Research Paper

Prospective genetic gain to improve salinity tolerance in a population of *Panicum coloratum* var. *coloratum* with two different selection methods

Ganancia genética potencial para mejorar la tolerancia a salinidad en una población de Panicum coloratum var. coloratum con dos diferentes métodos de selección

GABRIELA PITTARO¹, MAURO LIFSCHITZ^{2,3}, MIGUEL SÁNCHEZ⁵, DOLORES BUSTOS¹, JOSÉ OTONDO⁴ AND MARÍA ANDREA TOMÁS²

Abstract

Panicum coloratum var. coloratum is a subtropical grass for potentially increasing forage production in lowly productive environments where cattle-raising activities have been relocated. Heritability was estimated for characters related to salinity tolerance under saline and non-saline conditions to explore the possibility of improving tolerance by selection. From a base germplasm collected in a very harsh environment, heritability and gain after selection were calculated using 2 recombination units: individual and phenotypic family mean (PFM). Heritability estimates were very low for all characters both in saline and non-saline conditions, suggesting a complex genetic control of salinity tolerance, with a high proportion of non-additive genetic effects. Estimates were higher using individual selection than with PFM and expected genetic gains were higher for individual selection. When compared in both saline and non-saline conditions, predicted means were greater than for plants of cv. Klein, the most common cultivar in use. It appears that the analyzed germplasm would be a valuable source of genes to be included in breeding programs to increase salinity tolerance in Panicum coloratum.

Keywords: Familial selection, heritability, individual selection, phenotypic and genetic variability.

Resumen

Panicum coloratum var. coloratum es una gramínea forrajera subtropical adecuada para incrementar la producción de forraje en ambientes de baja productividad donde la ganadería ha sido relocalizada. En un estudio realizado en Buenos Aires y Córdoba, Argentina, se estimó la heredabilidad para caracteres relacionados a la tolerancia a salinidad en condiciones salinas y no salinas para explorar la posibilidad de mejorar la tolerancia por selección. A partir de un germoplasma base recolectado de un ambiente con condiciones restrictivas de crecimiento, la heredabilidad y la ganancia

Correspondence: M.A. Tomás, Instituto de Investigación de la Cadena Láctea (INTA-CONICET), Estación Experimental Agropecuaria Rafaela, Ruta 34, Km 227, Rafaela, Santa Fe,

Argentina. Email: tomas.maria@inta.gob.ar

¹Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto de Fisiología y Recursos Genéticos Vegetales, Unidad de Estudios Agropecuarios (INTA-CONICET), Córdoba, Argentina. <u>inta.gob.ar/ifrgv</u>

²Instituto de Investigación de la Cadena Láctea (INTA-CONICET), Estación Experimental Agropecuaria Rafaela, Rafaela, Santa Fe, Argentina. <u>inta.gob.ar/rafaela</u>

³Consejo Nacional de Investigaciones Científicas y Técnicas. conicet.gov.ar

⁴Instituto Nacional de Tecnología Agropecuaria, (INTA), EEA Cuenca del Salado, Buenos Aires. inta.gob.ar/cuencadelsalado

⁵Unidad de Estudios Agropecuarios, INTA-CONICET, Córdoba, Argentina. conicet.gov.ar

genética luego de un ciclo de selección fueron calculadas usando dos unidades de recombinación: individual y media fenotípica familiar (PFM en inglés). Las estimaciones de heredabilidad fueron bajas para todos los caracteres tanto en condiciones salinas como no salinas sugiriendo un complejo control genético de la tolerancia a salinidad con alta proporción de efectos genéticos no aditivos. Las estimaciones fueron superiores usando la selección individual en comparación con la media fenotípica familiar. La ganancia genética esperada fue mayor para la selección individual. Tanto en condiciones salinas como en no salinas, la media predicha fue superior al cv. Klein, el cultivar más común de la variedad. El germoplasma analizado sería un buen recurso para ser incluido en programas de mejoramiento para incrementar la tolerancia a salinidad en *Panicum coloratum*.

Palabras clave: Heredabilidad, selección familiar, selección individual, variabilidad fenotípica y genotípica.

Introduction

Nearly one billion hectares of the earth's land surface $(13.2 \times 10^9 \text{ ha})$ are made up of saline and/or sodic soils, and between 25 and 30% of irrigated lands are saltmaking them essentially commercially affected. unproductive or lowly productive (Shahid et al. 2018). In recent decades, due to the expansion of cropping, cattleraising activities have moved to less-productive areas, many of which are affected to some degree by salinity, sodicity or a combination of both (Kraemer et al. 2013). These areas are covered by native species adapted to harsh conditions, which generally produce poor quality forage. Sowing of a warm-season grass like *Panicum coloratum*, tolerant of halomorphic conditions, could represent a potential option for increasing the productivity of such areas (Rossi 2005).

Panicum coloratum is a cross-pollinated, perennial C₄ grass, native to South Africa (Tischler and Ocumpaugh 2004) and P. coloratum var. coloratum has been grown in Africa, Australia and Southern USA to improve pasture lands and for erosion control and re-vegetation (Conrad 1976; Lodge et al. 2009). It exhibits above-average forage quality compared with other warm-season grasses (Rouquette et al. 1974, Ricci 2007) and has high resistance to drought, frost (Petruzzi et al. 2003) and salinity stress (Taleisnik et al. 2002; Pittaro et al. 2015), along with tolerance of waterlogging (Imaz et al. 2013; 2015) and alkaline soils as well (Otondo 2011; Luna et al.

As in other allogamous forage grasses, P. coloratum cultivars are highly heterozygous and represent panmictic populations, where internal genotypic variability offers the possibility of selecting individuals with improved performance in specific traits (Vogel and Burson 2004). Breeding efforts allowed the development of experimental lines displaying the following attributes: improved seed retention (Young 1991); reduced postharvest dormancy (Tischler and Young 1987); differences in the length of the subcoleoptilar node (Tischler and Voigt 1995); and increased seed weight (Hussey and Holt

1986; Giordano et al. 2013). In P. coloratum var. makarikariense genetic variation in tolerance of abiotic stresses such as drought (Giordano et al. 2019) and salinity has been reported recently (Cardamone et al. 2018). However, breeding efforts to increase salinity tolerance in *P. coloratum* var. coloratum have still not been made.

