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**Forage crops** Full-length research article

# Multivariate analysis reveals genetic diversity in *Paspalum notatum* Flügge

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**ABSTRACT** - The objective of this study was to evaluate 94 *Paspalum notatum* genotypes over two growing seasons to estimate genetic dissimilarity through agronomic traits and the distance between genotypes. This information is used to create an ideotype from the best averages obtained for the set of characteristics evaluated. Seven apomitic, three sexual, and 81 hybrid genotypes were compared with native genotypes "André da Rocha", "Bagual", and cultivar "Pensacola" as controls. There is genetic variability in *P. notatum* for the studied characteristics, and distinct genotypes with superior characteristics can be used in new combinations between apomictic and sexual plants to obtain hybrids. The characters with the greatest relative contribution to the dissimilarity between the genotypes were tiller density, stem dry mass, and leaf dry mass yield. Thus, these characteristics are suitable criteria to infer genetic distance studies in *P. notatum*. The selection index based on the ideotype is an auxiliary tool in the breeding process. The ideotype must be based on characteristics of interest according to the objective of the breeding program, as well as on the breeder's prior knowledge in relation to culture.

Keywords: cluster analysis, intraspecific hybrids, plant breeding

# 1. Introduction

In South America, grasses of the genus *Paspalum* are widely used as livestock feed, with a large number of species exhibiting high yield potential and nutritional quality, heterogeneity arising from the different modes of reproduction, and differing ploidy levels within the genus (Sartor et al., 2011). In the southern region of Brazil, these species are constituents of the natural grasslands of the Pampa biome and have recognized forage potential (Pereira et al., 2012; Steiner et al., 2017).

*Paspalum notatum* Flügge stands out among other species because it has genotypes that have superior forage attributes for feeding ruminants. Knowledge of genetic variability and diversity within a species is essential for use of the genetic resource (Govindaraj et al., 2015). This species has a great potential for use in breeding programs as the species has a wide genetic diversity (Fachinetto et al., 2017), and the native genotypes show superior performance to the commercial cultivar "Pensacola", which is the only commercially available cultivar and is widely grown in the southern

region of the country (Steiner et al., 2017; Machado et al., 2019). New cultivars of *P. notatum*, with high production and adapted to edaphoclimatic conditions, enable more profitable and sustainable livestock production as they can be used in the recovery of degraded natural pasture areas or directly as cultivated perennial pastures (Machado et al., 2019).

Genetic variability is important for the selection of desirable traits and for breeding (Azimi et al., 2018). For these authors, the first step in plant breeding is phenotypic and genotypic characterization, aiming to access new genetic alterations and use them in breeding programs. Thus, the estimate of genetic distance between genotypes can be used to predict variability (Hosan et al., 2010) and, accompanied by the use of multivariate statistics, it allows grouping the genotypes with similar characteristics (Streck et al., 2017; Chiomento et al., 2021). The main objective of this study was to evaluate genotypes of *P. notatum* in two growing seasons to estimate genetic dissimilarity through agronomic traits and the distance between genotypes of *P. notatum*. This information is used to create an ideotype based on the best averages obtained for the set of characteristics evaluated.

# 2. Material and Methods

The study was carried out in Eldorado do Sul, Rio Grande do Sul, Brazil (latitude 30°29'26" S, longitude 51°06'42" W, altitude 62 m a.s.l.). The local climate is classified as (Cfa) according to the Köppen classification (Moreno, 1961): subtropical with no defined dry season and temperature of the hottest month above 22 °C. The average minimum and maximum annual temperatures in the region are 14.0 and 24.2 °C, respectively, and the annual average temperature is 19.6 °C. The average annual rainfall is 1398 mm. The soil is classified as an Ultisol (USDA Soil taxonomy). Prior to experiment establishment, soil samples (0-0.2 m) were collected and presented the following chemical characteristics: clay = 15%; pH (H<sub>2</sub>O) = 5.4; SMP index = 6.3; P (mg dm<sup>-3</sup>) = 15.6; K (mg dm<sup>-3</sup>) = 151.4; organic matter = 2.7%.

