Grass and Forage Science
The Journal of the British Grassland Society | The Official Journal of the European Grassland Federation

Evaluation seedling biomass and its components as selection criteria for improving salt tolerance in Buffel grass genotypes

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Abstract

Damage produced by salt stress (300 mM NaCl) under hydroponic greenhouse conditions in seedlings of eight Buffel grass (*Cenchrus ciliaris* L. Syn. *Pennisetum ciliare* Link) genotypes was estimated through different component characters of total fresh weight damage (DTFW). Coefficient of genetic determination (CGD) was estimated for each character as well as their direct and indirect contribution to damage to total fresh weight, considered as biomass production loss. Americana and Biloela were the most salt-tolerant cultivars, whereas sexual line and Texas 4464 were the genotypes most susceptible to salt stress. Damage to fresh weight of aerial part (DFWA) was the only component with a direct effect on DTFW and with a high CGD. Therefore, DFWA could be used in breeding programmes as an indirect selection criterion for tolerance and better productivity.

Keywords: *Cenchrus ciliaris,* forage, salt stress, breeding programme, selection criteria, path analysis

Introduction

Buffel grass (*Cenchrus ciliaris* L. Syn. *Pennisetum ciliare* Link) is an important gramineous forage species in arid and semiarid regions in the world. In Argentina, this species has been introduced as a forage resource in areas affected mainly by water stress. Salinity is an abiotic stress factor that contributes to severe production decline, affecting 34% of the cultivable area in Argentina (Ghassemi *et al.*, 1995).

A genetic improvement programme in Buffel grass is currently being conducted at INTA-IFFIVE with the aim of obtaining new salt-tolerant germplasm. For this purpose, obligate apomictic cultivars are available to be used as male parents in controlled hybridizations with an introduced sexual line (female parent).

Among Buffel grass apomictic cultivars, Texas 4464, Americana, and Biloela have been poorly studied in terms of their tolerance to water and salt stress. Texas 4464 has been reported as tolerant to water stress (Ayerza, 1981), and Biloela was found to be tolerant to salinity (Graham and Humphreys, 1970). In a study of salt stress on Buffel grass and its effects on productivity decline, Lanza Castelli (2006) recently conducted a hydroponic experiment under semicontrolled greenhouse conditions. They found that Texas 4464 was susceptible to salt stress (300 mM NaCl) at the seedling stage, whereas Americana exhibited lowest damage to aerial fresh weight, root length, and plant height with respect to the control treatment (0 mM NaCl).

Total fresh weight, indicative character of productivity in seedlings grown in hydroponic greenhouse conditions, is a complex polygenically controlled trait. Selection based solely on performance of total fresh weight could be misleading and of reduced efficiency. Thus, identifying and manipulating characters that contribute to total fresh weight is important to increase improvement efficiency. Accordingly, characters that are easily measured, highly heritable and successfully related to total fresh weight, such as biomass productivity, are of great importance in indirect selection for tolerance. Correlation studies using path analysis provide a better understanding of the association of different characters with total fresh weight.

Path analysis partitions linear correlation coefficients into direct and indirect effects that determine the expression of independent characters on a basic principal-dependent variable. This technique has been used

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Received 23 March 2010; revised 02 June 2010

doi: 10.1111/j.1365-2494.2010.00754.x

in *Pennisetum purpureum* by Daher *et al.* (2004), who found direct associations between seedling height, number of tillers and tiller diameter, and the dependent character dry-matter production.

With the aim of finding a parameter of early selection for salt tolerance, Buffel different genotypes were evaluated on the basis of total fresh weight and its component characters in salt stress conditions.

Materials and methods

Characterization of Buffel grass genotypes for their salt stress tolerance in greenhouse

An introduced sexual line (SL), considered as a sexual clone by Bashaw (1969, 1980)was kindly granted by the Germplasm Bank of the Texas A&M University (College Station, TX, USA), six obligated apomictic cultivars: Texas 4464 (T-4464), Nunbank, Biloela, Nueces, Americana, and Tarewinnabar, and an apomictic hybrid (H3/1) derived from the cross between the sexual line and Biloela were evaluated for their salt stress tolerance under hydroponic greenhouse conditions. Seeds of the different genotypes were grown in earthen pots (30 cm diameter), in a greenhouse under natural light and day/night temperature 30/15°C, and 30 days after sowing, seedlings were placed individually in holes of a styrofoam board (20 seedlings per board). The styrofoam boards were suspended in 10-L rectangular plastic trays (30 × 20 × 60 cm) filled with aerated Hoagland nutrient solution (Hoagland and Arnon, 1950). The experiment was conducted in a completed randomized design with two replicates per genotype and treatment.

The plants remained in these conditions for 12 days, and the salinization process was carried out by gradually adding NaCl (100 mM every 48 h) to the nutrient solution until the final concentration, 300 mM, was reached. Nutrient solution without NaCl was used as control.

According to results obtained by previous works of our team (Lanza Castelli, 2006; Griffa, 2010; Lanza Castelli *et al.*, 2010), after 17 days to reach the final concentration, the following characters were measured in the 64-day-old seedlings: total fresh weight of seedling (TFW), fresh weight of aerial part (FWA), fresh weight of root part (FWR), seedling height (SH), root length (RL), number of tillers (NT), and number of leaves (NL).