Panicum coloratum var. coloratum is widely known as Klein grass and Verde is the most popular cultivar used extensively in Texas and Central Argentina (Ruiz et al. 2004; Tischler and Ocumpaugh 2004). This material has been introduced to Argentina as cultivar Klein and has been sown in different soil and climatic conditions during the 20th century, resulting in long-lasting pastures given its wide adaptation to various environments (Burgos et al. 2018). Severe selection pressures like extreme droughts or frosts have produced races or local populations that are common in central Argentina. These local populations may become the starting point to initiate a breeding program aimed at generating germplasm tolerant of different stressful conditions, if they have retained enough genetic variation.

The breeding method most commonly used to improve quantitatively inherited characters in populations of outcrossing plants is recurrent phenotypic selection (Vogel and Burson 2004). However, whether selection is exerted on individual plants or in families depends on the structure of the population and the source of genetic variation (Nguyen and Sleper 1983). Then, estimates of variance components and their distribution in the traits of interest of the germplasm source provide priceless information to decide on selection strategy in any breeding program. In addition, estimating additive genetic effects and heritability may allow prediction of genetic gain as a result of applying selection at a given intensity (Casler et al. 2002).

In a previous study, plants from an old pasture of P. coloratum cv. Klein, which had survived in a saline site during periods of severe droughts and frosts were collected, cloned, multiplied and evaluated in a greenhouse setting (Pittaro et al. 2015). The collected plants were found to be more tolerant (33%) of salinity than plants produced from commercial seeds of the same cultivar (Pittaro et al. 2015), supporting the hypothesis that strong selection pressure had been exerted by the stressful environment. Although the number of plants collected at this stressful site was relatively low, analysis with random genetic markers (ISSRs) revealed significant genetic variability (Pittaro et al. 2015). Tolerance of salinity combined with genetic variation suggests that this material may be a valuable source of germplasm to start a breeding program to further increase salinity tolerance. Nonetheless, it is necessary to confirm that genetic variation for specific traits related to salinity tolerance exists, as there is a possibility that the intense selection pressure exerted by drought and frost might have substantially reduced it.

In any breeding program, quantitative genetic information regarding the base population or the source of germplasm is required to effectively design the breeding strategy (Vogel and Burson 2004). Then, it is necessary to acquire information on the extent, nature and distribution of genetic variation and heritability of the traits of interest in order to make predictions of the possible genetic progress to be achieved (Dudley and Moll 1969). Estimates of variance components may be achieved by analyzing the performance of half-sib (HS) families as has been reported in other forage grasses, e.g. wheatgrass, ochardgrass (Casler and Brummer 2008) and tall fescue (Majidi et al. 2009). However, whether selection is performed on an individual plant basis or based on means for families depends on the structure of genetic variation and its distribution in the available germplasm (Fehr 1987). In addition, flowering synchronicity should be evaluated before pursuing any breeding strategy to ensure that estimates are made on the actual mating population (Majidi et al. 2009).

Prior to launching a breeding program to increase salt tolerance in P. coloratum var. coloratum, we were interested in characterizing the germplasm that survived extreme environments in terms of flowering time, biomass accumulation and plant structure under field conditions. The objectives were: 1. to characterize genotypes according to flowering synchronicity, biomass production and plant structure to narrow down the population that would be actually breeding; 2. to estimate narrow-sense heritability and genetic gain per cycle of traits associated with salinity tolerance in saline and non-saline conditions in a prospective breeding population; 3. to compare attainable genetic progress towards increasing productivity in saline conditions from individual and familial selection; and 4. to compare the calculated probable means with the performance of a widely used cultivar.

Materials and Methods

Plant material and clone production

The plants used for the trials were initially collected from an established pasture on a typical Natracualf soil (35°25'0" S, 57°43'0" W), which survived saline conditions and severe droughts (Pittaro et al. 2015). Then, these plants, identified here as genotypes, were grown in a greenhouse and propagated to obtain clonal groups as described by Pittaro et al. (2015).

Evaluation of the P. coloratum genotypes under field conditions

Twelve replicates of each of 15 genotypes were planted in December 2013 in the experimental field at 'Chacra Experimental Integrada Chascomús, INTA-MAA', Buenos Aires Province. The historical annual precipitation at the site is 1,038 mm, while soil is characterized by an electrical conductivity (EC) of 4.7 dS/m and a pH of 9.5. The genotypes were planted at random at 1 m spacing in a plot of 200 m². In the first year (accumulated precipitation 661 mm), height and diameter of each individual plant were registered. Height was measured from the base of the plant to the ligula of the flag leaf and diameter as the length of the longitudinal projection of the plant canopy on the soil surface at the widest part. Fresh biomass accumulation of the plants was recorded, and subsamples were taken to the laboratory, weighed and dried to calculate moisture content of each sample. In the second year (mean annual precipitation 788 mm), the first growth was cut in early October and discarded, then flowering synchrony was evaluated at the end of November, using an arbitrary scale based on the number of open spikelets per panicle at the time of evaluation, when all genotypes presented panicles. All plants were evaluated and were categorized with the following taxonomy: 'late' - fewer than 50% open spikelets per panicle; 'intermediate' - 50% open spikelets per panicle; and 'early' - more than 50% open spikelets per panicle plus older anthers.

Evaluation of salinity tolerance in the offspring of synchronic clones of P. coloratum

Ten genotypes previously classified as early- and intermediate-flowering were clonally propagated in a greenhouse and transferred to an isolated polycross nursery in the experimental 'Chacra Experimental Sinsacate' situated in Córdoba (30°56' S, 64°05' W), 1,000 km from the collection site. The historical annual

precipitation at the site is 780 mm, with mean temperature 15.8 °C; soil is an Udic Argiustoll, developed on silty loess in a rolling landscape, poorly drained with neutral pH. Twelve replicates of each genotype were planted in a crossing block at a spacing of 1 m to facilitate pollen exchange throughout. In January 2015, after anthesis had completed, several inflorescences on each plant were enclosed in seed traps to facilitate harvest of seeds from each genotype to produce half-sib families (HS fam). The collected seeds were manually threshed and blown to remove glumes and other residuals in a laboratory. Seeds from all replicates of each genotype were bulked to constitute one HS fam. Fifty seeds of each HS fam were germinated in a petri box containing vermiculite.

