarias, Instituto Nacional de Tecnología Agropecuaria (IFRGV-CIAP-INTA), Camino 60 cuadras Km 5 ½, X5020ICA, Córdoba, Argentina
[b] Consejo de Nacional Investigaciones Científicas y Técnicas, Godoy Cruz 2290, C1425FQB, CABA, Argentina

ARTICLE INFO

*Article history:*
Received 30 December 2015
Received in revised form
10 February 2016
Accepted 11 February 2016
Available online 15 February 2016

*Keywords:*
*Cenchrus ciliaris*
Sexual hybrids
Salt stress
Seedling
Germination

ABSTRACT

As part of a breeding program for new salt-tolerant sexual genotypes of *Cenchrus ciliaris* L., here we evaluated the salt-stress response of two new sexual hybrids, obtained by controlled crosses, at seedling and germination stages. A seedling hydroponic experiment with 300 mM NaCl was performed and physiological variables and growth components were evaluated. While salt-treated sexual material did not show a decrease in productivity with respect to control plants, a differential response in some physiological characteristics was observed. Sexual hybrid 1-9-1 did not suffer oxidative damage and its proline content did not differ from that of control treatment. By contrast, sexual hybrid 1-7-11 suffered oxidative damage and accumulated proline, maintaining its growth under saline stress. At the germination stage, sexual hybrid 1-9-1 presented the highest Germination Rate Index at the maximum NaCl concentration assayed, suggesting an ecological advantage in this genotype. These new sexual resources are promising maternal parental with differential response to salt and could be incorporated in a breeding program of C. ciliaris in the search of new genotypes tolerant to salinity.

© 2016 Elsevier Masson SAS. All rights reserved.

## 1. Introduction

Buffelgrass (*Cenchrus ciliaris* L., Syn. *Pennisetum ciliare* Link) is an important gramineous forage species in arid and semiarid regions worldwide (Hanselka et al., 2004). In Argentina, it was introduced as a forage resource in areas affected mainly by water stress, showing good performance, and is adapted to harsh climatic conditions prevailing in the Argentine northwestern region (NOA) (Tessi et al., 2014). The saline soils characteristic of this vast region limit implantation, persistence and forage production (Ashraf et al., 2006). Buffelgrass has a mainly obligate apomictic reproductive mechanism (Snyder et al., 1955) and the use of obligate sexual or apomictic genotypes with high levels of sexuality is the only alternative for conventional crosses (Bray, 1978; Bashaw, 1980; Sherwood et al., 1980; Quiroga et al., 2013). Our working group characterized a sexual line that was used as the only maternal source, showing poor forage aptitude (Griffa et al., 2005) and significant susceptibility to salt stress (Griffa, 2010; Lanza Castelli et al., 2010). However, using this source of sexuality, two sexual genotypes genetically divergent from the female parental line have been obtained from hybridization with apomictic material (Quiroga et al., 2013). These new sexual hybrids showed some promising traits for higher quality forage and biomass yield, and therefore could be used as new female parents for breeding purposes (Quiroga et al., 2013).

Despite the potential importance of buffelgrass as forage resource for cattle production, few reports have characterized its biochemical and physiological response to salinity for genetic improvement purposes (Akram et al., 2006; Ashraf et al., 2006; Griffa, 2010; Lanza Castelli et al., 2010). Salt stress leads to the overproduction of reactive oxygen species (ROS), such as superoxide ($O^{-}_{2}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($HO^{-}$) (Apel and Hirt, 2004). The excess of ROS in plants is highly toxic and causes damage to proteins, membrane lipids, carbohydrates and

* Corresponding author. Instituto de Fisiología y Recursos Genéticos Vegetales, Centro de Investigaciones Agropecuarias, Instituto Nacional de Tecnología Agropecuaria (IFRGV-CIAP-INTA), Camino 60 cuadras Km 5 ½, X5020ICA, Córdoba, Argentina.
*E-mail address:* quiroga_mariana@yahoo.com.ar (M. Quiroga).

http://dx.doi.org/10.1016/j.plaphy.2016.02.016
0981-9428/© 2016 Elsevier Masson SAS. All rights reserved.

DNA, producing oxidative stress (Gill and Tuteja, 2010). Plants have a complex antioxidant enzymatic and non-enzymatic defense system designed to regulate ROS levels (Ashraf and Foolad, 2007; Ashraf, 2009); when the defense mechanism fails to inactivate ROS excess, oxidative damage occurs. Severity of oxidative damage in plants can be assessed by measuring Malondialdehyde (MDA) content, which reflects the product of peroxidation of membrane lipids (Pérez-López et al., 2009). MDA content has also been shown to be a biochemical indicator of salt tolerance in buffelgrass (Lanza Castelli et al., 2010; López Colomba et al., 2013). The strategies that plant may use for dealing with such stress can be indirectly detected by estimating the redox state of the plant; this is accomplished by evaluating the ability to reduce iron (FRAP) via the non-enzymatic antioxidant defense system (Benzie and Strain, 1996; Ou et al., 2002). Oxidative damage can also be estimated by measuring proline content because high concentrations of this osmo-compatible compound may protect plants from salt stress via detoxification of ROS, protection of membrane integrity, and stabilization of enzymes/proteins as well as through contribution to cellular osmotic adjustment (Ashraf and Foolad, 2007; Ashraf, 2009; Cha-Um and Kirdmanee, 2009).

A reduction in chlorophyll content in leaves under salt conditions has been reported in various plant species (Parida et al., 2004). This decline may be attributed to the destruction of chlorophyll pigments and instability of pigment-protein complexes, interference of salt ions with protein synthesis and structural components of chlorophyll (Munns, 2011). Thus, photosynthesis is one of the primary processes affected by salinity through a reduction of the maximum quantum efficiency of photosystem II (PSII) (Munns et al., 2006). Salt influences photosynthetic capacity and its effects vary with salt concentration, duration of stress and the assayed germplasm (Kalaji et al., 2011).

Salinity affects plant performance (Zhu, 2001; Yu et al., 2012) by reducing water availability to plants and interfering with ionic balance inside the cell, causing molecular damage, growth arrest and cell death (Zhu, 2001). For instance, relatively high $Na^+$ and $Cl^-$ concentrations can obstruct the absorption of $K^+$, $Ca^{2+}$, $Mg^{2+}$ and other ions, and reduce root and shoot growth (Yu et al., 2012). As a result, a high $K^+/Na^+$ ratio is an important criterion used for selecting for salt tolerance in other species (Al-Khateeb, 2006; Lopez and Satti, 1996; Monirifar and Barghi, 2009; Paz et al., 2012).

General symptoms of damage by salt stress in plants include growth inhibition, accelerated development, senescence and death during prolonged exposure (Jouyban, 2012). Damage to fresh weight of aerial part was found to be the principal component character with direct influence on productivity and a reliable indicator of early selection for salt-tolerant genotypes in buffelgrass (Griffa, 2010). Moreover, salinity effects vary with growth stage. Salt tolerance at germination and emergence, as well as in later growth stages, is among the traits that could confer performance advantages in saline environments. Germination and seedling establishment are considered to be the most critical stages of the plant life cycle under salt conditions (Ungar, 1978) and the capability to germinate under such conditions is essential for ensuring the natural resowing of pastures. In some species, plants are more sensitive to salt during germination and emergence than at later stages (Bazzigalupi et al., 2008). However, salinity tolerance at different growth stages seems to be controlled by independent genes (Jena and Mackill, 2008) and there is evidence that response at the seedling stage persists in adult plants (Khan and McNeilly, 2005; Griffa, 2010).

The aim of this work was to study the genotypic variation in response to salinity in a new sexual germplasm of buffelgrass during the seedling and germination stages.

