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Research article

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

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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.

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

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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