Genetic variation for salinity tolerance among HS in greenhouse.

The aim of this assay was to evaluate the performance of seedlings from 10 HS fam in saline and non-saline conditions, and compare them with commercial cv. Klein. Two-leaved seedlings were transplanted into forest tube pots (55 cm diameter and 20 cm height), containing a mixture of perlite and washed river sand (2:1), and placed in plastic trays. The experimental design was a split-plot completely randomized with 3 blocks and 12 seedlings per HS fam in total. Salinity treatments consisted of non-saline (EC 0.0 dS/m) and saline conditions (with the addition of 0.2 M NaCl to get EC 20.0 dS/m). Pots were irrigated twice a week with Hoagland solution (Hoagland and Arnon 1950) and gradually salinized with increasing NaCl concentrations (0.05, 0.1, 0.15 and 0.2 M) during a 2week period. Once 0.2 M NaCl was reached, the trays were placed in a system with automatic irrigation. Concentration of the solutions in each tray was controlled once a week by measuring EC of the drained water, using a digital conductivity meter (Altronix CTX-II, Buenos Aires, Argentina). Leaf number (LN) and tiller number (TN) per plant were counted twice a week. Leaf emergence rate (LR) and tillering rate (TR) were calculated as a function of time, i.e. x/day, where x is either number of leaves or tillers. Plants were harvested 45 days after transplanting to collect aboveground biomass, which was then dried in an oven until constant weight to obtain shoot dry weight (SDW). Numbers of leaves (L) and tillers (T) per plant were also counted at harvest. For all variables, the values in saline and non-saline conditions were calculated (saline/non-saline) on a per cent basis and were named as %SDW, %TR, %TN, %LR and %LN.

Statistics

A general mixed linear model was used to estimate variance components in HS fam of *P. coloratum* var. *coloratum* seedlings growing in both saline and nonsaline conditions with Infostat software with R interface (Di Rienzo et al. 2008).

The model used was:

$$Xij = \mu + Bi + Fj + (BF)ij + Eij$$

where:

Xij is the ijth observation of the ith block and the jth family;

μ is the general mean;

Bi is the fixed ith block effect;

Fj is the random jth family effect;

BFij is the random jth family by ith block interaction effect; and

E*ij* is the error term.

Narrow-sense heritability on an individual plant basis was estimated as follows:

$$h^2 = \sigma^2_A / \sigma^2_P$$

where:

 σ^2_A is the additive variance calculated as $4\sigma^2_F$ (σ^2_F : family variance component);

 σ^2_P is the phenotypic variance corresponding to the sum of $\sigma^2_F + \sigma^2_E + \sigma^2_W$, where σ^2_E is ambient variation (σ^2_E) plus the family by block interaction of both (σ^2_W) (Nguyen and Sleper 1983).

Genetic gain per cycle (AG) of individual plant selection (mass selection in one year at one location) was predicted as follows:

$$AG = c k h^2 \sigma_P$$

where:

c = parental control factor (<u>Sprague and Eberhart</u> 1977);

k = standardized selection differential;

 h^2 = narrow-sense heritability on an individual basis; and

 σ_P = phenotypic standard deviation among individual plants.

Parental control factor c=1 if selected plants are planted together in the field and both maternal and paternal selections are in place.

Heritability estimates were also calculated on a phenotypic family mean (PFM) basis as follows:

$$h^{2}_{PFM} = \sigma^{2}_{F}/(\sigma^{2}_{F} + \sigma^{2}_{E}/r + \sigma^{2}_{W}/r*n)$$

where:

 $\sigma^2_{\rm F}$ is the family variance;

 $\sigma_{\rm E}^2$ is the ambient variation plus the within block variance $\sigma_{\rm W}^2$;

r and n are the number of blocks and plants per block, respectively.

Genetic gain per cycle of familial selection with 30% intensity was calculated in the same way as $AG = c k h^2_{PFM}$ σ_{PFM} . Genetic progress was calculated under the assumption that superior parents were selected on the basis of the mean performance of their HS progenies, then replicated parents inter-mated in an isolated polycross (c = 2).

Standard error of heritability was calculated with Dickerson approximation (Dickerson 1969).

Individual and family selection methods

Two selection methods were used to calculate prospective means after the selected individuals were allowed to intermate: for individual selection, individuals were chosen according to their performance both in saline and nonsaline conditions; for familial selection, parents were chosen according to the performance of their progeny. To select individuals or families a principal component analysis (PCA) based on the Euclidean distance matrix (Cardamone et al. 2018) was calculated from 5 variables: SDW, LN, TN, LR and TR and individuals were ranked considering all variables.

The predicted values derived after selection both on individual and phenotypic family mean (PFM) bases were compared with the mean values in the base population to calculate the progress made. In addition, predicted values were compared with the mean values registered by seedlings of cv. Klein growing in saline and non-saline conditions.

Results

Variability in biomass accumulation, plant structure and flowering synchronicity in clones of P. coloratum grown in field plots

Biomass accumulation showed significant differences among genotypes growing in the field (P<0.05) as indicated in Figure 1. Genotype 2 had the highest biomass accumulation, while Genotypes 11, 6 and 10 showed the lowest values. When evaluating plant structure by the ratio of plant diameter:height (D:H), all genotypes ranged from 1 to 1.5 (Figure 1). Genotypes 1, 16, 6 and 10 showed a compact structure (ratio close to 1), while Genotypes 12 and 9 had the highest D:H, indicating a prostrate habit.

It was possible to classify genotypes on the basis of flowering time into 3 groups (early, intermediate and late) (Figure 1), with 5 genotypes identified as late-flowering, while the other 10 were grouped as early- and intermediate-flowering. The early- plus intermediateflowering group retained much of the phenotypic variation as it congregated genotypes differing in biomass production and plant structure.