## 2. Material and methods

### 2.1. Plant material

The genetic material used in this work was obtained from an active collection of buffelgrass located in the Experimental Area of IFRGV-INTA (Córdoba, Argentina). Five genotypes were evaluated to determine the genotypic variation in response to salt stress: original sexual line, female parent (S. line), two apomictic accessions (male parents), register numbers (RN) 153 and 136, and two sexual hybrids: 1-9-1 (S. line × RN 153) and 1-7-11 (S. line × RN 136).

### 2.2. Seedling hydroponic experiment

Forty plants of each parental line (S. line and apomictic males) and sexual hybrids were arranged in four plastic trays with aerated Hoagland's nutrient solution (Hoagland and Arnon, 1950). Two trays were allocated to the control (0 mM NaCl) and the two other trays, to the saline treatment of 300 mM NaCl; this amount was selected because buffelgrass genotypes were previously found to manifest symptoms of salt stress at this concentration (Griffa, 2010). Ten seedlings per genotype and condition were allowed to acclimate to hydroponic conditions for 7 days. Then nutrient solution was gradually salinized by adding 100 mM NaCl every 24 h, until reaching 300 mM NaCl, and the seedlings were kept in these conditions for 12 days. Leaf samples were collected and kept frozen at −20 °C until evaluation of physiological variables.

#### 2.2.1. Evaluation of physiological variables

Relative water content (RWC) was evaluated at 24 h of reaching 300 mM of NaCl and at the end of the assay (12 days) (Turner, 1986), $Na^+/K^+$ ratio and $Mg^{2+}$ concentration with High Performance Liquid Chromatography (HPLC) (Shimadzu). Parameters associated with oxidative stress were also measured 24 h after the nutrient solution contained 300 mM NaCl: MDA content (Heath and Packer, 1968), the ability to reduce iron (FRAP) (Benzie and Strain, 1996) and chlorophyll content (Tetley and Thimann, 1974). At the end of the assay, total soluble sugars (Fales, 1951), total protein (Bradford, 1976) and proline contents (Bates et al., 1973) were estimated.

#### 2.2.2. Maximum quantum efficiency of PSII (PSII), SPAD values (SPAD) and Foliar Temperature (FT)

Maximum quantum efficiency of PSII (Fm/Fv) was measured according to Bilger and Björkman (1990) using a modulated fluorescence system (FMS2, Hansatech Instruments, Pentney King's Lynn, UK), SPAD values using a chlorophyll meter Model CL-01 Chlorophyll Content Meter (Hansatech Instruments) (Xu et al., 2000), and Foliar Temperature (FT) using an Infrared Thermometer (IT-330 Horiba). All measurements were taken every 48 h.

#### 2.2.3. Growth components

The following growth components were evaluated in each of the seedlings per genotype and per treatment: total and aerial fresh weight (TFW and AFW, respectively), total and aerial dry weight (TDW and ADW, respectively), leaf fresh weight (LFW), root fresh and dry weight (RFW and DRW, respectively) and leaf area (LA).

### 2.3. Germination

Salt tolerance at the germination stage was evaluated according to López Colomba et al. (2013) for buffelgrass. Each treatment consisted of five trays, each tray containing 20 seeds of each genotype mentioned in point 2.1. Seeds were disinfected with commercial sodium hypochlorite (NaClO 55 g/L) at a concentration of 10% for 5 min and rinsed three times with distilled water. The

disinfected seeds were transferred to plastic trays lined with paper filter soaked with each salt treatment. The salt treatments used were: 0 mM (control), 75, 125, 175 and 225 mM NaCl. Seed incubation was performed in a growth chamber during 20 days 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 recorded every 48 h for a total of 20 days. The proportion of total germinated seeds (PGS) was calculated following López Colomba et al. (2013). The effect of treatments was evaluated using the germination rate index (GRI), which expresses germination speed, calculated as follows:

$$GRI = \Sigma\ Gi/Di$$

where Gi is the difference in number of germinated seeds between i count and the previous count and Di is the day of each count after planting (5, 10, 15 and 20 days) (Bazzigalupi et al., 2008).

#### 2.3.1. Statistical analysis

For comparisons between means in RWC, $Na^+/K^+$ ratio, FRAP, chlorophylls and proline contents, general linear mixed models were used and ANOVA was applied for a two-factor model with interaction between the factors genotype × treatment (0 and 300 mM NaCl), in a randomized complete block design. For $Mg^{2+}$ concentration, MDA, total soluble sugars, total protein content, PSII, SAPD values and FT two- and three-way interactions were applied between variables (e.g. Genotype × Treatment and Genotype × Treatment × Time of measurement). Di Rienzo, Guzmán and Casanoves test (DGC) (Di Rienzo et al., 2002) was performed using InfoStat statistical package (Di Rienzo et al., 2014).

To evaluate salt tolerance at the germination stage, generalized linear mixed models were used to compare means of PGS and GRI. An ANOVA with interaction between genotype × salt concentration was performed and a DGC test was applied for both variables using InfoStat (Di Rienzo et al., 2014).

## 3. Results

### 3.1. Seedling hydroponic assay

#### 3.1.1. Evaluation of physiological variables in 300 mM NaCl at 24 h

After 24 h of exposure to the final salt concentration, no genotype × treatment interaction was observed for RWC ($P = 0.19$), chlorophyll content ($P = 0.33$) or $Mg^{2+}$ concentration ($P = 0.09$). RWC and $Mg^{2+}$ content did not have significant differences for genotypes ($P = 0.62$ and 0.09, respectively) or treatment ($P = 0.09$ and 0.86, respectively). Average RWC was 93.91% for the control treatment (0 mM NaCl) and 86.94% for the salt treatment (300 mM NaCl), whereas mean $Mg^{2+}$ values were 4.06 nmol/g DW for control and 4.47 nmol/g DW for salt treatment. Chlorophyll content did not differ between genotypes ($P = 0.06$), but it did differ between treatments ($P = 0.004$), with a mean of 6700.30 μg/g DW in the control treatment, decreasing to 4160.85 μg/g DW in salt treatment.

The $Na^+/K^+$ ratio showed significant differences in genotype × treatment interaction ($P = 0.01$), with an increase being recorded in salinity in all genotypes (Table 1). Hybrid 1-7-11 and its parentals (S. line and RN 136) had the highest ratios.

The genotype × treatment interaction was significant for MDA content ($P = 0.04$) (Table 1). While most genotypes showed no differences among them or between treatments, treated hybrid 1-7-11 plants and their male parent (RN 136) showed an increase in MDA content (Table 1).

There was genotype × treatment interaction for FRAP values ($P = 0.03$) (Table 1). None of the sexual genotypes showed significant differences among them or between treatments, whereas the apomictic parental plants showed increased values under salt conditions.

#### 3.1.2. Evaluation of physiological variables after 12 days of exposure to 300 mM NaCl

Results of the physiological variables evaluated at the end of the assay are shown in Table 2. Significant differences between genotype and treatment were observed for RWC ($P = 0.01$), which increased only in the hybrid 1-7-11 and apomictic material RN 153 under salt conditions.

The $Na^+/K^+$ ratio presented significant genotype × treatment interaction ($P = 0.01$). Ratios increased in all genotypes in the saline treatment compared to the control, with the hybrid 1-7-11 showing a very pronounced increase.

The analysis of $Mg^{2+}$ content also showed interaction ($P = 0.02$), with S. line being the only genotype showing a decrease under salinity condition.

Of the tested osmo-compatible compounds, proline content was the only one presenting genotype × treatment interaction ($P = 0.05$). There were no differences among genotypes in the control treatment, whereas in salt treatment, proline content increased in hybrid 1-7-11, S. line and RN 153. Total soluble sugar content also exhibited a significant interaction ($P = 0.02$), with hybrid 1-7-11 being the only genotype with a decrease in soluble sugar content under salt conditions.

