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Productive potential of superior genotypes of *Paspalum notatum* Flügge in response to nitrogen fertilization

Potencial produtivo de genótipos superiores de Paspalum notatum Flügge em resposta à fertilização nitrogenada

MACHADO, Juliana Medianeira*[1]; DALLAGNOL, Miguel[2]; MOTTA, Eder Alexandre Minsk da[2]; PEREIRA, Emerson André[3]; BARBOSA, Marlon Risso[2]; NEME, Jackson Camargo[2]; KRYCKI, Karine Cristina[2]

[1]Universidade de Cruz Alta, Centro de Ciências Agrárias e da Saúde, Cruz Alta - RS, Brasil. [2]Universidade Federal do Rio Grande do Sul, Departamento de Zootecnia, Porto Alegre – RS, Brasil. [3]Universidade Regional do Noroeste do Estado do Rio Grande do Sul, Departamento de Estudos Agrários, Ijuí – RS, Brasil.
*Endereço para correspondência: julianam.machado@yahoo.com.br

SUMMARY

The *Paspalum* genus forms the natural pastures of the tropical and subtropical regions of America and includes several species of recognized forage potential. The aim of this study was to evaluate the influence of different levels of nitrogen fertilization on the agronomic traits and nitrogen use efficiency in superior genotypes of *Paspalum notatum* Flügge. Four nitrogen fertilization levels (0, 60, 180 and 360 kg N ha^{-1}) and nine genotypes were evaluated, six from the United States Department of Agriculture (USDA) (30N, 36N, 48N, 70N, 83N and 95N), collected in South America, two native genotypes of Rio Grande do Sul, Brazil (André da Rocha and Bagual) and cv. Pensacola. The experimental design was a randomized complete block in a 4 x 9 factorial, with four field replications. From the variability of the responses found for the agronomic traits, the genotypes 48N, André da Rocha and Bagual are indicated to continue in the breeding program because they reach the maximum productive efficiencies according to the doses of N tested. The genotype 36N obtained the highest efficiency of nitrogen utilization, being biologically viable with the lowest N dose, equivalent of 60 kg of N ha^{-1}.

Keywords: native species, nitrogen fertilization, genus *Paspalum*

RESUMO

O gênero *Paspalum* compõe as pastagens naturais das regiões tropicais e subtropicais da América com várias espécies de reconhecido potencial forrageiro. O objetivo deste estudo foi avaliar a influência de diferentes níveis de fertilização nitrogenada sobre os caracteres agronômicos e na eficiência de utilização de nitrogênio por genótipos superiores de *P. notatum* Flügge. Foram avaliados quatro níveis de fertilização nitrogenada (0, 60, 180 e 360 kg N ha^{-1}) e nove genótipos, seis provenientes do United States Departament of Agriculture (USDA) (30N, 36N, 48N, 70N, 83N e 95N), coletados na América do Sul, dois genótipos nativos do Rio Grande do Sul, Brasil (André da Rocha e Bagual) e a cv. Pensacola. O delineamento experimental utilizado foi o de blocos casualizados em esquema fatorial 4 x 9, com quatro repetições de campo. A partir da variabilidade de respostas encontradas para os caracteres agronômicos são indicados a prosseguir no programa de melhoramento genético os genótipos 48N, André da Rocha e Bagual por atingirem as máximas eficiências produtivas de acordo com as doses de N testadas.O genótipo 36N que obteve a maior eficiência de utilização

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de nitrogênio, demonstrando-se biologicamente viável com a menor dose de N, equivalente a 60 kg de N ha^{-1}.

Palavras-chave: espécie nativa, fertilização nitrogenada, gênero *Paspalum*

INTRODUCTION

Species of the genus *Paspalum* are the main constituents of natural pastures in the tropical and subtropical regions of South America (Sartor et al., 2011). In southern Brazil, these species predominate in the physiognomy and are critical for livestock production in the natural fields of the Pampa Biome. Studies have demonstrated the forage potential of different *Paspalum* species (Pereira et al., 2012; Motta et al., 2017; Steiner et al., 2017).

Among *Paspalum* species, *P. notatum* Flüggeis salient, with native genotypes of recognized forage potential, which surpasses that of the commercial cultivar Pensacola predominantly used in the southern region of the country (Fachinetto et al., 2012, Weiler et al., 2018).Knowledge of the productive potential of promising genotypes released as new cultivars coupled with the appropriate use of input technologies can be used to intensify livestock production systems. In this sense, the use of nitrogen (N) is one of the main factors responsible for increased biomass production in natural ecosystems and is an important agronomic tool to improve the performance of warm season grasses. In natural pasture areas of the Pampa Biome, where *P. notatum* is one of the predominant species, a positive biological and economic response was observed with application of up to 200 kg N ha^{-1} (Santos et al., 2008).

On the other hand, the high cost of N fertilization has hindered efforts to increase production on small farms, especially in marginal cultivation regions (Do Vale et al., 2012). In developed countries, where larger amounts of N are applied to soils, environmental problems occur owing to its high pollutant potential (Ahlgren et al., 2008).