For each quantitative character measured, damage (D) produced by salt stress was estimated using the equation:

$$D = [\Sigma(\mathrm{MXc} - \mathrm{Xis})/\mathrm{MXc}]/n$$

where MXc is the mean for control plants, Xis is the value of each salt-treated plant, and n is the number of replications. Once damage was estimated, each variable was defined as follows: damage to total fresh weight of seedling (DTFW), damage to fresh weight of aerial part (DFWA), damage to fresh weight of root (DFWR), damage to seedling height (DSH), damage to root length (DRL), damage to number of tillers (DNT), and damage to number of leaves (DNL). Data obtained for each 'damage' variable were analysed by ANOVA and Di Rienzo, Guzman, and Casanoves (DGC) (Di Rienzo *et al.*, 2002) multiple comparison of means test, using InfoStat (2009).

Path analysis

The path analysis was performed using the procedure of InfoStat (2009) to study correlations of DTFW with the variables: DFWA, DFWR, DSH, DRL, DNT, and DNL acting as predictors (causes) on DTFW considered as the response variable.

Estimation of the coefficient of genetic determination (CGD)

For each of the variables mentioned, the environmental (Ve) and genetic (Vg) variances were estimated. Ve was estimated from the variability among plants of the same clone and estimated for the mean square within clones. Vg was evaluated from the difference between mean squares among and within clones, divided by the number of replicates. The coefficient of genetic determination (CGD) was calculated as follows:

$$CGD = Vg/(Vg + Ve)$$

Results and discussion

Characterization of Buffel grass genotypes for their salt stress tolerance

Results of the ANOVA show significant differences between genotypes for all the variables analysed ($P < 0{\cdot}05$). The mean values of each variable indicated that the greatest damage induced by salt stress occurred in TFW, FWA, and NT (0·62, 0·67, and 0·57 respectively; Table 1). Lowest damage was observed in the variables RFW and SH (0·35 and 0·33 respectively).

According to the damage induced by salt stress, as measured by the damage variables, genotypes were grouped in three classes: (i) the most susceptible genotypes, the introduced sexual line and T-4464 that exhibited the highest damage; (ii) an intermediate group comprised of the genotypes Nunbank, H3/1, Nueces, and Tarewinnabar; and (iii) group composed of the least damaged cultivars, Americana and Biloela, which were the most tolerant to 300 mM NaCl at the

Table 1 Significance of the differences between mean values estimated in the parameters: total fresh weight of seedling damage (DTFW), fresh weight of aerial part damage (DFWA), root fresh weight damage (DRFW), root length damage (DRL), seedling height damage (DSH), number of tillers damage (DNT), and number of leaves damage (DNL), in Buffel grass genotypes under salt stress.

Genotypes	DTFW	DFWA	DRFW	DRL	DSH	DNT	DNL
Biloela	0·48 a	0·60 a	0·03 a	0·32 a	0·18 a	0·13 a	0·45 a
Americana	0·56 b	0·59 a	0·32 b	0·29 a	0·33 c	0·48 b	0·42 a
Hybrid H3/1	0·60 b	0·63 b	0·24 b	0·39 b	0·27 b	0·95 d	0·56 b
Nueces	0·59 b	0·66 b	0·38 b	0·38 b	0·43 d	0·90 d	0·46 a
Nunbank	0·62 b	0·66 b	0·26 b	0·40 b	0·25 b	0·69 c	0·57 b
Tarewinnabar	0·63 b	0·69 b	0·52 c	0·45 b	0·31 c	0·41 b	0·42 a
T-4464	0·72 c	0·77 c	0·47 c	0·57 c	0·48 d	0·42 b	0·49 a
Sexual line	0·76 c	0·82 c	0·60 c	0·51 c	0·45 d	0·63 c	0·90 c

*Different letters in columns indicate significant differences between genotypes ($P < 0·05$).

seedling stage and under hydroponic greenhouse conditions. The latter result agrees with results reported by Graham and Humphreys (1970) and Lanza Castelli (2006), in which Biloela and Americana were described as 'tolerant'.

Our results for the genotypes Nunbank, Nueces, Tarewinnabar, Biloela, Americana, T-4464 cultivars and the introduced sexual line are the first reports of the characterization of salt tolerance in Buffel grass.

Path analysis

All variables exhibited a statistically significant and positive correlation with DTFW ($P < 0·05$). DFWA was the only variable with a direct effect on DTFW ($P < 0·05$) (rd = 0·71, Figure 1) of seedlings subjected to salt stress. The effects of the variables DSH, DRL, DRFW, DNT, and DNL were indirect and through DFWA. Moreover, DFWA accounted for almost all of the variation in DTFW (total $R = 0·94$, Figure 1).

Coefficient of genetic determination (CGD)

For most of the characters, CGD was high (>60%), showing high genetic influence, except for DTFW, which had a CGD of 40% (Table 2). DFWA, which had a direct influence on DTFW, exhibited a high CDG (60%).