Total protein content also showed genotype × treatment interaction ($P = 0.02$), with values increasing in hybrid 1-9-1 and its parental RN 153 in the salt treatment. In contrast, protein content values decreased in RN 136 under salinity conditions. Protein content did not vary in either treatment for hybrid 1-7-11 and S. line.

#### 3.1.3. Maximum quantum efficiency of PSII, SPAD values (SPAD) and Foliar Temperature (FT)

PSII values were non-significant either for three-way interaction (genotype × treatment × time, $P = 0.88$) or two-way interaction (genotype × treatment, genotype × time, $P = 0.41$ and 0.48, respectively). While treatments did not differ ($P = 0.20$), with average values of 0.80 in control and 0.79 in salt, genotypes ($P = 0.03$) and time ($P = 0.03$) were the factors that showed differences for this variable. A decrease in PSII was observed over time, with RN 136 presenting the lowest values.

SPAD values did not show significant differences for three-way interaction ($P = 0.52$) or the two-way interactions genotype × treatment or genotype × time ($P = 0.66$ and 0.68, respectively). The factors treatment and time of measurement were significant ($P < 0.0001$). In the control treatment, SPAD varied with seedling growth, being low at the beginning (1.14), increasing with growth (7.44) and decreasing at the end of the assay (4.93). Under stress conditions, SPAD values showed no difference (1.60–2.97), but were significantly lower than those of the control at 12 days of assay.

ANOVA results for FT showed no genotype × treatment × time interaction ($P = 0.90$) or genotype × treatment ($P = 0.41$) or genotype × time ($P = 0.97$) interactions. Although no differences were observed among genotypes ($P = 0.21$), there were differences between treatments ($P = 0.002$) and time of measurement ($P = 0.01$). Average FT in salt treatment was higher (29.11 °C) than in control (28.61 °C). Regarding time of measurement, although FT increased at 48 h of sampling, it tended to stabilize at the end of the test (192 h).

#### 3.1.4. Growth components

Genotype × treatment interaction was highly significant ($P = 0.0001$) for total fresh weight and fresh weight of the aerial

**Table 1**
Evaluation of physiological variables of two sexual hybrids and their parentals at 24 h of 300 mM NaCl treatment. Mean values are presented and standard deviation is indicated in parentheses. Different letters in each column indicate significant differences ($P \leq 0.05$).

| Genotype | Treatment | $Na^+/K^+$ | MDA | FRAP |
|---|---|---|---|---|
| | | | (μmol/g DW) | (μmol/g DW) |
| RN 153 | 0 mM NaCl | 0.02 c | 39.48 b | 168.08 a |
| | | (0.01) | (4.37) | (23.42) |
| | 300 mM NaCl | 0.07 b | 40.03 b | 124.52 b |
| | | (0.02) | (4.37) | (18.22) |
| RN 136 | 0 mM NaCl | 0.03 c | 30.17 b | 169.56 a |
| | | (0.01) | (4.37) | (23.42) |
| | 300 mM NaCl | 0.13 a | 64.38 a | 55.16 b |
| | | (0.02) | (4.37) | (18.22) |
| Sexual line | 0 mM NaCl | 0.02 c | 31.51 b | 104.59 b |
| | | (0.01) | (6.18) | (18.22) |
| | 300 mM NaCl | 0.16 a | 39.26 b | 65.98 b |
| | | (0.06) | (4.37) | (18.22) |
| 1-7-11 (S. line × RN 136) | 0 mM NaCl | 0.03 c | 36.41 b | 104.37 b |
| | | (0.01) | (6.18) | (18.22) |
| | 300 mM NaCl | 0.17 a | 62.69 a | 80.71 b |
| | | (0.02) | (6.18) | (18.22) |
| 1-9-1 (S. line × RN 153) | 0 mM NaCl | 0.03 c | 26.69 b | 77.78 b |
| | | (0.01) | (4.37) | (18.22) |
| | 300 mM NaCl | 0.08 b | 41.47 b | 104.7 b |
| | | (0.02) | (4.37) | (18.22) |

**Table 2**
Evaluation of physiological variables at 12 days of 300 mM NaCl treatment of two sexual hybrids and their parentals. Mean values are presented and standard deviation is indicated in parentheses. Different letters in each column indicate significant differences ($P \leq 0.05$).

| Genotype | Treatment | RWC (%) | $Na^+/K^+$ | $Mg^{2+}$ | Proline | Sugars | Proteins |
|---|---|---|---|---|---|---|---|
| | | | | (nmol/g DW) | ($mol/mm^2$) | (μmol/g DW) | (μmol/g DW) |
| RN 153 | 0 mM NaCl | 77.78 b | 0.04 d | 3.46 a | 0.46 b | 1.36 b | 95.27 b |
| | | (2.62) | (0) | (0.51) | (0.19) | (0.15) | (18.45) |
| | 300 mM NaCl | 94.91 a | 0.23 b | 3.29 a | 1.28 a | 0.88 b | 220.15 a |
| | | (2.62) | (0.04) | (0.51) | (0.27) | (0.15) | (18.45) |
| RN 136 | 0 mM NaCl | 92.59 a | 0.03 d | 3.19 a | 0.26 b | 1.01 b | 192.01 a |
| | | (3.7) | (0) | (0.72) | (0.19) | (0.15) | (24.93) |
| | 300 mM NaCl | 89.58 a | 0.2 b | 3.11 a | 0.72 b | 0.78 b | 160.63 b |
| | | (3.70) | (0.04) | (0.51) | (0.19) | (0.15) | (29.88) |
| Sexual line | 0 mM NaCl | 91.67 a | 0.06 c | 3.62 a | 0.75 b | 1.33 b | 67.63 b |
| | | (2.62) | (0) | (0.51) | (0.19) | (0.15) | (18.45) |
| | 300 mM NaCl | 74.07a | 0.2 b | 0.42 b | 1.85 a | 0.76 b | 156.36 b |
| | | (3.70) | (0.04) | (0.72) | (0.27) | (0.15) | 25.18 |
| 1-7-11 (S. line × RN 136) | 0 mM NaCl | 75.87 b | 0.04 d | 0.95 b | 0.41 b | 2.03 a | 60.8 b |
| | | (2.62) | (0) | (0.72) | (0.27) | (0.15) | (18.45) |
| | 300 mM NaCl | 88.33 a | 0.45 a | 1.08 b | 1.25 a | 0.73 b | 118.88 b |
| | | (3.70) | (0.04) | (0.72) | (0.27) | (0.15) | (18.5) |
| 1-9-1 (S. line × RN 153) | 0 mM NaCl | 84.24 a | 0.05 c | 1.99 b | 0.4 b | 1.38 b | 124.18 b |
| | | (2.62) | (0) | (0.51) | (0.27) | (0.15) | (18.45) |
| | 300 mM NaCl | 97.14 a | 0.33 b | 2.08 b | 0.63 b | 1.23 b | 222.07 a |
| | | (3.70) | (0.13) | (0.51) | (0.19) | (0.15) | (18.45) |

part (TFW and AFW), with variation in response between hybrids (Fig. 1A and B). Weight of hybrid 1-7-11, RN 136 and RN 153 decreased under salt conditions, whereas weight of hybrid 1-9-1 and S. line (female parental) did not change under salt stress.

The analysis of total dry weight (TDW) and dry weight of the aerial part (ADW) (Fig. 1C and D) showed a high genotype × treatment interaction ($P = 0.0007$ and 0.0061, respectively). Sexual hybrids did not differ in their behavior and were not affected by salt, similarly to RN 153 and S. line. However, the latter had lower dry matter in both treatments than both hybrids. RN 136 was the only genotype affected under 300 mM NaCl treatment.

