Germplasm Evaluation and Frost Tolerance Improvement of *Setaria sphacelata* in Uruguay

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Introduction

Setaria sphacelata (Schumach.) Stapf & C.E. Hubb is a highly productive warm-season grass that has been evaluated in Uruguay since the 1970s (Mas 2007). It is one of the most productive and promising subtropical grasses for the Uruguayan climatic conditions. Some of the most remarkable attributes of interest are its palatability, ease to establish from seed, highly persistent, adapted to a wide range of soils, and some frost tolerance. Cultivars 'Narok' and 'Kazungula' were the only materials evaluated and no genetic improvement was conducted in Uruguay. These cultivars present some limitations, such as low seed yields and quality, and susceptibility to low temperatures. Looking to overcome these limitations, a germplasm collection from the USDA was introduced to Uruguay to explore the genetic variability and start a breeding program.

Materials and methods

Germplasm evaluation and selection

In November of 2008 three spaced-plant nurseries were installed at the experimental research stations of Palo a Pique, La Magnolia and Glencoe of the National Institute of Agricultural Research covering different agro ecological regions of Uruguay. In each nursery, 68 accessions introduced from the GRIN-USDA, and the cultivar 'Narok', were evaluated in a row-column experimental design with five replications and five plants per replication. During 2009 and 2010 every accession was evaluated for dry matter production, seed production, frost damage, winter survival, spring regrowth vigor, ploidy level, oxalate content, and morphological characterization. Dry matter production and regrowth in spring were made by visual score. To take this measure to grams per plant, plants were sampled from each score and an adjusted regression was applied. Seeds were harvested in a single-plant basis at an optimal time for each plant. The ploidy level of each accession was determined by flow cytometry at the Flow Cytometry Service (Institute for Biological Research Clemente Estable) using 'Narok' (tetraploid) as internal control. Oxalate content was determined by sampling leaves with 25 days of regrowth. Samples were oven dried and ground. The determination of oxalates was performed at the Laboratory of Industrial Quality Grain (INIA La Estanzuela), as described by Moir (1953). Data were analyzed using mixed models (SAS 2008), where repetition, row and column are random effects and accessions and sites as fixed effects. Main selection criteria were frost tolerance, winter survival, dry matter production, and seed production, resulting in 11 breeding lines (G1 to G11 in Table 1).

Evaluation of breeding lines

Breeding lines were established and evaluated in two sites (experimental research stations La Magnolia and Glencoe) in plots with a density of 25 plants per m^2 in a complete randomized block design with four replications per site. Cultivars 'Narok', 'Kazungula', 'Splenda', and 'Solander' were used as controls. Dry matter production and winter survival were measured during two growing seasons (2011-2012 and 2012-2013).

Results

Germplasm evaluation

Large variability was observed in several morphological features. The size of the leaves varied between 11.8 to 48.0 cm in length and from 0.5 to 1.4 cm in width. Generally, the color of panicle bristles and stigmas are used for cultivar description. We observed 31% of the plants with white bristles, 17% with yellow, 39% with orange, and 13% with brown. Smaller variation was observed for stigma colors where 95% were violet and 5% white. Regarding the ploidy level, four were diploids, 55 tetraploids, and the remaining 10 were mostly hexa- and octo-ploids. The oxalate contents also showed variation among accessions ranging 2.3-5.5%. Fifty-nine accessions (including 'Narok') had over 4%, which is considered the critical threshold (Jones and Ford 1972), and only 10 had lower oxalate contents. Dry matter and viable seeds production showed significant differences ($P<0.0001$) among accessions. For both traits, 10 accessions were better than 'Narok' which ranked 36 and 17 for dry matter and viable seeds production respectively. Significant differences ($P<0.0001$) among accessions were detected for frost tolerance during the first winter, where half of the accessions died.

Evaluation of breeding lines

The 11 breeding lines developed based on the selection criteria described above, showed significant differences for their dry matter and harvestable seed production during the first season of evaluation. The most important improvement was achieved for frost tolerance, where most of the breed-

Table 1. Winter survival (%) for the 11 breeding populations and cultivars 'Narok', 'Kazungula', 'Splenda', and 'Solander' evaluated in two sites after the first winter.

Breeding population	Winter survival (%)
'Kazungula'	76.9 a†
G9	73.3 ab
G6	73.0 ab
G5	72.7 ab
G8	71.0 ab
G3	69.1 ab
G2	66.4 abc
G1	65.4 abc
G10	65.0 abc
G4	64.3 abc
G7	58.4 bc
G11	50.0 c
'Splenda'	29.7 d
'Narok'	28.0 d
'Solander'	20.3 d

†Means with a common letter are not significantly different (P >0.05). Least significant difference Tukey = 18.36

ing lines showed much higher tolerance and winter survival than 'Narok', 'Splenda', and 'Solander' (Table 1). However, in these experiments 'Kazungula' showed similar winter survival than the breeding lines.

Conclusions

Large variability was observed for all the traits, and for most there were accessions with better performance than 'Narok', which is the only available cultivar in Uruguay. Breeding lines were created after cross-pollinating plants from these accessions pursuing different breeding goals. Preliminary information from the breeding lines showed that great improvement was achieved for winter survival, which directly affects the dry matter production and persistence of the plants. More information and evaluations are still needed to fully analyze the genetic gain achieved in several traits under different environments for the Uruguayan conditions.

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