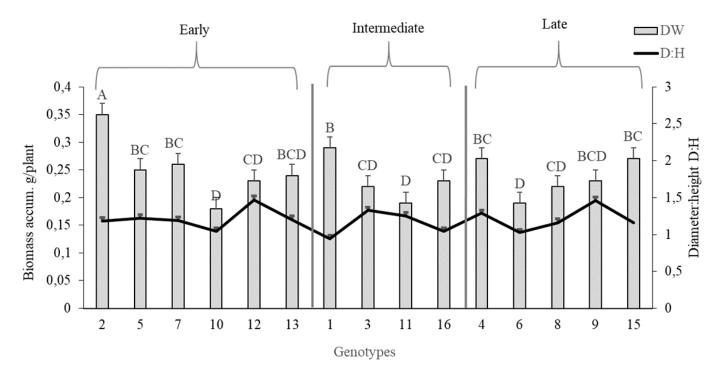


Figure 1. Biomass accumulation (DW; g/plant) and diameter: height (D:H) ratio in genotypes of Panicum coloratum growing in the field. Vertical lines separate groups according to flowering synchronicity into late-, intermediate- and early-flowering. Each column shows the mean \pm s.d. Different letters on columns indicate significant differences at P<0.05 by Fisher's LSD test.

Performance of half-sib families in the greenhouse

Half-sib families from a crossing block of replicated genotypes belonging to the late- and intermediateflowering groups were evaluated in the greenhouse in saline and non-saline conditions. No interaction between salinity condition and HS-family was evident for any of the evaluated traits. The parameters SDW and TN were negatively affected by salinity (P = 0.0001 and P = 0.01, respectively), while LN and LR were similar in both saline and non-saline conditions (P = 0.029 and P =0.140, respectively). Only TR was higher in saline than non-saline conditions (P = 0.0001). Differences among families were evident in SDW (P = 0.0194), while no differences were observed for TN. TR. LR and LN. The most productive family regarding shoot biomass both in saline and non-saline conditions was HS 3 (data not shown). In addition, this family showed the highest salinity tolerance (saline/non-saline) for %SDW, %TN, %LR and %TR (P = 0.0145; P = 0.0161; P = 0.0019; P= 0.0021, respectively).

Estimates of genetic parameters

Variance components were estimated from HS family values in both saline and non-saline conditions. Estimates of heritabilities were calculated for all evaluated variables on both individual plant and phenotypic family mean (PFM) bases. Given that the base population was already proven tolerant of salinity, estimations of heritability were calculated in saline and non-saline conditions (Table 1). Estimates of heritabilities were low in non-saline and saline conditions for SDW, TN, TR and LN (h²<0.25), on both individual plant and PMF bases. For LR, heritability estimate was higher than the variables mentioned before $(h^2 = 0.521)$ but only in non-saline conditions and on an individual plant basis, while it was close to null in saline conditions. Heritability estimates calculated on an individual plant basis were always higher than on a PFM basis in both saline and non-saline conditions (Table 1).

Regarding expected genetic gains, progress was greater when calculated on an individual plant basis (Figure 2), by selecting individual plants in both saline and non-saline conditions for all variables measured. Progress in SDW, TN and LR is expected to be greater when selected in non-saline conditions, while higher expectations would occur in variables such as LN and TR when selected under saline conditions. However, genetic gains were negligible for all variables in saline conditions for both individual and familial selection procedures (Figure 2).

Table 1. Narrow-sense heritability (h²) for individual and familial selection methods in saline and non-saline conditions, for shoot dry weight (SDW), leaf number (LN), tiller number (TN), leaf emergence rate (LR) and tillering rate (TR) of *Panicum coloratum*.

		$_{-}$ h^2		
		Individual selection	Familial selection (PFM)	
Non-saline	SDW	0.134 ± 0.016	0.025 ± 0.65	
	LN	0 ± 0	0 ± 0	
	TN	0.19 ± 0	0.037 ± 0.52	
	LR	0.521 ± 0.32	0.095 ± 0.47	
	TR	0.22 ± 0.226	0.040 ± 0.52	
Saline	SDW	0.059 ± 0.23	0.010 ± 0.58	
	LN	0.090 ± 0.29	0.015 ± 0.47	
	TN	0 ± 0	0 ± 0	
	LR	0 ± 0	0 ± 0	
	TR	0.110 ± 0.27	0.019 ± 0.64	

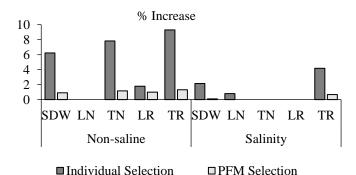
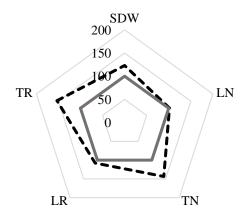


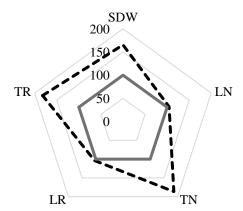
Figure 2. Percentage increase in predicted population of *Panicum coloratum* using individual and PFM selection methods, in non-saline and saline conditions for: shoot dry weight (SDW), leaf number (LN), tiller number (TN), leaf emergence rate (LR) and tillering rate (TR).

To visualize the prospective increase in performance through selection, we compared the predicted means from individual selection with the values obtained when growing seedlings of cv. Klein under the same conditions as described before, in saline and non-saline conditions. Means were plotted as radar graphs, where values for cv. Klein seedlings represent 100%. In both saline and nonsaline conditions (Figure 3A; Figure 3B), the means predicted after selection were higher than the means of cultivar Klein in the same condition. Means after selection would be higher for TN (43.6%), TR (53.1%) and SDW (23.0%) than for cv. Klein seedlings under saline conditions. A more remarkable advance would be observed when selection was performed on plants growing in nonsaline conditions. That is, the predicted population showed a better performance than cv. Klein (86.4% for TN, 82.2% for TR and 65.3% for SDW; Figure 3B).