Fresh weight of leaf (LFW) (Fig. 2A) exhibited differences in genotype × treatment interaction ($P < 0.0001$), with hybrid 1-9-1 presenting similar values to those of its maternal line, without significant differences between treatments. Hybrid 1-7-11 behaved similarly to RN 153, with LFW decreasing in salt. RN 136 showed the highest LFW values in both treatments, but decreased in seedlings grown under salt conditions.

Fresh and dry root weight (RFW and RDW) (Fig. 2 B and C) showed differences in the interaction ($P = 0.0112$ and 0.0031, respectively). Sexual hybrids, S. line and RN 153 did not differ between treatments, although the latter showed higher values for RFW in both treatments. RN 136 presented higher RFW and RDW under control conditions and was the only genotype significantly affected by 300 mM NaCl. However, RN 153 had the highest RFW value in salt, but its RDW did not differ from the other genotypes.

Leaf area (LA) (Fig. 2 D) showed differences in the interaction ($P < 0.0001$). Hybrid 1-9-1 was characterized by a smaller LA under control conditions, as its female parent, but was the only genotype that did not exhibit LA variation under salt conditions. In the

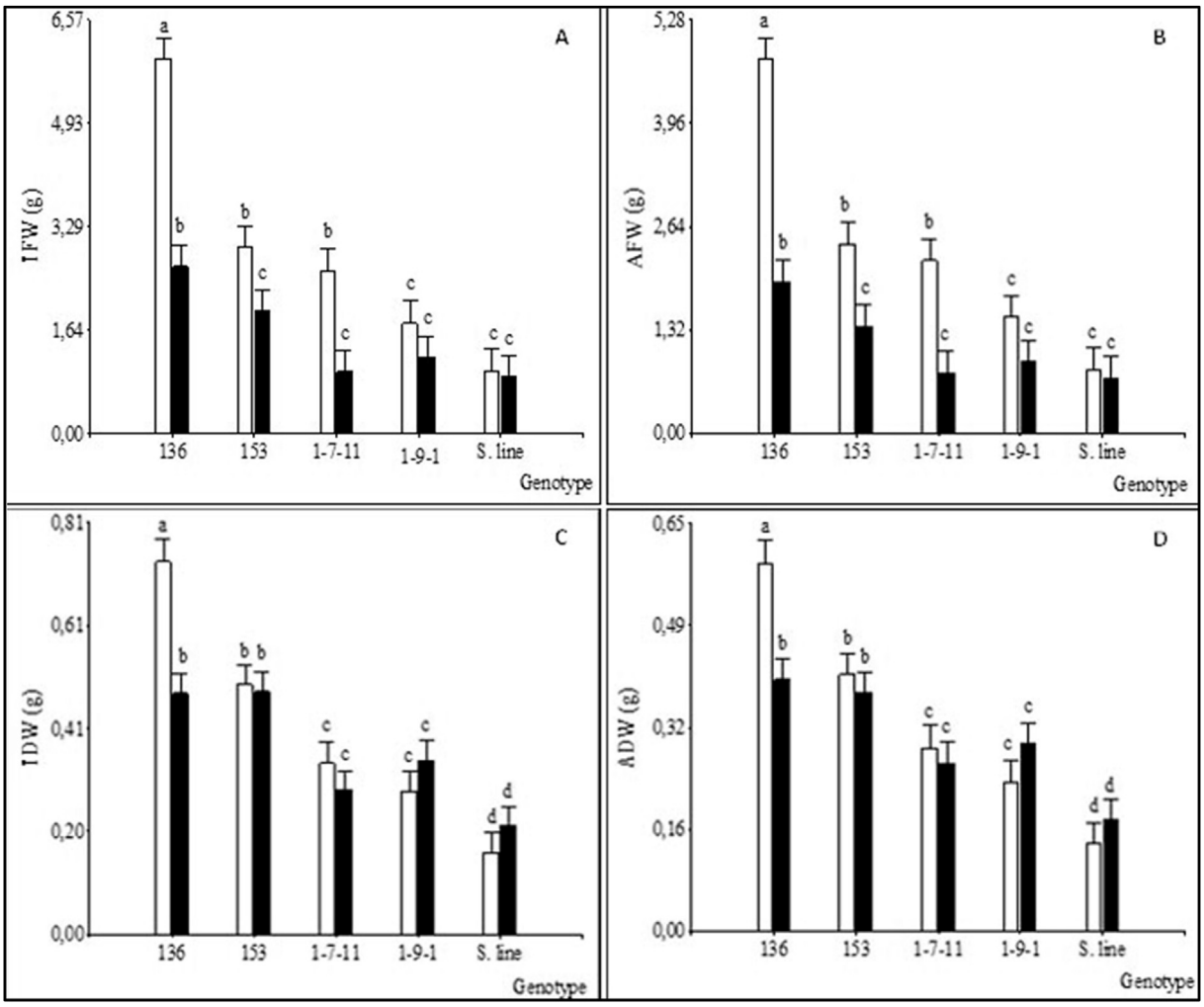


**Fig. 1.** Evaluation of growth components in two sexual hybrids (1-9-1 and 1-7-11) and their respective parents (S. line, RN 153 and 136) at 12 days of hydroponic seedling assay. A: total fresh weight (TFW); B: aerial fresh weight (AFW) C: total dry weight (TDW); D: aerial dry weight (ADW). Each data bar shows the mean and the error bars show standard deviation. White and black bars indicate 0 mM and 300 mM NaCl, respectively. Different letters indicate significant differences ($P \leq 0.05$).

remaining genotypes LA decreased at 300 mM NaCl, with hybrid 1-7-11 being the most affected genotype.

### 3.2. Germination

#### 3.2.1. Proportion of germinated seeds (PGS)

The genotype × treatment interaction was significant ($P = 0.0002$) for PGS (Fig. 3). A decrease in PGS was observed at a concentration of 125 mM NaCl, affecting S. line and RN 153. At 175 mM NaCl, sexual hybrids and their maternal line were the least affected by salt of all apomictic materials, but with differences from the control condition. At the highest tested concentration (225 mM), no difference was observed among genotypes.

#### 3.2.2. Germination rate index (GRI)

A high interaction between genotype and treatment ($P < 0.0001$) (Fig. 4) was observed for GRI. GRI decreased with increasing salt concentration. At 0 mM NaCl, RN 153 and 136 had the highest GRI. A reduction in GRI was observed at 75 mM NaCl in all genotypes, but it was lower in RN 136. At 125 mM NaCl, GRI decreased sharply in RN 153 with respect to the remaining genotypes. Hybrid 1-9-1 had the highest GRI at 175 mM NaCl. At 225 mM NaCl, there were no differences in GRI among genotypes.