A completely randomized block design with four replications was established at the experimental station. There was a total of 94 genotypes of *P. notatum*. Clones were transplanted into the field with a spacing of 1 m in and between rows. Three of the genotypes were tetraploid sexual genotypes sourced from the Instituto de Botánica del Nordeste (IBONE - UNNE), Corrientes, Argentina, named "C44X" (Quarin et al., 2001), "Q4188", and "Q4205" (Quarin et al., 2003). Seven tetraploid apomitic genotypes ("30N", "36N", "48N", "70N", "83N", "95N" and "V4") were sourced from the United States Department of Agriculture (USDA) from collections made in South America. Additionally, the native "André da Rocha" and "Bagual" genotypes were evaluated (collected in the 1980s - Rio Grande do Sul, Brazil), and the only commercially available cultivar was Pensacola (Table 1).

In addition, 81 hybrids were evaluated (Table 2), result of crosses between sexual plants – females (coming from IBONE - UNNE) and apomictic genotypes – males (coming from the USDA germplasm bank), according the methodology described by Burton (1948), adapted by Weiler et al. (2018). The genotypes from USDA used in these crosses were selected in a preliminary evaluation for forage production (Fachinetto et al., 2012). The F1 seeds were germinated in petri dishes containing Germest paper, then seedlings were transferred to speedling trays containing commercial substrate when they presented the first fully expanded leaf. After tillering, four tillers were separated and planted in pots, obtaining four clones. The experimental area was fertilized with nitrogen, phosphorus, and potassium, according to the technical indications for perennial grasses of hot season, following the recommendations of the CQFS (2004). Urea was applied at a rate equivalent to 160 kg N ha<sup>-1</sup> (CQFS, 2004).

During the first growing season, two evaluations were carried out (03/15/2013 and 04/26/2013) and in the second growing season, four evaluations (11/12/2013, 12/17/2013, 01/09/2014, and 02/02/2014). The plants were cut to a residual height of 5 cm when they reached an average height of 20 cm. Material was separated into leaves (leaf blades), stems (stems and sheaths), and inflorescences, then placed in a forced-air oven at 60 °C, until constant weight.

| Genotype       | Origin                                    | Ploidy level |  |
|----------------|---|--------------|--|
| C44X           | Corrientes – Argentina                    | Tetraploid   |  |
| Q4188          | Corrientes – Argentina                    | Tetraploid   |  |
| Q4205          | Corrientes – Argentina                    | Tetraploid   |  |
| 30N            | Santa Fé – Argentina                      | Tetraploid   |  |
| 36N            | Santa Fé – Argentina                      | Tetraploid   |  |
| 48N            | Mercedes – Argentina                      | Tetraploid   |  |
| 70N            | Córdoba – Argentina                       | Tetraploid   |  |
| 83N            | Corrientes – Argentina                    | Tetraploid   |  |
| 95N            | Corrientes – Argentina                    | Tetraploid   |  |
| V4             | Barra do Quaraí/RS – Brazil               | Tetraploid   |  |
| André da Rocha | André da Rocha/RS – Brazil                | Tetraploid   |  |
| Bagual         | Região das Missões/RS – Brazil Tetraploid |              |  |
| Pensacola      | Viamão/RS – Brazil                        | Diploid      |  |

**Table 1** - Identification and ploidy levels of the genotypes used in the study of genetic diversity in *Paspalum* notatum collection

**Table 2** - Identification of intraspecific hybrids of Paspalum notatum

| Progeny | Pare  | No. of hybrids |    |
|---------|-------|----------------|----|
| A       | C44X  | 30N            | 1  |
| В       | C44X  | 36N            | 9  |
| 2       | C44X  | 70N            | 1  |
| )       | C44X  | 83N            | 6  |
| Е       | C44X  | 95N            | 4  |
| F       | Q4188 | 30N            | 11 |
| G       | Q4188 | 70N            | 1  |
| H       | Q4188 | 83N            | 9  |
| [       | Q4188 | 95N            | 11 |
| ſ       | Q4188 | V4             | 2  |
| К       | Q4205 | 36N            | 7  |
|         | Q4205 | 95N            | 14 |
| М       | Q4205 | V4             | 5  |

