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Evaluating General Combining Ability for Multiple Traits in Tetraploid Bahiagrass

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ABSTRACT

Recurrent selection based on combining ability has been successfully used in tetraploid bahiagrass (*Paspalum notatum* Flüggé) to accumulate heterotic effects and exploit hybrid vigor. However, its efficiency depends on an accurate selection of the best genotypes to form a new recombinant population. The objective of this work was to assess the general combining ability of female parents of bahiagrass based on the performance of their progeny for agronomic and morphological traits using a mixed model approach, biplot analysis and selection index. There were evaluated 29 half-sib families generated by crossing 29 sexual tetraploid genotypes from a sexual synthetic tetraploid population and a group of apomictic tetraploid genotypes. Agronomic and morphological traits were analyzed using a mixed model approach (BLUP). The multi-trait analysis was based on a biplot analysis and a selection index using the family BLUPs. BLUP analysis showed significant differences among families for most of the evaluated traits. Sexual female parents of families 5, 9, 8, 28, 21 and 16 were identified as those with greater general combining ability. Biplot showed variability among families and allowed identifying six sexual parents with greater general combining ability. The same sexual parents that exhibited greater general combining ability by BLUP were identified with greater general combining ability by biplot. Selection index was variable and allowed identifying the same best sexual parents that BLUP and biplot. The three analysis methods were equally effective to estimate general combining ability of a group of sexual parents of tetraploid bahiagrass based on the performance of their progeny.

Keywords: progeny test, general combining ability, BLUP, biplot analysis, selection index.
INTRODUCTION

The most important forage species used for beef cattle production systems in the tropics and subtropics reproduce asexually by apomixis (Jank et al. 2014). Among them the genera *Panicum*, *Urochloa*, *Paspalum* and *Cenchrus* (Moser et al. 2004) have the greatest economic value. Apomixis allows cloning superior genotypes by seed without loss of vigor or change in genotype through generations (Hanna and Bashaw 1987), which provides the opportunity to exploit heterosis.

The most popular breeding method in apomictic species has been direct selection from wild accessions. However, finding new accessions that combine multiple traits of interest is a limitation (Miles 2007). Hybridization arose as a new way to improve apomictic species once sexual plants with the same ploidy level of apomictic were found in nature or were artificially created (Vogel and Burson 2004). The main objective of this breeding approach is to release the natural diversity contained in apomictic ecotypes and fix superior F₁ hybrids (Miles 2007).

*Paspalum notatum* Flüggé, bahiagrass, is the primary constituent of rangelands of South America and it is cultivated as forage and turf around the world (Gates et al. 2004). In the United States, bahiagrass is the main pasture used in the beef cattle production systems of the Coastal Plain region (Blount and Acuña 2009). This species has tetraploids that reproduce by apomixis and diploids that reproduce sexually (Ortiz et al. 2013). The apomictic tetraploid genotype is the most abundant cytotype in nature (Gates et al. 2004). A sexual synthetic tetraploid population (SSTP) has been recently created and it was characterized by high phenotypic and genotypic diversity (Zilli et al. 2018; 2019). This new diverse sexual tetraploid germplasm opens the door to develop new breeding approaches at tetraploid level with the objective to exploit heterosis. A recent work reported that in tetraploid bahiagrass heterosis is mainly due to the accumulation of
dominant favorable alleles (Marcón et al. 2019), so identification of superior parents with contrasting dominant allele frequencies for traits of interest is crucial to exploit hybrid vigor.

Considering the importance of heterosis in apomictic species and that heterotic parents are required, Miles (2007) proposed recurrent selection based on combining ability to improve a sexual synthetic tetraploid population of *Brachiaria* by the accumulation of heterotic effects over selection cycles. And then obtain superior apomictic hybrids by crossing the improved sexual genotypes with apomictic ones. Based on the scheme proposed by Miles (2007), Marcón et al. (2020) carried out recurrent selection based on general combining ability to improve the SSTP of bahiagrass. This breeding approach consisted in a four-step process where (1) plants of the SSTP where crossed by a group of elite apomictic genotypes used as testers, (2) superior sexual parents were selected on the basis of the performance of their testcross progeny, (3) the selected sexual parents were polycrossed to form a recombinant superior population, and (4) sexual plants of the recombined population were crossed by superior apomictic plants.