## 4. Discussion

### 4.1. Seedling hydroponic assay

Salinity is the major environmental factor limiting plant growth and productivity. Salt stress increases the level of $Na^+$ ions inside the cell due to non-specific ion uptake. Salt exclusion and sequestration are two of the major mechanisms identified in salt-tolerant plants that maintain an appropriate $Na^+$ level in the cytosol (Anower Rokebul et al., 2013). Selective uptake of $K^+$ as opposed to $Na^+$ is also considered one of the key physiological mechanisms contributing to salt tolerance in many plant species (Asgari et al., 2012). Maintenance of relative water content (RWC) is important because salinity induces osmotic stress and reduces water availability to plants (Munns and Tester, 2008). In the new sexual materials evaluated in this work we found variation in these variables. Hybrid 1-9-1 presented the lowest $Na^+/K^+$ ratio and was able to keep the RWC, whereas this genotype showed an increase in RWC and $Na^+/K^+$ ratio. The behavior of hybrid 1-7-11 could be related to high levels of $Na^+$ inhibiting $K^+$ uptake, thereby increasing this ratio (Benito et al., 2014). This may be attributed to the fact that $Na^+$ produces a disturbance in the ion balance in plants by an increase in the $Na^+$ uptake (Çiçek and Çakirlar, 2002). Therefore, hybrid 1-7-11

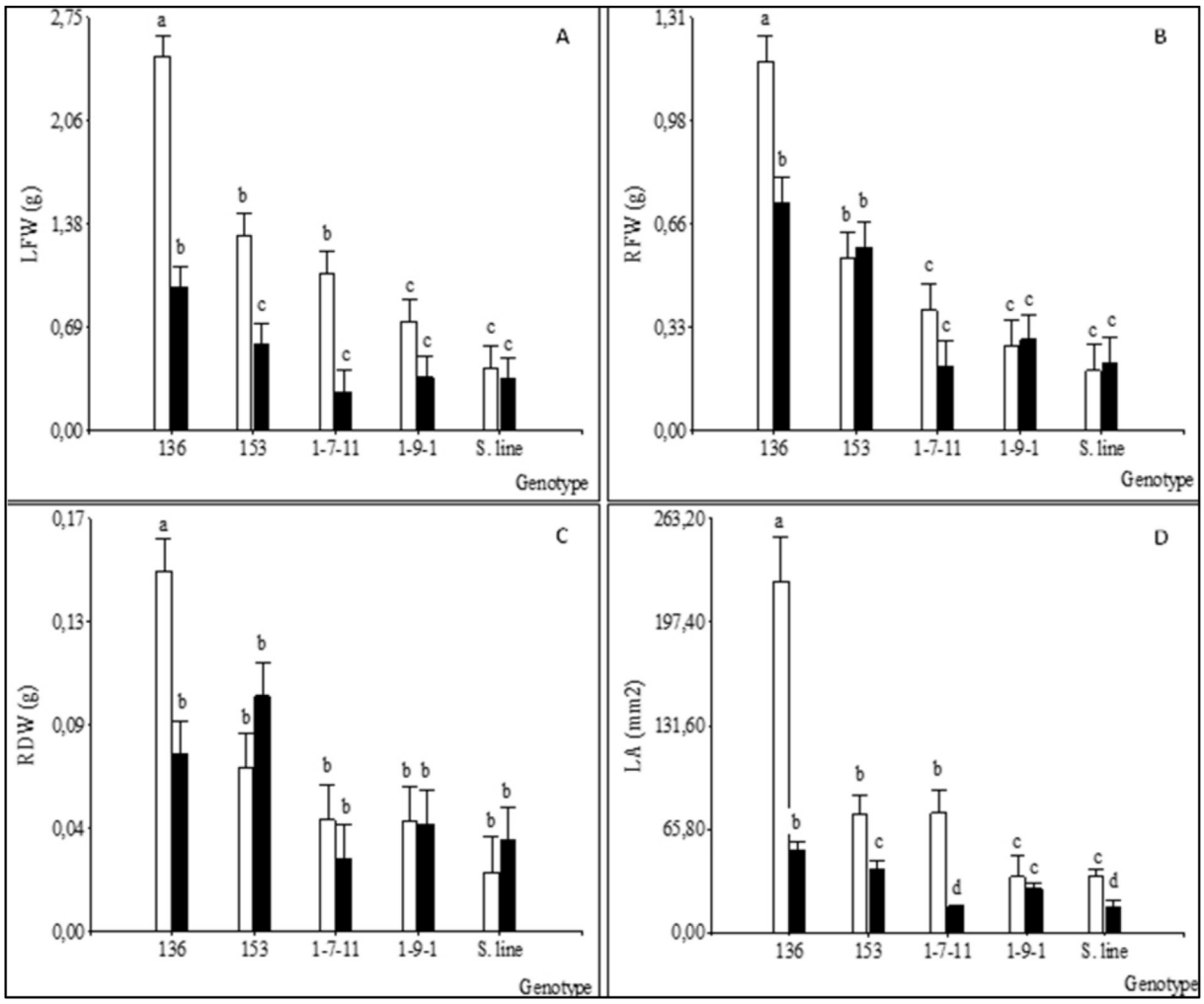


**Fig. 2.** Evaluation of growth components in two sexual hybrids (1-9-1 and 1-7-11) and their respective parents (S. line, RN 153 and 136) at 12 days of hydroponic seedling assay. A: leaf fresh weight (LFW); B: root fresh weight (RFW); C: root dry weight (RDW); D: leaf area (LA). Each data bar shows the mean and the error bars show standard deviation. White and black bars indicate 0 mM and 300 mM NaCl, respectively. Different letters indicate significant differences ($P \leq 0.05$).

treated with a high solute concentration could have absorbed more water and consequently would have had high RWC in its cells. This mechanism is called osmotic adjustment, and is usually observed in plants exposed to water deficit and salinity (Suriya-Arunroj et al., 2004).

To help with this adjustment, plants also accumulate organic compounds known as osmo-compatible compounds, like proline and soluble sugars (sucrose, mannitol and sorbitol). Proline can also interact with some enzymes and stabilize their structure and function (Štajner et al., 2012). Among the evaluated sexual hybrids we found a differential response in proline content, with hybrid 1-7-11 exhibiting an increase. This result suggests that proline could be used as osmo-compatible compound (Štajner et al., 1995; Szabados and Savouré, 2010). Soluble sugar content was reduced in this hybrid, suggesting a use of proline for sustaining growth and not as an osmo-compatible compound, as reported in previous works (Gadallah, 1999; Parida et al., 2004).

With respect to protein content, the technique used cannot discriminate whether variation in the total content observed between hybrids is due to a de novo synthesis of proteins or their cleavage (Bradford, 1976). Therefore, while total protein variability was found between hybrids, to understand the behavior of this variable, an additional trial designed to evaluate the physiological changes associated with adaptation to salt stress would be necessary.

Chlorophyll concentration in stressed tissue can be construed as an index of tissue tolerance to NaCl (Lutts et al., 1996). However, in some Poaceae species (e.g. durum wheat) chlorophyll content remains unaffected by salt (Carillo et al., 2008; Pompeiano et al., 2014). In agreement with those reports, in our study, chlorophyll and $Mg^{2+}$ contents did not show significant changes in the sexual hybrids between treatments, and seem to be insensitive to the salinity tested. This is consistent with findings reported for other monocots including rice, wheat, and maize (Rao and Gnanam, 1990; Lutts et al., 1996; Shabala et al., 1998).

In our results, PSII, SPAD values and FT decreased with increasing time of salt exposure, suggesting that such stress could reduce photosynthesis. This effect could be due to lower stomatal conductance, depression of specific metabolic processes in carbon uptake, inhibition in photochemical capacity, or a combination of these factors (Seemann and Critchley, 1985; Dubey, 1997; Jamil et al., 2007). In addition, salinity has been found to affect reaction centres of PSII either directly (Masojidek and Hall, 1992) or via an accelerated senescence (Kura-Hotta et al., 1987). Further studies related to PSII photochemistry, stomatal conductance and $CO_2$ capture in these new sexual resources are necessary to determine

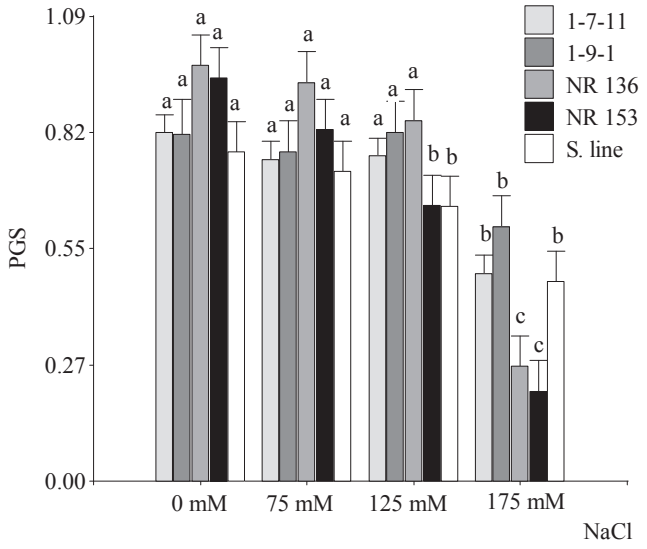


**Fig. 3.** Proportion of germinated seeds (PGS) in two sexual hybrids (1-9-1 and 1-7-11) and their respective parents (S. line, RN 153 and 136) at different concentrations of NaCl (mM). Each data bar shows the mean and the error bars show standard deviation. Different letters indicate significant differences ($P \leq 0.05$).