The measured characteristics were: plant height (PH, cm), tiller population density (TPD, tillers plant<sup>-1</sup>), accumulated total dry mass (ATDM, kg DM plant<sup>-1</sup>), accumulated leaf dry mass (ALDM, kg DM plant<sup>-1</sup>), accumulated stem dry mass (ASDM, kg DM plant<sup>-1</sup>), and accumulated inflorescence dry mass (AIDM, kg DM plant<sup>-1</sup>). Plant height was measured before each observation from soil level to the average bend of the leaves. To determine the TPD, the total number of tillers per plant was counted. The ATDM, ALDM, ASDM, and AIDM are the total accumulated DM of each component across the entire evaluation period. The leaf:stem ratio (LSR) was calculated using the quotient between the ALDM and ASDM.

Initially, analysis of variance (ANOVA) and F test were conducted. We used the following statistical model (Equation 1):

$$Y_{ii} = \mu + G_i + B_i + e_{ii}, \tag{1}$$

in which  $Y_{ij}$  is the observed value of the *i*-th genotype in the *j*-th block,  $\mu$  is the general mean;  $G_i$  is the effect of the *i*-th genotype,  $B_j$  is the effect of the *j*-th block, and  $e_{ij}$  is the experimental error associated

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with the  $Y_{ij}$  observation. The normality of errors was verified by the Shapiro-Wilk test (Shapiro and Wilk, 1965).

The correlation coefficients were expressed by the genotypic correlations in Equation 2:

$$r_{g(XY)} = \frac{COV_{g(XY)}}{\sqrt{\sigma_{g(X)}^2 \cdot \sigma_{g(Y)}^2}},$$
(2)

in which  $COV_{g(XY)}$  is the genetic covariance between the traits X and Y,  $\sigma_{g(X)}^2$  is the genetic variance of trait X, and  $\sigma_{g(Y)}^2$  is the genetic variance of trait Y.

To graphically express the functional relationship between the correlation estimates of the characteristics, the proximity between them was proportional to the absolute value between their correlations, performed through the Rbio software (Bhering, 2017). The thickness of the edges was controlled by applying a cut-off value of 0.50, which meant that only | rij |  $\geq$  0.50 had their edges highlighted. Finally, positive correlations were represented in blue, while negative correlations were represented in red.

Subsequently, a multivariate analysis was performed, generating the Mahalanobis Distance matrix (D<sup>2</sup>), and a cluster analysis, used to assess the genetic variability performed by Tocher's method (Rao, 1952). The quantification of the relative contribution of the characters (S.j) was obtained according to Singh (1981).

To assist the recommendation of crosses between genotypes based on the magnitude of the genetic distance, an index was used in relation to the ideotype (ideal genotype proposed by the breeder) based on the best behaviors verified in each character evaluated in the present study. The ideotype was based on the highest averages for the characters ATDM, ALDM, AIDM, LSR, PH, and TPD and on the lowest mean for the ASDM characteristic. Mahalanobis distances were then estimated between the 94 genotypes studied and the created ideotype. In this way, the genotypes were classified according to the distance from the ideotype with the best genotypes being those that had the shortest distances from the ideotype (Bertan et al., 2006) with GENES software (Cruz, 2016).

## **3. Results**

The univariate analysis of variance for agronomic characteristics (Table 3) showed genotypic differences for all variables analyzed. The significance of the genotype effect indicated the presence of genetic variability and indicates that is possible to include these characters in genetic distance studies. The variation coefficients ranged from a minimum of 1.06% (TPD) to a maximum of 19.41% (LSR), demonstrating experimental precision.

The genetic correlation was determined for the genotypes and is presented through the correlation network analysis (Figure 1). The correlation coefficients ranged from 0.27 (AIDM and PH) to 0.96 (ATDM and ALDM). The characters ALDM, ASDM, AIDM, TPD, and PH, which are the primary components of forage production, showed high genotypic correlations of 0.96 (P $\leq$ 0.01), 0.91 (P $\leq$ 0.01), 0.77 (P $\leq$ 0.01), 0.66 (P $\leq$ 0.01), and 0.52 (P $\leq$ 0.01) with ATDM, respectively. These results show that, for the genotypes analyzed, the characteristics ALDM, ASDM, AIDM, AIDM, TPD, and PH are important characters for future breeding of *P. notatum* to increase ATDM.