The testcross progeny test was successful in improving tetraploid bahiagrass for forage yield since it allowed to obtain superior hybrid families by the accumulation of dominant favorable alleles (Marcón et al. 2020). However, the success of this method depends on the accuracy of selection of the best parents to form a new recombinant population (Miles 2007; Hallauer et al. 2010). Marcón et al. (2020) selected the best parents based on their general combining ability which was estimated by using analysis of variance. Nonetheless, this type of analysis considers the genotypes as fixed, so lot of information might be missed by unbalanced data (Little et al. 1996). Loss of information could lead to a wrong estimation which might reduce the selection efficiency.
In order to make a more efficient election of the best recombinant parents in recurrent selection schemes based on progeny test of apomictic forages, Worthington and Miles (2015) proposed to use best linear unbiased predictor (BLUP) to predict genetic effects such as general and specific combining ability. BLUP is a predictor of random effects and it is part of the mixed model approach that may be more informative than analysis of variance (Bernardo 2010). BLUP for estimating genetic parameters has been extensively used in sexual crops such as *Zea mays* (Bernardo 1999), however, in apomictic forage species there are few studies. In *Urochloa decumbens*, Matías et al. (2020) were able to estimate specific combining ability of a group of sexual genotypes using indexes based on BLUP predictions.

Considering the importance of the election of the best recombinant parents in recurrent selection methods, it would be of great interest to evaluate a more efficient way of selecting female parents with greater general combining ability in tetraploid bahiagrass. The objectives of the present study were to (i) evaluate agronomic and morphological traits of a half-sib progeny test of bahiagrass obtained by crossing sexual genotypes of the SSTP and a group of elite apomictic genotypes, and (ii) assess the general combining ability of female parents of bahiagrass based on the performance of their progeny for multiple traits using a mixed model approach, biplot analysis and selection index.

**MATERIALS AND METHODS**

**Plant material**

A group of 29 half-sib families of tetraploid *P. notatum* was used in this study. These families were generated by crossing 29 sexual tetraploid genotypes from a diverse sexual synthetic tetraploid population (SSTP) recently created (Zilli et al. 2018; 2019), and a group of five elite apomictic genotypes as described in Marcón et al. (2020). The 29 sexual genotypes were
previously selected from a group of 306 genotypes belonging to the SSTP based on their spring-
summer growth (Marcón et al. 2020). Four of the five apomictic genotypes used as male parents
(UF3, UF13, UF93 and UF122) were introduced from the University of Florida and the fifth is the
cultivar Boyero UNNE (Urbani et al. 2017). Half-sib progeny, 24 individuals per each of 29
families, were transplanted to a field near the city of Corrientes in November 2015, on 1-m centers
in a randomized complete block designed with three replications.

Agronomic and morphological evaluations

A group 12 of agronomic and morphological traits was measured in all half-sib families to
then select the best sexual parents based on their progeny behavior. Initial vigor was visually
estimated 45 days after transplanting, using a 1 to 5 scale, where 1 represented the plants exhibiting
the least aboveground vigor, and 5 represented the plants with the greatest vigor. Summer and
winter growth were estimated using the same 1-5 scale described above in April and August 2016,
respectively. Forage yield of all families was evaluated in November 2016 and March 2017 by
harvesting each family at 5-cm stubble height using a sickle bar mower. The fresh weight of the
harvested material was recorded, and a subsample was collected and dried at 60 °C for 48 h. The
dried subsample was weighed, and the dry weight of harvested biomass was calculated. Cold
tolerance was evaluated three days after the first frost event on June 2016, with the minimum
temperature reaching -2.4 °C, using a visual scale that varied from 1 to 5, where 1 represented the
least cold tolerant plant and 5 the most tolerant plant. Flowering index was estimated in April
2016 using a visual scale that ranged from 0 to 4, where 0 = plants without inflorescences, 1 = 1-
5 inflorescences per plant, 2 = 6-10 inflorescences per plant, 3 = 11-20 inflorescences and 4 =
more than 20 inflorescences per plant. Reproductive tiller/vegetative tiller ratio (RT/VT) was
estimated on April 2016 using a visual scale from 0 to 5, where 0 represented plants without
reproductive tillers or with fewer than 5 inflorescences, 1 represented a high RT/RV ratio and 5 represented a low RT/RV ratio and with more than 5 inflorescences. Plant height was measured from the base of the plant to the top of the canopy in October 2016. Leaf blade length (cm) was measured on the youngest fully expanded leaf and leaf blade width (mm) at the widest point of the leaf blade. Both leaf traits were evaluated in November 2016. Seed yield was evaluated in January 2017. For this, seed heads of each family were harvested by hand and dried at 30 ºC for 48 h. Dried seed heads were then manually threshed, and seeds were separated using a seed blower (Seedburo Equipment Company 1022W).