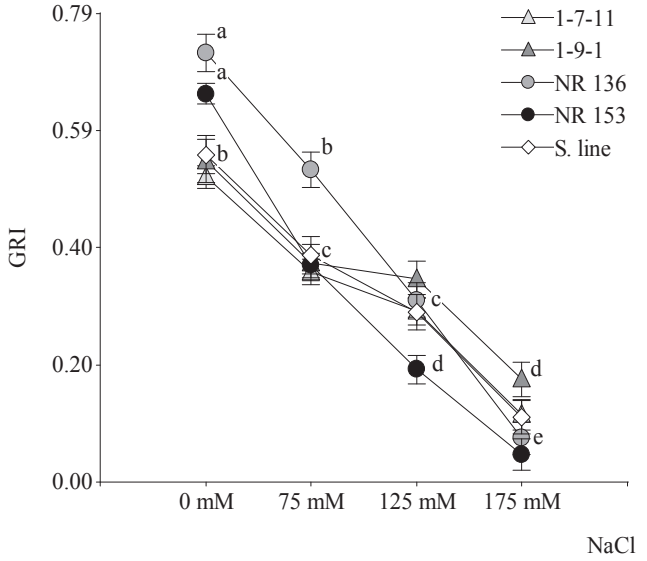


**Fig. 4.** Germination rate index (GRI) in two sexual hybrids (1-9-1 and 1-7-11) and their respective parents (S. line, RN 153 and 136) at different NaCl concentrations (mM). Each symbol shows the mean and the error bars show standard deviation. Different letters indicate significant differences ($P \leq 0.05$).

the effect of salinity on PSII.

As we mentioned in Introduction, MDA is a cytotoxic product of membrane lipid peroxidation that is generally taken as an index for measuring oxidative stress level (Meloni et al., 2003; Tommasino et al., 2012). MDA content was also found to be a good salinity tolerance marker in *Chloris gayana* (Luna et al., 2000; Ribotta, 2011) as well as in *C. ciliaris* (Lanza Castelli et al., 2010). In our experiment, MDA showed variability in salt treatment, with hybrid 1-7-11 presenting higher peroxidation of lipid membranes than hybrid 1-9-1. Membrane damage might be caused by high $H_2O_2$ levels, which might accelerate the Haber–Weiss reaction, resulting in hydroxyl radical ($OH^-$) formation and therefore, lipid peroxidation (Mittler, 2002).

In order to scavenge ROS, plants use antioxidant defense machinery composed of an enzymatic and non-enzymatic system (Mittler, 2002). The latter is represented by a series of antioxidant molecules, such as ascorbic acid, glutathione, anthocyanin and phenolic compounds (Cervilla et al., 2007). FRAP was used in this work to obtain information on total non-enzymatic antioxidant activity. FRAP content showed no differences between sexual hybrids in the salt treatment, suggesting that the difference found in MDA content cannot be explained by non-enzymatic activity. Studies focusing on enzymatic antioxidant activity should be performed in these hybrids (Tommasino et al., 2012).

Regarding growth components, previous studies involving adult buffelgrass plants conducted under field conditions revealed variability between sexual hybrids (Quiroga et al., 2013). In the present work, which is the first report of growth components at the seedling stage, variability was also observed, although it was lower under NaCl stress. Accordingly, variability for Leaf Area (LA) was only observed, with hybrid 1-9-1 showing no reduction in LA even under salt stress. Maintenance of LA in salt may be important in terms of providing tissue for translocation of ions away from regions of active growth (Munns, 1993; Hester et al., 2001). Additionally, in this hybrid (1-9-1) dry weight, which is considered the character of highest agronomic relevance and the main component of forage yield (Moore et al., 2004), was maintained in salt. Although hybrid 1-9-1 under salinity conditions has improved productivity with respect to the S. line, the variability observed in LA might have contributed to the maintenance of dry matter productivity under salt stress (Hester et al., 2001). In fact, the primary effect of salt stress is water deficit, in which morphometric variables that influence transpirational water loss, such as leaf area, may be associated with salt tolerance (Hester et al., 2001).

While salt-treated sexual material did not showed a decrease in productivity with respect to control plants, variability was observed in response to some physiological characteristics. Sexual hybrid 1-9-1 did not sense saline stress at 24 h or 12 days of exposure to 300 mM NaCl, because it did not suffer oxidative damage, as their parents, and its osmotic balance did not differ. Instead, S. line and RN 153 had to increase their proline content to maintain osmotic balance. By contrast, sexual hybrid 1-7-11 did detect stress, which was evident in oxidative damage, and generated a rapid osmotic response, which allowed it to maintain growth under NaCl stress.

### 4.2. Germination

Seedling establishment is a critical process in plant life, especially in the presence of adverse environmental factors (Bohnert and Jensen, 1996). When compared with glycophytes, halophytes can survive high salt levels during germination (Malcolm et al., 2003; Debez et al., 2004). However, several studies showed that even halophytes are relatively sensitive to salinity during the stages of germination and seedling emergence (Khan and Ungar, 1997; Tobe et al., 2000; Khan and Abdullah, 2003; Malcolm et al., 2003; Debez et al., 2004). In this study, salinity promoted a decrease in germination of all evaluated materials, with 225 mM NaCl concentration causing a drastic PGS reduction in all genotypes (16%). These results suggest that the osmotic stress imposed by such concentration could be enough to slow water uptake and reduce the required water content for germination (Läuchli and Epstein, 1990; Maas and Grattan, 1999; Läuchli and Grattan, 2007). Moreover, both seed germination capacity under salt conditions and germination velocity in these adverse conditions are important in buffelgrass. Plants that exhibit these characteristics would have an ecological advantage, since seedlings would establish rapidly (Pujol

et al., 2000); hybrid 1-9-1 showed to have such advantages up to a 175 mM NaCl concentration.

To sum up, these new sexual resources are promising maternal parentals with differential response to salinity, which would allow them, along with apomictic parentals, to increase the probability of occurrence of new salt-tolerant combinations in their progenies. These new material could be incorporated in a breeding program and released as new cultivars of *C. ciliaris* in the search of new genetic resources tolerant to salinity conditions.

## Contribution

The new sexual resources are promising maternal parentals with differential response to salinity, which would allow them, along with apomictic parentals, to increase the probability of occurrence of new salt-tolerant combinations in their progenies. These new material could be incorporated in a breeding program and released as new cultivars of *C. ciliaris* in the search of new genetic resources tolerant to salinity conditions.

## References

Akram, N.A., Shahbaz, M., Athar, H., Ashraf, M., 2006. Morpho-physiological responses of two differently adapted populations of *Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L. to salt stress. Pak. J. Bot. 38, 1581–1588.

Al-Khateeb, S.A., 2006. Effect of calcium/sodium ratio on growth and ion relations of alfalfa (*Medicago sativa* L.) seedling grown under saline condition. J. Agron. 5, 175–181.