Saline individual selection **A**-



B-Non-saline individual selection



Predicted Population

Figure 3. Radar plot showing a comparison of *Panicum coloratum* cv. Klein indicated as 100% and the predicted means after 30% selection intensity in % shoot dry weight (SDW), % leaf appearance rate (LR), % tillering rate (TR), % tiller number (TN) and % leaf number (LN) in (A) saline and (B) non-saline conditions with the individual selection method.

Discussion

In many forage species, progress in breeding has been accomplished by means of phenotypic recurrent selection (Vogel and Pedersen 1993; Marcón et al. 2020). This approach has been used to increase forage biomass (Dhaliwal 2009) and other traits such as seedling vigor (Tischler et al. 1989) and seed weight (Giordano et al. 2013). Relocation of cattle-raising activities into lessproductive environments, frequently subjected to restrictions in growth by salinity, heavy soils, frequent droughts or flooding, has moved breeding objectives to plant survival and forage production under restrictive conditions (Griffa et al. 2010). In this study, we explored the possibility of initiating a breeding program involving the subtropical forage grass Panicum coloratum var. coloratum to increase forage production when growing in saline conditions. We considered using a set of plants collected in a very restricted environment as a source germplasm, assuming the selected individuals were already adapted to these conditions. The collected plants were analyzed and proved to be variable with neutral molecular markers (Pittaro et al. 2015). To analyze the possible outcome of the breeding program, we studied the base germplasm to choose mating genotypes according to phenology, estimated the variability related to additive effects and estimated the progress to be expected after selection. Finally, we evaluated the chance that the prospective germplasm we would obtain through selection might surpass the performance of the most common and widely known cultivar of P. coloratum var. coloratum (cv. Klein).

Variability among genotypes under field conditions.

Genotypes of P. coloratum transplanted to the field in a saline soil were highly variable for traits such as biomass production and plant structure (Figure 1). Phenotypic variation confirmed that P. coloratum is an allogamous species, quite variable in morphology and molecular markers (Burgos et al. 2018). As with other subtropical forage grasses, e.g. Chloris gayana (Ortega and Taleisnik 2003) and Cenchrus ciliaris (Tommasino et al. 2012), P. coloratum is adapted to thrive under stressful conditions. Even though individuals studied here were collected in a very stressful environment and had been exposed to quite strong selection pressure that might have been expected to reduce genetic variation, differences in phenology, plant structure and biomass production were apparent even in the reduced number of genotypes.

The synchrony in reproductive structures is of fundamental importance for the reproductive success of the plant and a point to consider when selecting genotypes to cross in a breeding program (Oleques et al. 2017). In the genotypes analyzed here, we identified 3 groups for flower synchrony (early, medium and late) when growing in the field (Figure 1). Then, individuals grouped as earlyand intermediate-flowering were crossed and their progeny tested for salinity tolerance under controlled conditions.

Phenotyping and genetic variability to salinity tolerance in half-sib P. coloratum.

To pursue our breeding objective, i.e. to increase salinity tolerance, we then evaluated the extent of additive genetic variance among individuals and estimated the possible gain to be achieved after selection. To do that, we analyzed half-sib families from the individuals resulting from crosses.

Agronomic aspect of salinity tolerance. Both growth and biomass yield were adversely affected by salinity, a common feature in most plant species (Ashraf 2004), although not all traits were equally affected in P. coloratum. In our study, seedlings showed a higher tillering rate in saline conditions than in a non-saline situation. Tiller outgrowth may be related to a high appearance rate of low rank tillers associated with stressful conditions determined by several morphogenetic components (Gastal and Lemaire 2015). Regarding genetic variation related to these traits, heritability estimates were in general very low. Contrary to other studies that showed estimates of heritability and additive genetic component estimates increased as stress conditions increased (Kacser and Burns 1981; Blum 1988), in this study, estimates were higher in non-saline than in saline conditions. However, Mohammadi et al. (2014) showed that narrow-sense heritability estimates were higher in saline than in non-saline conditions for traits related to salinity tolerance in rice. In addition, P. coloratum var. makarikariense, a botanical variety of the same species, displayed high values for heritability of morphological traits in saline (Cardamone 2020) and drought conditions (Giordano 2018). On the contrary, our results suggested that additive variation for salinity tolerance had already been exploited in this source of germplasm as the population had been subjected to strong selection pressure in a very harsh environment; as a result, heritability estimates under saline conditions were very low. It is well accepted that estimates of heritability of any given character refer only to the germplasm evaluated under the particular experimental conditions (Falconer and Mackay 1996). Low heritability may be caused by low additive effects and/or high dominant gene action (Falconer and Mackay 1996). Salinity tolerance was suggested to have a complex pattern of inheritance related to a high non-additive component, associated with low heritabilities in highly saline concentrations in sorghum (Azhar and McNeilly 1988) and pearl millet (Kebebew and McNeilly 1996) adjudged to non-additive components. It was suggested that changes in genetic variation related to increases or decreases in salt concentrations might be driven by the action of different genes that contribute to the same trait in different environments (Rao and McNeilly 1999).

Selection methods and growth conditions. Phenotypic recurrent selection is the most efficient and commonly used form of plant breeding in perennial forage grasses (Vogel and Burson 2004). Estimates of heritable genetic variation and the degree of genetic progress within breeding populations are helpful to breeders in determining the probable effectiveness of pursuing selection strategies over time. In characters exhibiting high heritability, selection is more likely to be effective (Degefa et al. 2014). However, the outcome may differ depending on the recombination unit on which selection is performed. Depending on whether selection is based on values expressed by individual members of the population (Mariotti and Collavino 2014) or on the family mean, recombination would involve different sets of alleles. In PFM selection, the parents of the best families are polycrossed in isolation. Nguyen and Sleper (1983) recommend family selection over individual selection, when estimates of heritability are low because of large environmental effects. We compared the outcomes from these 2 selection methods estimating heritability on both individual plant and PFM bases and calculating genetic gain after 30% selection intensity. These methods have been used in breeding P. coloratum and other forage grasses (Giordano et al. 2013; Simeão et al. 2013; Cardamone et al. 2018). Both methods showed low narrow-sense heritability values for almost all parameters evaluated, but, in general, individual-based values were higher than family-based with minor differences. Accordingly, genetic gain per cycle was low for all parameters using both selection methods, although the standardized selection differential was higher in individual selection (data not shown).