**Mixed model approach analysis and narrow-sense heritability**

Data from the half-sib family evaluations were analyzed using a mixed model approach (Piepho et al. 2008; Bernardo 2010) according to the following model:

\[ y_{ij} = \mu + f_i + b_j + e_{ij}, \]

where \( y_{ij} \) is the observation of the plot within block \( j \) that received the family \( i \); \( \mu \) is an overall constant associated with all observations; \( f_i \) is the random effect of family \( i \); \( f_i \sim N(0, \sigma_f^2) \); \( b_j \) is the fixed effect of block \( j \); and \( e_{ij} \) is the experimental error associated with \( y_{ij} \); \( e_{ij} \sim N(0, \sigma_e^2) \).

The parameters \( \sigma_f^2 \) and \( \sigma_e^2 \) refer to the variance components associated with family, and error, respectively.

The statistical model was fitted using the lme4 package (Bates et al. 2015) in the R software (R Core Team 2019). The predictions of the genetic values and standard errors of the 29 half-sib families of bahiagrass for all evaluated traits were estimated from the best linear unbiased predictor (BLUP) in order to identify the best female parents. The confidence intervals for these predictions based on t-statistics were performed at 0.05 significance level and plotted in a caterpillar plot.
(Wickham 2016). The variance components were estimated using the residual maximum likelihood (REML) method (Patterson and Thompson 1971) and their significance were assessed by the likelihood ratio test using the lmerTest R package (Kuznetsova et al. 2017).

Narrow sense heritability ($h^2$) on family-mean basis for a group of agronomic and morphological traits was calculated using the standard estimator as $\frac{\sigma_f^2}{\sigma_{ph}^2}$, where $\sigma_f^2$ is the genetic variance among half-sib families, which corresponds to $\frac{1}{4}$ of the additive genetic variance, and $\sigma_{ph}^2$ is the average phenotypic variance (Falconer and Mackay 1996). The analysis was carried out with R software (R Core Team 2019).

**Biplot analysis and correlation test**

A genotype by trait biplot analysis (Yan and Kang 2003) was performed based on average BLUPs of the 29 half-sib families for a group of agronomic and morphological traits that showed statistical differences in order to describe the female parent profile. The biplot graphical representations were plotted using the GGEBiplot R package (Dumble 2017).

Furthermore, the pairwise genetic correlations of the traits were estimated from the BLUPs of the families using the Pearson correlation coefficient, and graphical visualization of the correlation matrix was done using the corrplot R package (Taiyun and Viliam 2017).

**Selection index**

A selection index was created to combine all traits in a single value and to select the most promising families. A different weight was assigned to each trait based on their importance for the breeding programs. The greatest weights were assigned to cold tolerance (0.2) and winter growth and initial growth (0.15) since the main objective was to select plants able to expose green forage
during the cold season and start growing quickly after planting. The weight 0.1 was given to summer growth, spring forage yield and seed yield since the selected plants should grow rapidly during the hot season. Seed yield is also important for natural reseeding and commercialization. Plant height, leaf blade length and leaf blade width were assigned with the 0.05 coefficient since tall plants with long and wide leaves were expected to produce more forage with better nutritive value. The 0.025 coefficient was given to RT/VT ratio and flowering index by their importance in seed yield. Furthermore, the mean family BLUPs were scaled from 0 to 1 for each trait.

The value of the selection index for each family was calculated as follow:

\[ I_i = \sum_{k=1}^{12} w_k \bar{z}_{ik} = w_1 \bar{z}_{i1} + w_2 \bar{z}_{i2} + \cdots + w_{12} \bar{z}_{i12}, \]

where \( \bar{z}_{ik} \) is the scaled mean family BLUP of the \( k \)-th trait, and \( w_k \) is the weight associated with the \( k \)-th trait, as aforementioned. Therefore, the selection index varied from 0 to 1, where 1 represented the best family for all evaluated traits and 0 represented the worst family.

**RESULTS**

**Mixed model approach analysis and narrow-sense heritability**

Narrow sense heritability (\( h^2 \)) on family-mean basis varied from 0.56 to 0.86 (Fig 1). Flowering index and plant height showed the greatest values of \( h^2 \) (0.86 and 0.83, respectively) whereas the RT/VT ratio and winter regrowth exhibited the lowest values (0.56 and 0.65, respectively).