Anower Rokebul, M., Mott, I.W., Peel, M.D., Wu, Y., 2013. Characterization of physiological responses of two alfalfa half-sib families with improved salt tolerance. Plant Physiol. Biochem. 71, 103–111.

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress and signal transduction. Annu. Rev. Plant Biol. 55, 373–399.

Asgari, H.R., Cornelis, W., Van Damme, P., 2012. Salt stress effect on wheat (*Triticum aestivum* L.) growth and leaf ion concentration. Int. J. Agron. Plant. Prod. 6, 195–208.

Ashraf, M., 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol. Adv. 27, 84–93.

Ashraf, M., Foolad, M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59, 206–216.

Ashraf, M.Y., Akhtar, K., Hussain, F., Iqbal, J., 2006. Screening of different accessions of three potential grass species from Cholistan desert for salt tolerance. Pak. J. Bot. 38 (5), 1589–1597.

Bashaw, E.C., 1980. Apomixis and its application in crop improvement. Hybrid. crop plants (hybridizationof 45–63.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant soil 39 (1), 205–207.

Bazzigalupi, O., Pistorale, S.M., Andrés, A.N., 2008. Tolerancia a la salinidad durante la germinación de semillas provenientes de poblaciones naturalizadas de agropiro alargado (*Thinopyrum ponticum*). Cien. Inv. Agric. 35 (3), 277–285.

Benito, B., Haro, R., Amtmann, A., Cuin, T.A., Dreyer, I., 2014. The twins $K^+$ and $Na^+$ in plants. J. Plant Physiol. 171 (9), 723–731.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of Plasma (FRAP) as a measure of "Antioxidant power". The FRAP assay. Anal. Biochem. 239, 70–76.

Bilger, W., Björkman, O., 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth. Res. 25 (3), 173–185.

Bohnert, H.J., Jensen, R.G., 1996. Strategies for engineering water stress tolerance in plants. Trends Biotechnol. 14, 89–97.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1), 248–254.

Bray, R.A., 1978. Evidence for facultative apomixis in *Cenchrus ciliaris*. Euphytica 27 (3), 801–804.

Carillo, Mastrolonardo, G., Nacca, F., Parisi, D., Verlotta, A., Fuggi, A., 2008. Nitrogen metabolism in durum wheat under salinity: accumulation of proline and glycine betaine. Funct. Plant Biol. 35, 412–426.

Cervilla, L.M., Blasco, B., Ríos, J.J., Romero, L., Ruiz, J.M., 2007. Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to boron toxicity. Ann. Bot. 100 (4), 747–756.

Cha-Um, S., Kirdmanee, C., 2009. Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. Pak. J. Bot. 41 (1), 87–98.

Çiçek, N., Çakirlar, H., 2002. The effect of salinity on some physiological parameters in two maize cultivars. Bulg. J. Plant Physiol. 28 (1–2), 66–74.

Debez, A., Hamed, K.B., Grignon, C., Abdelly, C., 2004. Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritima*. Plant soil 262 (1–2), 179–189.

Di Rienzo, J.A., Guzmán, A.W., Casanoves, F., 2002. A multiple-comparisons method based on the distribution of the root node distance of a binary tree. J. Agric. Biol. Environ. Stat. 7 (2), 129–142.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2014. InfoStat Versión. Universidad Nacional de Córdoba, Argentina. Grupo InfoStat, FCA.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2014. InfoStat Versión. Grupo InfoStat, FCA. Universidad Nacional de Córdoba, Argentina. URL. http://www.infostat.com.ar.

Dubey, R.S., 1997. Photosynthesis in plants under stressful conditions. In: Pessarakli, M. (Ed.), Handbook of Photosynthesis. Marcel Dekker, New York, pp. 859–875.

Fales, F.W., 1951. The assimilation and degradation of carbohydrates by yeast cells. J. Biol. Chem. 193 (1), 113–124.

Gadallah, M.A.A., 1999. Effects of proline and glycinebetaine on *Vicia faba* responses to salt stress. Biol. Plant 42 (2), 249–257.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.

Griffa, S.M., 2010. Caracterización bioquímica y molecular de germoplasma, evaluación de la tolerancia a la salinidad y obtención de híbridos en buffelgrass. Tesis Doctoral. Universidad Nacional de Córdoba, Córdoba, Argentina, p. 137.

Griffa, S.M., Ribotta, A., Muñoz, N., López Colomba, E., Ojeda, M., Luna, C., Grunberg, K., Díaz, D., Biderbost, E., 2005. Análisis de la herencia de caracteres de interés agronómico en *Cenchrus ciliaris* L. In: XXXIV Congreso Argentino de Genética. pp.150. 11 al 14 de Septiembre, Trelew, Chubut, Argentina.

Hanselka, C.W., Hussey, M.A., Ibarra, F.F., 2004. Buffelgrass. Warm-Season (C4). Grasses 45, 477–502.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125 (1), 189–198.

Hester, M.W., Mendelssohn, I.A., McKee, K.L., 2001. Species and population variation to salinity stress in *Panicum hemitomon, Spartina patens*, and *Spartina alterniflora*: morphological and physiological constraints. Environ. Exp. Bot. 46 (3), 277–297.

Hoagland, D.R., Arnon, D.I., 1950. The Water-culture Method for Growing Plants without Soil. Circular. California Agricultural Experiment Station, 347 (2nd edit).

Jamil, M., Lee, K.J., Kim, J.M., Kim, H.S., Rha, E.S., 2007. Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. Sci. Agric. 64 (2), 111–118.

Jena, K.K., Mackill, D.J., 2008. Molecular markers and their use in marker-assisted selection in rice. Crop Sci. 48 (4), 1266–1276.

Jouyban, Z., 2012. The Effects of Salt stress on plant growth. Tech. J. Engin. App. Sci. 2 (1), 7–10.

Kalaji, H.M., Bosa, K., Kościelniak, J., Żuk-Gołaszewska, K., 2011. Effects of salt stress on photosystem II efficiency and $CO^2$ assimilation of two Syrian barley landraces. Environ. Exp. Bot. 73, 64–72.

Khan, M.A., Abdullah, Z., 2003. Salinity–sodicity induced changes in reproductive physiology of rice (*Oryza sativa*) under dense soil conditions. Environ. Exp. Bot. 49 (2), 145–157.

Khan, A.A., McNeilly, T., 2005. Triple test cross analysis for salinity tolerance based upon seedling root length in maize (*Zea mays* L.). Breed. Sci. 55 (3), 321–325.

Khan, M., Ungar, I., 1997. Effects of thermoperiod on recovery of seed germination of halophytes from saline conditions. Am. J. Bot. 84 (2), 279–279.

Kura-Hotta, M., Satoh, K., Katoh, S., 1987. Relationship between photosynthesis and chlorophyll content during leaf senescence of rice seedlings. Plant Cell Physiol. 28, 1321–1329.

Lanza Castelli, S., Grunberg, K., Muñoz, N., Griffa, S., López Colomba, E., Ribotta, A., Biderbost, E., Luna, C., 2010. Oxidative damage and antioxidant defenses as potential indicators of salt-tolerant *Cenchrus ciliaris* L. genotypes. FLORA 205, 622–626.

Läuchli, A., Epstein, E., 1990. Plant responses to saline and sodic conditions. Agric. Salin. Assess. Manag. 71, 113–137.

Läuchli, A., Grattan, S.R., 2007. Plant growth and development under salinity stress. In: Advances in Molecular Breeding toward Drought and Salt Tolerant Crops. Springer, Netherlands, pp. 1–32.

Lopez, M.V., Satti, S.M.E., 1996. Calcium and potassium-enhanced growth and yield of tomato under sodium chloride stress. Plant Sci. 114 (1), 19–27.