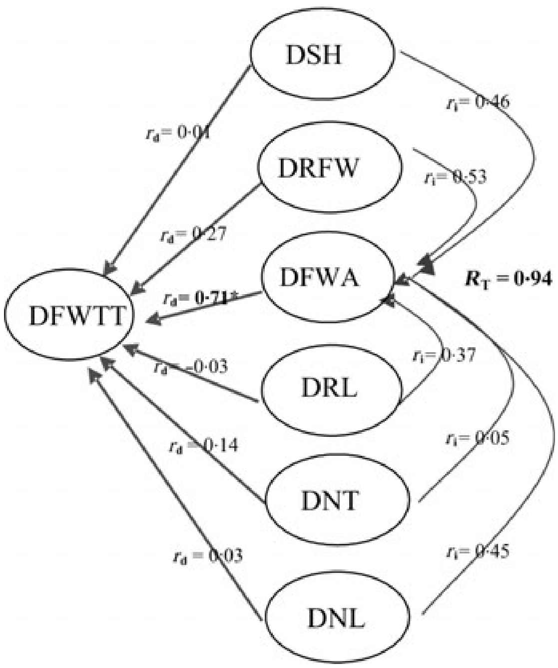

Figure 1 Direct and indirect effects of the variables damage to seedling height (DSH), damage to root fresh weight (DRFW), damage to root length (DRL), damage to number of tillers (DNT), and damage to number of leaves (DNL), through damage to fresh weight of aerial part (DFWA) on damage to total plant fresh weight (DTFW) and their direct (rd) and indirect (ri) phenotypic correlations ($P < 0·05$).

Table 2 Environmental variances (Ve), genetic variances (Vg), and estimation of coefficients of genetic determination (CGD) in total fresh weight of seedling damage (DTFW), fresh weight of aerial part damage (DFWA), root fresh weight damage (DRFW), root length damage (DRL), seedling height damage (DSH), number of tillers damage (DNT), and number of leaves damage (DNL) in Buffel grass genotypes.

Character	Ve	Vg	CGD
DTFW	0·010	0·008	0·44
DFWA	0·003	0·004	0·57
DRFW	0·030	0·033	0·52
DSH	0·010	0·011	0·52
DRL	0·002	0·009	0·85
DNT	0·050	0·086	0·63
DNL	0·002	0·036	0·95

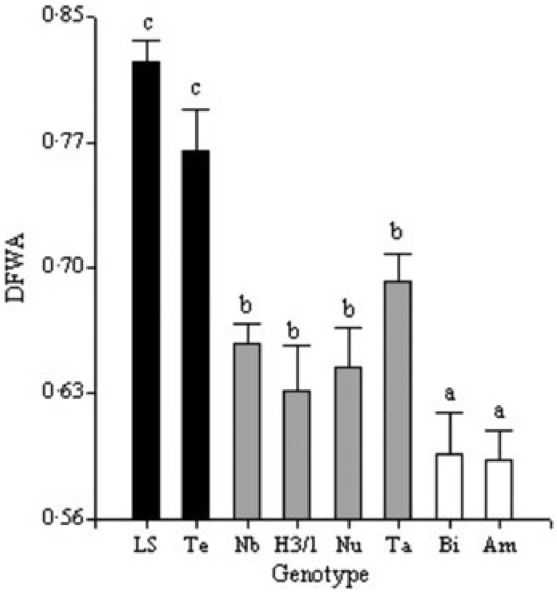

Figure 2 Differentiation of Buffel grass genotypes according to damage caused by salt stress with NaCl to fresh weight of aerial part (DFWA). Susceptible genotypes: sexual line (SL) and Texas 4464 (Te). Moderately tolerant genotypes: Nunbank (Nb), hybrid 3/1 (H3/1), Nueces (Nu), and Tarewinnabar (Ta). Tolerant genotypes: Biloela (Bi) and Americana (Am). Different letters above each column indicate significant differences ($P < 0{\cdot}05$) between genotypes. Error bars indicate standard error.

In the path analysis, DPFA was the principal component character of direct influence on productivity and discriminated the genotypes evaluated into three groups (Figure 2), which is in agreement with characterization of tolerance for most variables.

Characterization of the Buffel grass germplasm for salt stress tolerance is valuable for genetic improvement purposes. In this study, Biloela and Americana were the most tolerant to salinity and the genotypes Nunbank, Nueces, Tarewinnabar, and hybrid 3/1 were intermediate. Therefore, all of these genotypes are proposed as potential parents for future crossings to achieve greater success in obtaining new germplasm with increased tolerance to salt stress.

Damage to the aerial part of total fresh weight was the only component character that had a direct effect on damage to total fresh weight and also it exhibited a coefficient of genetic determination >50%. Hence, the character damage to fresh weight of aerial part may be a reliable indicator, with high genetic expression, for the early selection of Buffel grass salt-tolerant genotypes, increasing the efficiency in selection for greater productivity under salt stress conditions.

Acknowledgments

This work was funded by The Instituto Nacional de Tecnología Agropecuaria, Argentina.

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