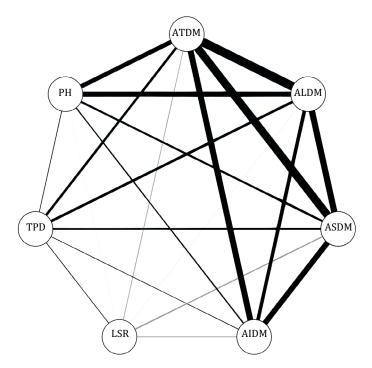
The relative contribution of quantitative traits to the genetic dissimilarity of genotypes (Figure 2) according to Singh's method (1981) showed that TPD, ASDM, and ALDM were the ones most discriminated against within the group of genotypes analyzed, and accounted for 66.9, 16.9, and 10.8% of the observed variation, respectively. In addition, these characteristics were strongly correlated with ATDM and accounted for >94.0% of the total discrimination of these genotypes.

The cluster analysis by the Tocher's method (Table 4) formed twelve distinct groups. The large number of groups is indicative of the great variability that exists between the studied genotypes. Group 1 had 48 genotypes (51.06%) and group 3 had 20 genotypes (21.3%), the other 10 groups

| Source of variation | DF  | Mean square  |           |           |          |  |
|---------------------|-----|--------------|-----------|-----------|----------|--|
| Source of variation | DF  | ATDM         | ALDM      | ASDM      | AIDM     |  |
| Blocks              | 3   | 5718.34      | 670.57    | 480.39    | 429.00   |  |
| Genotypes           | 93  | 53537.78*    | 19893.40* | 5816.12*  | 1351.20* |  |
| Residual            | 279 | 54.48        | 11.53     | 2.90      | 1.40     |  |
| Minimum             | -   | 10.4         | 8.7       | 1.3       | 0.2      |  |
| Maximum             | -   | 589.0        | 349.2     | 183.0     | 77.0     |  |
| Mean                | -   | 185.18       | 109.13    | 50.57     | 26.14    |  |
| P-value             | -   | 0.0001       | 0.0001    | 0.0001    | 0.0001   |  |
| CV (%)              | -   | 3.99         | 3.11      | 3.37      | 4.52     |  |
|                     |     | Mean square  |           |           |          |  |
|                     |     | LSR          | РН        | TI        | PD       |  |
| Blocks              | 3   | 5.88         | 217.14    | 798.12    |          |  |
| Genotypes           | 93  | 7.52*        | 97.69*    | 11580.55* |          |  |
| Residual            | 279 | 0.26         | 0.27      | 1.21      |          |  |
| Minimum             | -   | 0.50         | 6.70      | 17.00     |          |  |
| Maximum             | -   | 10.10        | 33.00     | 263.00    |          |  |
| Mean                | -   | 2.62 18.23 1 |           | 103       | 3.82     |  |
| P-value             | -   | 0.0001       | 0.0001    | 0.0001    |          |  |
| CV (%)              | -   | 19.41        | 2.86      | 1.        | 06       |  |

#### Table 3 - Summary of the analysis of variance for the characters studied in Paspalum notatum

ATDM - accumulated total dry mass (kg of DM plant<sup>-1</sup>); ALDM - accumulated leaf dry mass (kg of DM plant<sup>-1</sup>); ASDM - accumulated stem dry mass (kg of DM plant<sup>-1</sup>); AIDM - accumulated inflorescence dry mass (kg of DM plant<sup>-1</sup>); LSR - leaf:stem ratio; PH - plant height (cm); TPD - tiller population density (tillers plant<sup>-1</sup>); DF - degree of freedom. \* Significance: P<0.05.