López Colomba, E., Tommasino, E., Luna, C., Griffa, S., Carloni, E., Ribotta, A., Quiroga, M., Grunberg, K., 2013. Differential salt-stress response during germination and vegetative growth *in vitro* selected somaclonal mutants of *Cenchrus ciliaris* L. S. Afr. J. Bot. 87, 157–163.

Luna, C., Garcia-Seffino, L., Arias, C., Taleisnik, E., 2000. Oxidative stress indicators as selection tools for salt tolerance. Plant Breed. 119 (4), 341–345.

Lutts, S., Kinet, J.M., Bouharmont, J., 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann. Bot. 78 (3), 389–398.

Maas, E.V., Grattan, S.R., 1999. Crop yields as affected by salinity. In: Skaggs, R.W., van Schilfgaarde, J. (Eds.), Agricultural Drainage ASA,CSSA, SSSA, Agron. Monogr, 38, pp. 55–108. Madison, WI.

Malcolm, C.V., Lindley, V.A., O'Leary, J.W., Barrett-Lennard, E.G., 2003. Halophyte and glycophyte salt tolerance at germination and the establishment of halophyte shrubs in saline environments. Plant Soil 253, 171–185.

Masojidek, J., Hall, D.O., 1992. Salinity and drought stresses are amplified by high irradiance in sorghum. Photosynthetica 27 (1–2), 159–171.

Meloni, D.A., Oliva, M.A., Martinez, C.A., Cambraia, J., 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot. 49 (1), 69–76.
Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7 (9), 405–410.
Monirifar, H., Barghi, M., 2009. Identification and selection for salt tolerance in alfalfa (*Medicago sativa* L.) ecotypes via physiological traits. Not. Sci. Biol. 1 (1), 63–66.
Moore, K.J., Boote, K.J., Sanderson, M.A., 2004. Physiology and Developmental Morphology. Buffelgrass Warm-season (C4) grasses. pp. 6, 179-216.
Munns, R., 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ. 16 (1), 15–24.
Munns, R., 2011. Plant adaptations to salt and water stress: differences and commonalities. Adv. Bot. Res. 57, 1–32.
Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651–681.
Munns, R., James, R.A., Laüchli, A., 2006. Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. 57, 1025–1043.
Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J.A., Deemer, E.K., 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. J. Agric. Food. Chem. 50 (11), 3122–3128.
Parida, A.K., Das, A.B., Sanada, Y., Mohanty, P., 2004. Effects of salinity on biochemical components of the mangrove, *Aegiceras corniculatum*. Aquat. Bot. 80 (2), 77–87.
Paz, R.C., Rocco, R.A., Reinoso, H., Menéndez, A.B., Pieckenstain, F.L., Ruiz, O.A., 2012. Comparative study of alkaline, saline, and mixed saline and alkaline stresses with regard to their effects on growth, nutrient accumulation, and root morphology of *Lotus tenuis*. Plant Growth Regul. 31, 448–459.
Pérez-López, U., Robredo, A., Lacuesta, M., Sgherri, C., Muñoz-Rueda, A., Navari-Izzo, F., Mena-Petite, A., 2009. The oxidative stress caused by salinity in two barley cultivars is mitigated by elevated $CO_2$. Physiol. Plant. 135 (1), 29–42.
Pompeiano, A., Giannini, V., Gaetani, M., Vita, F., Guglielminetti, L., Bonari, E., Volterrani, M., 2014. Response of warm–season grasses to N fertilization and salinity. Sci. Hortic. 177, 92–98.
Pujol, J.A., Calvo, J.F., Ramirez-Diaz, L., 2000. Recovery of germination from different osmotic conditions by four halophytes from southeastern Spain. Ann. Bot. 85 (2), 279–286.
Quiroga, M., Grunberg, K., Ribotta, A., López Colomba, E., Carloni, E., Tommasino, C. Luna, Griffa, S., 2013. Obtaining sexual genotypes for breeding in buffel grass. S. Afr. J. Bot. 88, 118–123.
Rao, R.K., Gnanam, A., 1990. Inhibition of nitrate and nitrite reductase activities by salinity stress in *Sorghum vulgare*. Phytochemistry 29 (4), 1047–1049.
Ribotta, A., 2011. Selección y caracterización de clones parentales diploides para la obtención de nuevo germoplasma con tolerancia incrementada a la salinidad en rhodesgrass (*Chloris gayana* Kunth). Tesis para optar al título de Master en Ciencias Agropecuarias. Universidad Nacional de Córdoba, Córdoba, Argentina, p. 142.
Seemann, J.R., Critchley, C., 1985. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. Planta 164 (2), 151–162.
Shabala, S.N., Shabala, S.I., Martynenko, A.I., Babourina, O., Newman, I.A., 1998. Salinity effect on bioelectric activity, growth, $Na^+$ accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. Funct. Plant Biol. 25 (5), 609–616.
Sherwood, R.T., Young, B.A., Bashaw, E.C., 1980. Facultative apomixis in buffelgrass. Crop Sci. 20 (3), 375–379.
Snyder, L.A., Hernandez, A.R., Warmke, H.E., 1955. The mechanism of apomixis in *Pennisetum ciliare*. Bot. Gaz. 209–221.
Štajner, D., Mimica-Dukić, N., Gasić, O., 1995. Adaptability to drought in sugar beet cultivars. Biol. Plant 37 (1), 107–112.
Štajner, D., Orlović, S., Popović, B.M., Kebert, M., Stojnić, S., Klašnja, B., 2012. Chemical parameters of oxidative stress adaptability in beech. J. Chem. 2013.
Suriya-Arunroj, D., Supapoj, N., Toojinda, T., Vanavichit, A., 2004. Relative leaf water content as an efficient method for evaluating rice cultivars for tolerance to salt stress. Sci. Asia 30, 411–415.
Szabados, L., Savouré, A., 2010. Proline: a multifunctional amino acid. Trends Plant Sci. 15 (2), 89–97.
Tessi, N.P., Avila, J.M., Edith, R., Armando, R.H., Ferrando, C.A., 2014. Buffel grass Generalidades, implantación y manejo para recuperación de áreas degradadas. In: INTA (Ed.), Chamical. La Rioja, pp. 1–20.
Tetley, R.M., Thimann, K.V., 1974. The metabolism of oat leaves during senescence. I. Respiration, carbohydrate metabolism and the action of cytokinins. Plant Physiol. 54, 294–303.
Tobe, K., Li, X., Omasa, K., 2000. Seed germination and radicle growth of a halophyte, *Kalidium caspicum (*Chenopodiaceae. Ann. Bot. 85 (3), 391–396.
Tommasino, E., Griffa, S., Grunberg, K., Ribotta, A., López Colomba, E., Carloni, E., Grunberg, K., Luna, C.M., 2012. Malondialdehyde content as a potential biochemical indicator of tolerant *Cenchrus ciliaris* L. genotypes under heat stress treatment. Grass Forage Sci. 67 (3), 456–459.
Turner, N., 1986. Adaptation to water deficits: a changing perspective. Funct. Plant. Biol. 13 (1), 175–190.
Ungar, I.A., 1978. Halophyte seed germination. Bot. Rev. 44 (2), 233–264.
Xu, W., Rosenow, D.T., Nguyen, H.T., 2000. Stay green trait in grain sorghum: relationship between visual rating and leaf chlorophyll concentration. Plant Breed. 119 (4), 365–367.
Yu, S., Wang, W., Wang, B., 2012. Recent progress of salinity tolerance research in plants. Russ. J.Genet. 48, 497–505.
Zhu, J.K., 2001. Plant salt tolerance. Trends Plant Sci. 6, 66–71.