Phenotypic family mean selection is recommended over individual selection for characters with low heritability (Basigalup 2007). When environmental deviations of individuals within the family tend to cancel each other out in determining the mean value of the family, the mean phenotypic value of the family approaches the genotypic mean (Falconer and Mackay1996). In this study, the experimental system allowed control of growth conditions and reduction in environmental variance (Broman and Sen 2009; Quero et al. 2013). Therefore, the low heritability values found here are due to limited genetic variability explained by the narrow genetic base in the population and not to environmental variation. Extreme conditions of drought, frosts and salinity at the site where the plants were collected might have exerted a strong selection pressure and reduced genetic variation among surviving individuals. As a result, heritability estimates were low for all characters, and minor differences were found depending on the method for calculating them (PFM or individual basis) (Table 1). In this situation, the recommendation for pursuing selection in this germplasm would be to perform individual selection, since it is more practical, easier and more straightforward than the PFM-based one (Falconer and Mackay 1996; Rose et al. 2008).

One interesting feature of this germplasm was that, while genetic gain was low for all characters and considering 2 selection methods, predicted means after selection were higher than the means observed in plants of cv. Klein for all parameters evaluated, in both nonsaline and saline growth conditions (Figure 3). This constitutes further evidence that, in the collected germplasm which had been exposed to extreme conditions, only tolerant genotypes with limited variation survived, making them promising germplasm not only to be introduced in a breeding program to improve salinity tolerance, but also to study mechanisms and genes involved in salinity tolerance in forage grasses.

Conclusion

Although germplasm of Panicum coloratum var. coloratum studied here showed variability in morphology and phenology as well as molecular markers, variation in salinity tolerance was limited and estimates of narrow-sense heritability were low, even when calculated on a PFM basis. Estimates of genetic advance were also low, indicating that no further progress could be made in salinity tolerance by recurrent selection in this material. However, the predicted population of *P. coloratum* showed higher dry weight, tiller number, tillering rate and leaf production rate than cv. Klein under saline conditions. We consider the collected germplasm represents a source of salinity-tolerance genes in establishing a breeding program for this species. This material might also be valuable in studies to understand mechanisms of salinity tolerance in this and other species.

Acknowledgments

The authors thank Rosalba Pemán from Oscar Pemán y Asociados Semillas for financial support and for providing the experimental site.

References

(Note of the editors: All hyperlinks were verified 15 April 2021.)

- Ashraf M. 2004. Some important physiological selection criteria for salt tolerance in plants. Flora 199 (5):361-376. doi: 10.1078/0367-2530-00165
- Azhar FM; McNeilly T. 1988. The genetic basis of variation for salt tolerance in Sorghum bicolor (L.) Moench, seedlings.

- Plant Breeding 101(2):114-121. doi: 10.1111/j.1439-0523. 1988.tb00275.x
- Basigalup DH. 2007. Mejoramiento genético y desarrollo de variedades. In: Basigalup DH, ed. El cultivo de alfalfa en la Argentina. Ediciones INTA, Buenos Aires, Argentina. p. 83-103. ISBN: 978-987-521-242-8. bit.ly/38ui2EN
- Blum A, ed. 1988. Plant breeding for stress environment. CRC Press. ISBN: 08-493-63888. bit.ly/3tmqqyv
- Broman KW; Sen S. 2009. Experimental design and power. In: Gail M; Krickeberg K; Samet J; Tsiatis A; Wong W, eds. A guide to OTL mapping with R/qtl. Statistics for biology and health. Springer, New York, NY. doi:10.1007/978-0-387-92125-9 6
- Burgos E; Thompson C; Giordano M; Thomas MA. 2018. Prebreeding studies in Panicum coloratum var. coloratum: Characterization using agro-morphological traits and molecular markers. Tropical Grasslands-Forrajes Tropicales 6:82-92. doi: 10.17138/tgft(6)82-92
- Cardamone L. 2020. Caracterización agronómica, variabilidad genética y ganancia por selección en caracteres relacionados con tolerancia a salinidad en una colección de Panicum coloratum var. makarikariense. Ph.D. Thesis. Universidad Nacional de Córdoba.
- Cardamone L; Cuatrin A; Grunberg K; Tomas MA. 2018. Variability for salt tolerance in a collection of Panicum coloratum var. makarikariense during early growth stages. Tropical Grasslands-Forrajes Tropicales 6:134-147. doi: 10.17138/tgft(6)134-147
- Casler M; Brummer EC. 2008. Theoretical expected genetic gains for among and within family selection methods in perennial forage crops. Crop Science 48(3):890-902. doi: 10.2135/cropsci2007.09.0499
- Casler MD; Fales SL; McElroy AR; Hall MH; Hoffman LD; Undersander DJ; Leath KT. 2002. Half-sib family selection for forage yield in orchardgrass. Plant Breeding 121(1):43-48. doi: 10.1046/j.1439-0523.2002.00665.x
- Conrad BE. 1976. Forage and animal production programs for South Texas. In: Holt EC; Lewis RD, eds. Grasses and Legumes in Texas - development, production and utilization. Vol 6. Texas Agricultural Experiment Station Research Monograph. p. 420-431. bit.ly/2Oc46sd
- Degefa I; Petros Y; Andargie M. 2014. Genetic variability, heritability and genetic advance in Mungbean (Vigna radiata L. Wilczek) Accessions. Plant Science Today 1(2):94-98. doi: 10.14719/pst.2014.1.2.54
- Dhaliwal A. 2009. Recurrent phenotypic selection for increased winter productivity in annual ryegrass (Lolium multiflorum Lam.). M.Sc. Thesis. Auburn University. bit.ly/3cn0ziq
- Di Rienzo JA; Casanoves F; Balzarini MG; Gonzalez L; Tablada M; Robledo CW. 2008. InfoStat, Grupo infostat, FCA, Universidad Nacional de Córdoba, Argentina. Versiones 2008. infostat.com.ar
- Dickerson GE. 1969. Techniques for research in quantitative animal genetics. In: Chapman AB, ed. Techniques and procedures in animal science research. American Society of Animal Science, Albany, NY.