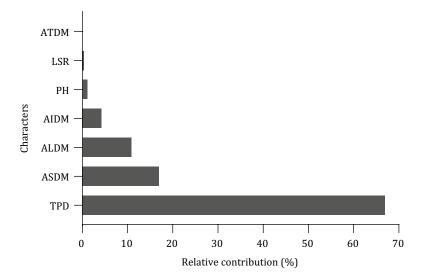
PH - plant height (cm); TPD - tiller population density (tillers plant<sup>-1</sup>); ATDM - accumulated total dry mass (kg of DM plant<sup>-1</sup>); ALDM - accumulated leaf dry mass (kg of DM plant<sup>-1</sup>); ASDM - accumulated stem dry mass (kg of DM plant<sup>-1</sup>); AIDM - accumulated inflorescence dry mass (kg of DM plant<sup>-1</sup>); LSR - leaf:stem ratio.

Figure 1 - Genotypic correlation network between seven agronomic characters of 94 genotypes of Paspalum *notatum* collection.

consisted of the remaining 26 genotypes, making up 27.7%. Therefore, it is possible to verify the presence of genetic variability between genotypes.

Groups 1 and 3 obtained low values for the characters TPD (71 and 138 tillers plant<sup>-1</sup>) and ALDM (72 and 123 kg DM plant<sup>-1</sup>), in addition to intermediate ASDM (33 and 47 kg DM plant<sup>-1</sup>), respectively. Group 4 included 42.8% of the male parents used in the crossings to obtain the hybrids and obtained higher values than groups 1 and 3 for characters TPD (181 tillers plant<sup>-1</sup>), ALDM (216 kg DM plant<sup>-1</sup>), and ASDM (91 kg DM plant<sup>-1</sup>). Forage breeding programs aim for genotypes with high ALDM and TPD and low ASDM.

For this experiment, the *P. notatum* ideotype was based on desirable forage characteristics that a plant must have, such as high ATDM, ALDM, AIDM, LSR, PH, and TPD. Although the genotypes showed little



PH - plant height (cm); TPD - tiller population density (tillers plant<sup>-1</sup>); ATDM - accumulated total dry mass (kg of DM plant<sup>-1</sup>); ALDM - accumulated leaf dry mass (kg of DM plant<sup>-1</sup>); ASDM - accumulated stem dry mass (kg of DM plant<sup>-1</sup>); AIDM - accumulated inflorescence dry mass (kg of DM plant<sup>-1</sup>); LSR - leaf:stem ratio.

**Figure 2** - Relative contribution, according to Singh (1981), of the characters to (di)similarity between the *Paspalum notatum* genotypes.

| Group | Genotype   |  |  |  |  |  |
|-------|--|--|--|--|--|--|
| 1     | 212, 315, 521, 215, 425, 1036, 115, 1021, 736, 226, 712, 612, 625, 114, 512, 825, 812, 936, 232, 325, Q4205, 626, 721, 83N, 415, 312, 1121, 912, 416, 124, 1026, 426, 126, 326, 412, 112, 732, 421, 1636, 526, 826, 214, 537, PENSACOLA, 1126, BAGUAL, 925, C44X |  |  |  |  |  |
| 2     | 532, 615, 632, 1136  |  |  |  |  |  |
| 3     | 225, 921, Q4188, 821, 836, 125, 237, 436, 432, 536, 111, 36N, 121, ANDRE DA ROCHA, 636, 926, 136, 621, 321, 337  |  |  |  |  |  |
| 4     | 221, V4  |  |  |  |  |  |
| 5     | 116, 316, 515, 70N, 236, 227   |  |  |  |  |  |
| 6     | 48N, 95N, 132, 30N   |  |  |  |  |  |
| 7     | 137, 216, 122  |  |  |  |  |  |
| 8     | 725, 726   |  |  |  |  |  |
| 9     | 332, 336   |  |  |  |  |  |
| 10    | 437  |  |  |  |  |  |
| 11    | 127  |  |  |  |  |  |
| 12    | 525  |  |  |  |  |  |

**Table 4** - Groups formed by the Tocher optimization method of 94 genotypes of *Paspalum notatum*, based on the<br/>Mahalanobis distance matrix (D<sup>2</sup>)

variability in PH, ATDM correlated in an intermediate way with PH, which allowed the inclusion of this characteristic in the construction of the ideotype. In contrast, high ASDM is an unwanted characteristic that breeding programs aim to reduce.