The BLUPs of the families and their prediction confidence intervals showed that some of those were statistically superior to the mean for all evaluated traits (Fig 2), except for summer forage yield. For cold tolerance, families 21, 28, 5, 25 and 29 were statistically over the mean. Families 17, 25, 16, 14, 27, 2, 3, and 29 exceeded flowering index mean value. Regarding initial
growth, families 8, 16 and 17 were statistically over the mean (Fig 2). Families 21, 5, 8, 28, 29, 1 and 9 were statistically over the mean leaf blade length, meanwhile families 18, 16, 3, 19 and 22 were over the mean leaf blade width. For plant height, families 8, 9, 21, 5, 15 and 28 exceeded statistically the mean. Families with greater RT/VT ratio were 5, 9 and 21. Regarding seed yield and summer forage yield, just two families were over the mean value (Fig 2). Families 9, 5, 16 and 8 exhibited the greater summer growth values. For winter growth, families 9, 1 and 21 were statistically over the mean (Fig 2).

Biplot analysis and correlation test

Analyzing the biplot obtained using the mean BLUP values of each family, high variability among families was observed and that all traits contributed almost equally to this variation, except for seed yield (Fig 3). Family 9 and 16 stood out for their high summer growth and RT/VT and flowering index and leaf blade width, respectively. While the families 21, 5, 8 and 28 exhibited good values for winter growth, cold tolerance, plant height, leaf blade length and spring forage yield (Fig 3). However, no multivariate pattern defines the best family for all the traits simultaneously. Combining BLUP and biplot analysis, sexual parents of families 9, 5, 8, 21, 16 and 28 exhibited greater general combining ability.

In addition, most of the genetic correlations between the traits were positive (Fig 4). The greatest positive values were between leaf blade length and plant height (0.85), initial growth and summer growth (0.85), RT/RV and summer growth (0.83), plant height and winter growth (0.82), spring forage yield and leaf blade length (0.79), spring forage yield and plant height (0.78), plant height and RT/RV (0.78), RT/RV and winter growth (0.73), plant height and cold tolerance (0.73) and leaf blade length and RT/RV (0.73) (Fig 4).
Selection index

A selection index was constructed to combine all evaluated traits into a single value so as to weigh all families taking into account all traits. Selection index values ranged from 0.18 to 0.82 among families, and 12 families exhibited values over 0.5 (Fig 5). The families with greatest values were 9, 5, 28, 8, 21, 25, 16, 1, 27 and 29 (Fig 5). Based on BLUP analysis, biplot and selection index, the sexual parents of families 9, 5, 8, 28, 21, and 16 exhibited greatest general combining ability.

DISCUSSION

New breeding schemes such as recurrent selection based on general combining ability have been successfully used in improving tetraploid bahiagrass. However, one of the challenges of these methods is efficiency to select superior parents to form a new recombinant population. For this reason, we employed the BLUP procedure in order to estimate accurately the general combining ability of sexual parents based on their progeny performance.

BLUP is a predictor of random effects with desirable properties for plant breeding purposes and it is part of the mixed model approach (Bernardo 2010). This analysis has been successfully used in major crops, such as maize, to select superior parents based on the performance of their progeny and continue with the breeding process (Bernardo 1999). In single crosses of *Urochloa humidicola*, an apomictic forage species, this analysis allowed identifying the most promising sexual and apomictic parents based on their general combining ability for forage yield and quality (Figueiredo et al. 2019). In this study which consisted of a progeny test belonging to a recurrent selection scheme of tetraploid bahiagrass, the mixed model analysis showed significant differences among families for most of the evaluated traits allowing identification of the best families for each
trait (Fig 2). No family stood out for all traits. However, it was possible to select six families that showed higher BLUP values for the most important traits in forage breeding. These results suggest that in apomictic forage breeding, the use of mixed model analysis for identifying superior parents or families was successful. On the other hand, in this work, cold tolerance, winter growth, initial growth, spring forage yield, summer growth and seed yield were considered the most important traits. In warm-season grasses biomass production is limited by short days and cold temperatures (Moser et al. 2004) so cold tolerance and winter growth were considered important. A good forage also must establish quickly after sowing or planting and grow rapidly during the growing season (Burton 1986). Thus, initial growth, spring forage yield and summer growth were among the most important forage characteristics. Another important aspect in forage breeding is cultivar increase or reseeding (Burton 1986) so seed yield played an important role. All these traits showed a large variation among families (Fig 2) and also exhibited a medium to high narrow-sense heritability (Fig 1) which means that it would be possible to improve tetraploid bahiagrass for those traits.