- Dudley J; Moll R. 1969. Interpretation and use of estimates of heritability and genetics variances to plant breeding. Crop Science 9(3):257–262. doi: 10.2135/cropsci1969. 0011183X000900030001x
- Falconer DS; Mackay TFC. 1996. Introduction to Quantitative Genetics. 4th Edn. Addison Wesley Longman, Harlow.
- Fehr WR. 1987. Principles of cultivar development. Vol 1. Theory and Technique. Macmillan Publishing Company, New York, Iowa State University. ISBN: 0-02-949920-8. bit.ly/3sZuUum
- Gastal F; Lemaire G. 2015. Defoliation, shoot plasticity, sward structure and herbage utilization in pasture: Review of the underlying ecophysiological processes. Agriculture 5(4):1146-1171. doi:10.3390/agriculture5041146
- Giordano MC; Berone GD; Tomás MA. 2013. Selection by seed weight improves traits related to seedling establishment in Panicum coloratum L. var. makarikariense. Plant Breeding 132(6):620-624. doi: 10.1111/pbr.12106
- Giordano, M. 2018. Plasticidad fenotípica en Panicum coloratum: variabilidad genética, heredabilidad y expresión diferencial de la plasticidad. Ph.D. Thesis. Universidad de Buenos Aires, Buenos Aires, Argentina.
- Giordano MC; Grimoldi AA; Tomás MA. 2019. Phenotypic plasticity to drought in seedlings of the warm season grass Panicum coloratum is related to collection site. Annals of Applied Biology 175(2):164-171. doi: 10.1111/aab.12525
- Griffa S; Ribotta A; López Colomba E; Tommasino E; Carloni E; Luna C; Grunberg K. 2010. Evaluation of seedling biomass and its components as selection criteria for improving salt tolerance in Buffel grass genotypes. Grass and Forage Science 65(3):358-361. doi: 10.1111/j.1365-2494.2010.00754.x
- Hoagland DR; Arnon DI. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347. University of California, Berkeley, CA, USA. goo.gl/Mee8fK
- Hussey MA; Holt EC. 1986. Selection for increased seed weight in Kleingrass. Crop Science 26(6):1162-1163. doi: 10.2135/cropsci1986.0011183X002600060017x
- Imaz J; Giménez D; Grimoldi A; Striker G. 2015. Ability to recover overrides the negative effects of flooding on growth of tropical grasses Chloris gayana and Panicum coloratum. Crop and Pasture Science 66(1):100-106. doi: 10.1071/CP14172
- Imaz JA; Giménez DO; Grimoldi AA; Striker GG. 2013. The effects of submergence on anatomical, morphological and biomass allocation responses of tropical grasses Chloris gayana and Panicum coloratum at seedling stage. Crop and Pasture Science 63(12):1145-1155. doi: 10.1071/CP12335
- Kacser H; Burns JA. 1981. The molecular basis of dominance. Genetics 97(3-4):639–666. <u>bit.ly/3ryHxMw</u>
- Kebebew F; McNeilly T. 1996. The genetic basis of variation in salt tolerance in Pearl Millet, Pennisetum americanum (L.) Leeke. Journal of Genetics & Breeding 50:129-136. (Italy). ISSN: 0394-9257. bit.ly/3t8gZSJ
- Kraemer FB; Chagas CI; Marré G; Palacín EA; Santanatoglia O. 2013. Desplazamiento de la ganadería por la agricultura en una cuenca de la pampa ondulada: efectos sobre el

- escurrimiento superficial y erosión hídrica. Ciencia del Suelo (Argentina) 31(1):83-92. bit.ly/3bDoThf
- Lodge GM; Boschma SP; Harden S. 2009. Replacement series studies of competition between tropical perennial and annual grasses and perennial grass mixtures in northern New South Wales. Crop and Pasture Science 68:526-531. doi: 10.1071/CP08374
- Luna DF; Aguirre A; Pittaro G; Bustos D; Ciacci B; Taleisnik E. 2016. Nutrient deficiency and hypoxia as constraints to Panicum coloratum growth in alkaline soils. Grass and Forage Science 72(4):640-653. doi: 10.1111/gfs.12263
- Majidi MM; Mirlohi A; Amini F. 2009. Genetic variation, heritability and correlations of agro-morphological traits in tall fescue (Festuca arundinacea Schreb.). Euphytica 167(3):323-331. doi: 10.1007/s10681-009-9887-6
- Marcón F; Martínez EJ; Zilli AL; Rodríguez GR; Brugnoli EA; Acuña CA. 2020. Recurrent phenotypic selection and recurrent selection based on combining ability in tetraploid bahiagrass. Crop Science 60:1386-1397. doi: 10.1002/csc2.20137
- Mariotti JA; Collavino NG. 2014. Los caracteres cuantitativos en la mejora genética de los cultivos. 1ra Edn. Orientación gráfica editora, Buenos Aires, Argentina. ISBN: 978-987-1922-09-3. bit.ly/3cyk08r
- Mohammadi R; Mendioro MS; Diaz G Q; Gregorio GB; Singh RK. 2014. Genetic analysis of salt tolerance at seedling and reproductive stages in rice (Oryza sativa). Plant Breeding 133(5):548-559. doi: 10.1111/pbr.12210
- Nguyen HT; Sleper DA. 1983. Theory and application of halfsib matings in forage grass breeding. Theoretical and Applied Genetics 64(3):187-196. doi: 10.1007/BF00303763
- Oleques SS; Overbeck GE; De Avia RS Jr. 2017. Flowering phenology and plant-pollinator interactions in a grassland community of Southern Brazil. Flora 229:141-146. doi: 10.1016/j.flora.2017.02.024
- Ortega L; Taleisnik E. 2003. Elongation growth in leaf blades of Chloris gayana under saline conditions. Journal of Plant Physiology 160(5):517–522. doi: 10.1078/0176-1617-00827
- Otondo J. 2011. Efectos de la introducción de especies megatérmicas sobre características agronómicas y edáficas de un ambiente halomórfico de la Pampa Inundable. M.Sc. Thesis. Universidad de Buenos Aires. bit.ly/2OGH6RW
- Petruzzi HJ; Stritzler NP; Adema EO; Ferri CM; Pagella JH. 2003. Mijoperenne-Panicum coloratum. Informe Técnico N51 EEA INTA Anguil, La Pampa, Argentina, Sitio argentino de producción animal. bit.ly/3bArNDq
- Pittaro G; Cáceres L; Bruno C; Tomás A; Bustos D; Monteoliva M; Ortega L; Taleisnik E. 2015. Salt tolerance variability among stress-selected Panicum coloratum cv. Klein plants. Grass and Forage Science 71:683-698. doi: 10.1111/gfs. 12206
- Ouero GE; Borsani O; Gutiérrez L; Melchiorre M; Monza J; Lascano R. 2013. Sistemas de fenotipado para la evaluación de las respuestas al estrés salino en Lotus. Agrociencia Uruguay 17(1):11-21 scielo.edu.uy/scielo.php?script=sci arttext&pid =S2301-15482013000100002