The genotypes with intermediate characters relative to the others evaluated by the Tocher grouping method were those that were closest to the *P. notatum* ideotype. In the ranking (Table 5), the genotypes with the shortest distance in relation to the ideotype (1st, 122; 2nd, 132; 3rd, 137; 4th, 216; 5th, 725) are indicated as they are most likely to advance the breeding program.

Due to the large number of genotypes in the present study, the 10 most promising genotypes included hybrids 122, 127, 132, 137, 216, 332, and 725 and genotypes 36N, 48N, and 95N from the USDA.

In the present study, in addition to some of the hybrids showing superiority in relation to the others, analysis highlighted that the female parents Q4188 and Q4205 were responsible for 42.8% of superior hybrids identified that approached the stipulated ideotype.

| Genotype          | Distance | Ranking | Genotype | Distance | Ranking | Genotype  | Distance | Ranking |
|-------------------|----------|---------|----------|----------|---------|-----------|----------|---------|
| 122               | 5190.5   | 1st     | 226      | 22871.1  | 33rd    | 912       | 35011.4  | 65th    |
| 132               | 7606.6   | 2nd     | 736      | 22941.4  | 34th    | 83N       | 35396.4  | 66th    |
| 137               | 8014.6   | 3rd     | V4       | 24029.0  | 35th    | 325       | 35792.3  | 67th    |
| 216               | 8307.2   | 4th     | 337      | 24091.6  | 36th    | 1026      | 36188.1  | 68th    |
| 725               | 8404.2   | 5th     | 1036     | 24271.8  | 37th    | 236       | 36335.9  | 69th    |
| 127               | 9534.9   | 6th     | 115      | 25965.9  | 38th    | 626       | 37040.9  | 70th    |
| 332               | 10661.0  | 7th     | 215      | 25994.4  | 39th    | 1121      | 38652.0  | 71st    |
| 48N               | 11232.4  | 8th     | 521      | 26241.0  | 40th    | 416       | 38753.7  | 72nd    |
| 95N               | 11440.7  | 9th     | 116      | 27201.8  | 41st    | Bagual    | 39183.2  | 73rd    |
| 36N               | 12298.0  | 10th    | 1021     | 27626.0  | 42nd    | 732       | 39323.6  | 74th    |
| 726               | 12439.7  | 11th    | 712      | 27665.5  | 43rd    | 412       | 40014.9  | 75th    |
| 926               | 12495.5  | 12th    | 212      | 28025.6  | 44th    | Pensacola | 40085.6  | 76th    |
| 321               | 12789.8  | 13th    | 612      | 28279.5  | 45th    | 70N       | 40245.5  | 77th    |
| 436               | 14416.5  | 14th    | 316      | 28849.2  | 46th    | 426       | 40503.4  | 78th    |
| 225               | 14418.7  | 15th    | 936      | 28983.7  | 47th    | 126       | 40837.3  | 79th    |
| Q4188             | 14890.3  | 16th    | 315      | 29018.31 | 48th    | 537       | 41630.8  | 80th    |
| 821               | 15704.2  | 17th    | 721      | 29318.68 | 49th    | 437       | 42018.7  | 81st    |
| 125               | 15767.9  | 18th    | 421      | 29344.7  | 50th    | 326       | 42061.3  | 82nd    |
| 237               | 15906.6  | 19th    | 227      | 29553.1  | 51st    | 1636      | 42585.3  | 83rd    |
| 921               | 16366.9  | 20th    | 415      | 29569.8  | 52nd    | 112       | 43136.7  | 84th    |
| 111               | 16670.9  | 21st    | 425      | 30901.8  | 53rd    | 214       | 43940.1  | 85th    |
| 30N               | 16767.3  | 22nd    | 812      | 31017.1  | 54th    | 826       | 45780.3  | 86th    |
| 636               | 16870.2  | 23rd    | 312      | 31111.4  | 55th    | 526       | 46274.1  | 87th    |
| 836               | 17663.1  | 24th    | 512      | 31834.0  | 56th    | 1126      | 47693.5  | 88th    |
| 136               | 18450.1  | 25th    | 625      | 31921.4  | 57th    | C44X      | 48029.2  | 89th    |
| 221               | 19833.7  | 26th    | 515      | 31965.8  | 58th    | 925       | 48272.9  | 90th    |
| 336               | 19854.8  | 27th    | 825      | 32047.7  | 59th    | 632       | 50267.9  | 91st    |
| André da<br>Rocha | 20574.2  | 28th    | Q4205    | 32473.5  | 60th    | 615       | 52029.2  | 92nd    |
| 432               | 21086.7  | 29th    | 114      | 32877.7  | 61st    | 1136      | 52499.8  | 93rd    |
| 536               | 21326.3  | 30th    | 525      | 33362.5  | 62nd    | 532       | 53183.2  | 94th    |
| 621               | 21852.8  | 31st    | 232      | 33629.9  | 63rd    |           |          |         |
| 121               | 21889.8  | 32nd    | 124      | 34840.1  | 64th    |           |          |         |