The same progeny test used in this work was previously evaluated by Marcón et al. (2020) with the aim of identify the best sexual parents based on their general combining ability. They only evaluated seasonal forage yield and estimated the general combining ability based on means comparisons by analysis of variance. Comparing the number of families selected in this study by BLUP and those selected by Marcón et al. (2020) it was observed that using BLUPs a smaller number of families were selected. This might be related with the number of traits evaluated since in this work 12 variables were evaluated, while Marcón et al. (2020) only tested seasonal forage yield. In addition, BLUP considers the genotypes as random effects while analysis of variance as fixed so lot of information might have been missed and resulted in a less efficient selection.
A biplot analysis was also conducted to combine all traits in a single analysis and make a more precise selection (Fig 3). Biplot did not show a clear pattern of the best families for all traits. Nonetheless, it allowed selecting six families that highlighted for some of the most important forage traits. This analysis was also effective in a recurrent selection based on specific combining ability scheme of *Urochloa decumbens*, where it allowed to select the best sexual parents for agronomic and nutritional traits (Matías et al. 2020). These data are indicating that in apomictic species the use of multivariate analysis based on BLUP were successful to identify sexual parents with greater general or specific combining ability based on their progeny behavior.

In addition, a selection index was carried out to combine all traits in a single value by assign different weight to each trait based on their importance for the breeding program. In this work, this selection tool was effective since it allowed us to select the 10 sexual parents that exhibited greater general combining ability based on their progeny behavior. It was also effective to estimate specific combining ability of a group of sexual genotypes of *Urochloa decumbes* taking into account a group of agronomic and nutritive traits (Matías et al. 2020). These results are indicating that selection index based on mean BLUP might be use as a tool to estimate general and specific combining ability in recurrent selection schemes.

Analyzing data generated by BLUP, biplot and selection index, it was observed that the three methods allowed detecting the same superior female parents, which may be demonstrating that these three analysis were successful to estimate general combining ability in tetraploid bahiagrass. In addition, the joint analysis tool allowed to increase the efficiency of the selection of the best parents which might be because the multivariate approaches taking account more properly the correlation between traits.
There were high positive correlations among plant height and leaf blade length with agronomic characteristics such as winter growth, cold tolerance, spring forage yield and RT/VT (Fig 4). Zilli et al. (2019) also found positive correlations among plant height and leaf blade length and between plant height and winter regrowth in the sexual synthetic tetraploid population of bahiagrass. However, in this study the correlation values were higher. Furthermore, these traits exhibited a high narrow-sense heritability (Fig 4), which means that plant height and leaf blade length may be use to select indirectly plants that tolerate and growth during the winter season, the most critic growing period for *Paspalum* species (Acuña et al. 2011; Marcón et al. 2018). In addition, taller plants with long leaves are related to an erect growth habit which is associated with greater biomass production (Acuña et al. 2011), so selecting on both traits may also result in an increase in forage yield.

In conclusion, BLUP, biplot and selection index were successful in estimating general combining ability of a group of sexual parents of tetraploid bahiagrass based on the performance of their progeny. The three methods were equally effective since they identified the same best female parents that could be used to continue with the breeding program. The joint use of those methods allowed identifying in a more efficient way sexual parents with greater general combining ability, so in apomictic species a more accurate selection may be carried out by using more than one analysis. High positive correlated traits with high narrow-sense heritability may be indicating that it is possible to select some traits indirectly by recurrent selection methods such as recurrent selection based on combining ability.

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Fig 1 Narrow sense heritability ($h^2$) of a group of agronomic and morphological traits from 29 families of *P. notatum* obtained by crossing sexual tetraploid genotypes from a sexual synthetic tetraploid population and a group of apomictic tetraploid genotypes.
Fig 2 Prediction of genotypic values of 29 half-sib families of *P. notatum* obtained by crossing 29 sexual genotypes from a sexual synthetic tetraploid population and a group of apomictic tetraploid genotypes for 12 agronomic and morphological traits using BLUP (best linear unbiased prediction). The vertical red line is the mean for each trait.
Fig 3 Biplot for agronomic and morphological traits assessed in 29 half-sib families of *P. notatum* obtained by crossing 29 sexual genotypes from a sexual synthetic tetraploid population and a group of apomictic tetraploid genotypes. The axes represent the first and second principal components.
**Fig 4** Pearson correlation values between 11 agronomic and morphological traits evaluated in 29 half-sib families of *P. notatum*.

**Fig 5** Selection index based on 11 agronomic and morphological traits in 29 half-sib families of *P. notatum*. 