- Rao SA; McNeilly T. 1999. Genetic basis of variation for salt tolerance in maize (Zea mays L). Euphytica 108:145–150. doi: 10.1023/A:1003612411293
- Ricci H. 2007. Producción primaria de cuatro gramíneas tropicales perennes cultivadas en la llanura deprimida de Tucumán. ISSN-13 N° 978-987-05-3688-8
- Rose LW; Das MK; Taliaferro CM. 2008. Estimation of genetic variability and heritability for biofuel feedstock yield in several populations of switchgrass. Annals of Applied Biology 152:11-17. ISSN 0003-4746. doi: 10.1111/j.1744-7348.2007.00186.x
- Rossi C. 2005. Mejoramiento de la condición forrajera en bajos alcalino-sódicos con especies subtropicales en la cuenca del salado. Informe de resultado del proyecto. Sitio Argentino de Producción Animal. bit.ly/3eqr5Kt
- Rouquette FM; Holt EC; Ellis WC. 1974. Nutrient characteristics of kleingrass at various stages of maturity. II. In vivo and in vitro evaluations of selected varieties. Agronomy Journal 66(4):510-513. doi: 10.2134/agronj1974.00021962006600040010x
- Ruiz MA; Babinec FJ; Adema EO; Rucci T. 2004. Producción de forraje y contenido de proteína de gramíneas estivales introducidas en Chacharramendi (La Pampa). Revista Argentina de Producción Animal 24(1-2):41-51. bit.ly/ <u>3rC</u>4e2v
- Shahid SA; Zaman M; Heng L. 2018. Soil Salinity: Historical Perspectives and a World Overview of the Problem. In: Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques. Springer, Cham. pp 43–53 doi: 10.1007/978-3-319-96190-3 2
- Simeão Resende RM; Casler MD; Vilela de Resende MD. 2013. Selection methods in forage breeding: a quantitative appraisal. Crop Science 53(5):1925-1936. doi: 10.2135/cropsci2013.03.0143
- Sprague GF; Eberhart SA. 1977. Corn breeding. In: Sprague GF, ed. Corn and Corn Improvement. 2nd Edn. American Society of Agronomy, Madison, Wisconsin, USA. p. 305-362.
- Taleisnik E; Pérez H; Córdoba A; Moreno H; García Seffino L; Arias C; Grunberg K; Bravo S; Zenoff A. 2002. Salinity effects

- on the early development stages of Panicum coloratum: Cultivar differences. Grass and Forage Science 53(3):270–278. doi: 10.1046/j.1365-2494.1998.00139.x
- Tischler CR; Ocumpaugh WR. 2004. Kleingrass, Blue Panic, and Vine Mesquite. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C4) grasses. American Society of Agronomy, Madison, Wisconsin, USA. p. 623-649. doi: 10.2134/agronmonogr45.c18
- Tischler CR; Voigt PW. 1995. Modifying seedling photomorphogenesis in Kleingrass by recurrent selection. Crop Science 35(6):1613-1617. doi: 10.2135/cropsci1995 .0011183X003500060016x
- Tischler CR; Voigt PW; Holt EC.1989. Adventitious root initiation in Kleingrass in relation to seedling size and age. Crop Science 29(1):180-183. doi: 10.2135/cropsci1989.0011183X0029000 10039x
- Tischler CR; Young BA. 1987. Development and characteristics of a kleingrass population with reduced postharvest seed dormancy. Crop Science 27(6):1238-1241. doi: 10.2135/cropsci1987.0011183X002700060030x
- Tommasino E; Griffa S; Grunberg K; Ribotta A; López Colomba E; Carloni E; Quiroga M; Luna CM. 2012. Malondialdehyde content as a potential biochemical indicator of tolerant Cenchrus ciliaris L. genotypes under heat stress treatment. Grass and Forage Science 67:456-459. doi: 10.1111/j.1365-2494.2012.00851.x
- Vogel KP; Burson BL. 2004. Breeding and genetics. In: Moser LE; Burson BL; Sollenberger LE, eds. Warm-season (C₄) grasses. American Society of Agronomy, Madison, Wisconsin, USA. p. 51-94. doi: 10.2134/agronmonogr45.c1
- Vogel KP; Pedersen JF. 1993. Breeding systems for crosspollinated perennial grasses. Agronomy & Horticulture. Faculty Publications, University of Nebraska, Lincoln. 984. Plant Breeding Reviews 11. bit.ly/3rwIMvI
- Young BA. 1991. Heritability of resistance to seed shattering in kleingrass. Crop Science 31(5):1156-1158. doi: 10.2135/ cropsci1991.0011183X003100050015x

(Received for publication 9 October 2020; accepted 3 March 2021; published 31 May 2021)

© 2021



Tropical Grasslands-Forrajes Tropicales is an open-access journal published by International Center for Tropical Agriculture (CIAT), in association with Chinese Academy of Tropical Agricultural Sciences (CATAS). This work is licensed under the Creative Commons Attribution 4.0 International (CC BY 4.0) license.