Table 5 - Index selection based on distance from the ideotype of the 94 genotypes of Paspalum notatum

# 4. Discussion

Studies in the genus *Paspalum*—Grupo Plicatula (Huber et al., 2016; Motta et al., 2017)—and *P. notatum* (Weiler et al., 2018; Machado et al., 2019) confirm the high correlation between ATDM and ALDM. This suggests that the laborious work of separating the morphological components can be avoided, saving time and resources. According to Carvalho et al. (2004), genetic correlations are important in simultaneous selection for more than one characteristic, and the magnitude determines the direction of the correlated response of an association of an inheritable nature. The causes of genetic correlations are mainly pleiotropism and gene connections (Falconer and Mackay, 1996).

Multivariate analysis techniques can be used to assess the presence of genetic variability in a group of genotypes, allowing to select descriptors with greater potential for discrimination between genotypes in a germplasm bank (Azevedo et al., 2011), as well as the simultaneous use of several characters for the selection of the best genotypes. Thus, it allows breeders to identify favorable genetic combinations before crosses are carried out (Nardino et al., 2017). To verify the proximity between the genotypes of *P. notatum*, group analysis was performed, and because the composition of groups is important in the selection of genotypes, new combinations can be based on the magnitude of the dissimilarity between the genotypes.

The characteristics with the greatest relative contribution are those related to the higher nutritional quality of the forage plant, with emphasis on leaf blades, which is preferentially grazed by livestock (Hodgson, 1990). In grasses, a tiller constitutes the primary growth unit, consequently impacting forage production (Volenec and Nelson, 1983). Selection to reduce stem production in the improvement of forages (Pereira et al., 2011) increases total feed quality, because the contribution of high fiber stem material is reduced. Total dry mass production is the main characteristic for genotype discrimination in forage breeding programs (Machado et al., 2019). However, in the present study, this characteristic did not contribute to the genetic dissimilarity between the genotypes (Figure 2).

The genotypes 30N, 36N, 48N, 70N, 83N, 95N, André da Rocha, and Bagual were selected by Fachinetto et al. (2012) for enhanced forage production. In later work, the genetic variability of these genotypes for morphological characteristics was confirmed (Fachinetto et al., 2017). Subsequently, nitrogen responses to 0, 60, 180, and 360 kg N ha<sup>-1</sup> were evaluated by Machado et al. (2019). Genotype 36N showed the highest efficiency of nitrogen use with the lowest N rate (60 kg of N ha<sup>-1</sup>), while 48N, André da Rocha, and Bagual had the maximum forage production. The information obtained in the present study is important for demonstrating that the genotypes already tested in other studies are in different groups, based on the characteristics evaluated here. This allows new parental combinations that may have a promising future to be identified.

For this reason, the selection index, which is based on the distance from the ideotype (Table 5), is reported. This tool makes it possible to recommend the most promising genotypes to obtain superior hybrids (Bertan et al., 2006). The ideotype was defined by Donald (1968) and has been used over the years to explore more direct and less empirical methods, aimed at the selection of superior genotypes (Sedgley, 1991). A major advantage of this method is that it allows specific characteristics to be selected in breeding programs, which complement the traditional genetic improvement process of plant selection and breeding (Sedgley, 1991).

Here, the *P. notatum* ideotype considered characteristics that have been explored in forage breeding programs, with emphasis on forage production in the selection of genotypes in genus *Paspalum* (Pereira et al., 2012; Weiler et al., 2018; Machado et al., 2019). Previously, Bertan et al. (2006) utilized a selection index approach for wheat (*Triticum aestivum* L.) that implemented the recommendation of the most promising genotypes based on the ideotype defined by the breeder. Similarly, this tool was used as an aid in decision making for the selection process in this study. The use of this tool is recommended, especially when a large number of genotypes are evaluated in a breeding program. In *P. notatum*, as well as in wheat (Bertan et al., 2006), the selection index based on the ideotype can

be used as an auxiliary analysis for genetic distance studies, increasing the efficiency in the choice of parents for future crossings (Silveira and Machado, 2020).

The results obtained indicate that there is genetic variability between the genotypes of *P. notatum* for all the characteristics evaluated. To help the plant selection process for *P. notatum*, multivariate analyzes are a useful tool to assist in the characterization and discrimination of genotypes.

A new approach was used in this study, which defined a *P. notatum* ideotype that can be used as an auxiliary tool in the identification and selection of superior genotypes in breeding programs. Total dry mass production was strongly associated with leaf dry mass production, indicating that the selection of superior genotypes can be made based on this characteristic. Tiller population density, stem dry mass, and leaf dry mass production were the characters with the greatest relative contribution to the genetic divergence between the studied genotypes. Based on the results, it is possible to make new gene combinations to increase forage production.

# **5.** Conclusions

There is genetic variability in *P. notatum* for all seven characteristics analyzed. The superior genotypes can be used in new combinations between apomictic and sexual plants to create superior hybrids.

The selection index, based on the ideotype, can be used as an auxiliary tool in the selection process. The characteristics of greater relative contribution were tiller population density, stem dry mass production, and the leaf dry mass production. These characters can be used in genetic distance studies in *P. notatum*.

# **Conflict of Interest**

The authors declare no conflict of interest.

# **Author Contributions**

Conceptualization: J.M. Machado, E.A.M. Motta, R.L. Weiler and M. Dall'Agnol. Data curation: J.M. Machado, D.C. Silveira and M. Dall'Agnol. Formal analysis: R.L. Weiler and D.C. Silveira. Investigation: J.M. Machado, E.A.M. Motta, M.R. Barbosa, R.L. Weiler, C. Simioni, E.A. Pereira and M. Dall'Agnol. Methodology: J.M. Machado, E.A.M. Motta, M.R. Barbosa, R.L. Weiler, C. Simioni, E.A. Pereira. Project administration: J.M. Machado and M. Dall'Agnol. Resources: M. Dall'Agnol. Supervision: J.M. Machado and M. Dall'Agnol. Validation: J.M. Machado, R.L. Weiler and D.C. Silveira. Visualization: J.M. Machado, E.A.M. Motta, M.R. Barbosa, R.L. Weiler and D.C. Silveira. Visualization: J.M. Machado, E.A.M. Motta, M.R. Barbosa, R.L. Weiler, C. Simioni, D.C. Silveira, A. Mills and M. Dall'Agnol. Writing-original draft: J.M. Machado, E.A.M. Motta, M.R. Barbosa, R.L. Weiler, C. Silveira, A. Mills and M. Dall'Agnol. Writing-review & editing: J.M. Machado, E.A.M. Motta, M.R. Barbosa, R.L. Weiler, C. Silveira, M. R. Barbosa, R.L. Weiler, C. Silveira, A. Mills and M. Dall'Agnol. Writing-review & editing: J.M. Machado, E.A.M. Motta, M.R. Barbosa, R.L. Weiler, C. Silveira, A. Mills and M. Dall'Agnol. Writing-review & editing: J.M. Machado, E.A.M. Motta, M.R. Barbosa, R.L. Weiler, C. Silveira, A. Mills and M. Dall'Agnol. Writing-review & editing: J.M. Machado, E.A.M. Motta, M.R. Barbosa, R.L. Weiler, C. Silveira, A. Mills and M. Dall'Agnol. Writing-review & editing: J.M. Machado, E.A.M. Motta, M.R. Barbosa, R.L. Weiler, C. Silveira, A. Mills and M. Dall'Agnol.

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