Among the future challenges of ruminant production is reduction in the use of non-renewable resources through the development of more productive and sustainable livestock. In order to achieve this goal, it is essential to develop forage cultivars adapted to various soil and climate conditions, with higher biomass production and efficient use of fertilizers. Therefore, the evaluation of different *P. notatum* Flügge genotypes in response to different N fertilization levels should be performed within a forage breeding program. Obtaining more productive genotypes with more efficient Nuse will enable more profitable and sustainable livestock production.

The aim of this study was to evaluate the influence of different levels of N fertilization on agronomic traits and N use efficiency of superior *P. notatum* Flügge genotypes.

MATERIAL AND METHODS

The experiment was conducted at the Agronomic Experimental Station belonging to the Federal University of Rio Grande do Sul. The climate is humid subtropical (Cfa), according to the Köppen classification (Moreno, 1961).During the experimental period, precipitation was 649.8 mm (accumulated in 5 months) in the first productive cycle and 1429.5 mm (accumulated in 11 months) in the second cycle, and the average temperature was 19.4 °C and 22.4 °C, respectively.

The soil is classified as Typical Dystrophic Red Argisol (Embrapa, 2013).According to the Soil Chemistry and Fertility Commission (CQFS) - RS / SC (2004), soil samples for forage

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cultivation were collected at 0–10 cm depth and had the following chemical: characteristics: clay = 15%; pH (H_2O) = 5.4; SMP index = 6.3; P (mg dm^{-3}) = 15.6; K (mg dm^{-3}) = 151.4; M.O. = 2.7%.The area was fertilized at the time of seeding with phosphorus and potassium, according to the technical indications for warm season perennial grasses, following the recommendations of CQFS (2004).

In the fall of 2011, clones of the genotypes were generated and kept in 1L pots with commercial substrate in a greenhouse. Subsequently, the seedlings were transplanted to the field on 10/12/2011 as plots formed by five rows of 1.5 m in length, spaced 0.15 m apart, totaling 50 plants per plot. The experimental design was a randomized block design in a 4 × 9 factorial scheme, with four field replications. The N fertilization treatments included four levels equivalent to 0, 60, 180 and 360 kg N ha^{-1}, and nine *P. notatum* genotypes (30N, 36N, 48N, 70N, 83N, 95N, André da Rocha, Bagual, and cultivar Pensacola) were tested. Genotypes 30N, 36N, 48N, 70N, 83N, and 95N were obtained from the United States Department of Agriculture (USDA) and derived from collections conducted in South America. In addition, the native genotypes André da Rocha and Bagual (collected in 1980 in the state of Rio Grande do Sul, Brazil) and cultivar Pensacola were used as witnesses
(Table 1).

Table 1 Identification of *Paspalum notatum* genotypes

Genotypes	Identification	Ploidylevel
30N	Santa Fé – Argentina	Tetraploid
36N	Santa Fé – Argentina	Tetraploid
48N	Mercedes – Argentina	Tetraploid
70N	Cordoba – 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	Missões/RS – Brazil	Tetraploid
Pensacola	Viamão/RS – Brazil	Diploid

N was applied as urea and fractionated in three applications per production cycle. In the first production cycle, the applications were performed on 02/24, 03/16, and 04/26/2012.In the second cycle, applications occurred on 10/24, 11/12, and 12/11/2012.All genotypes were selected in the preliminary evaluation of forage production (Fachinetto et al., 2012). The evaluation was performed by cuts, using two squares of0.25 m^2 per plot.

The cuts were performed whenever the majority of genotypes reached 20 cm of canopy height, such that the post-cut residue height was 5 cm. Seven cuts were made during the evaluation period (03/16, 04/26, 11/12, and 12/11/2012; 01/22,02/20, and 03/18/2013).After the cuts, the samples were transported to the laboratory for morphological separation of leaf blades, stems, and stems and inflorescences. Subsequently, the samples were placed in a forced air oven at 65 °C until a constant mass was achieved.

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The measured variables included accumulated total dry mass production (TDMP, kg ha^{-1}), accumulated leaf blade dry mass production (LBDMP, kg ha^{-1}), and accumulated stem dry mass production (SDMP, kg ha^{-1}). The leaf: stem ratio (LSR) was calculated by the ratio between LBDMP and SDMP. Nitrogen use efficiency (NUE, kg of MS kg^{-1} of N applied) was calculated according to the following equation: NUE = (TDMP in N fertilized plot – TDMP in unfertilized plot) / (applied N level) (Marriott; Haystead, 1993; Silveira et al., 2013).

Data were subjected to analysis of variance and the F test at 5% probability. When differences between treatments were detected, comparison of means was performed by the Scott-Knott test at 5% probability. In addition, the variables were subjected to Pearson correlation analysis. For N fertilization levels, regression analysis was performed. Data were evaluated using the GENES statistical package (Cruz, 2007).

RESULTS AND DISCUSSION

The *Paspalum* genotypes and N levels were correlated ($p < 0.05$) for the TDMP, LBDMP, LSR, and NUE variables. The TDMP and LBDMP parameters fit linear and quadratic regression models (Figure 1).

For TDMP, genotypes 30N, 36N, 70N, 83N, and 95N fit the linear regression model. The results indicated that these *P. notatum* genotypes responded positively up to the maximum N level tested (360 Kg N ha^{-1}) and demonstrated that the soil N supply did not meet the needs of the genotypes.

For each kg of N applied, there was a conversion of 11.6, 7.8, 23.4, 12.0, and 16.2 kg TDMP for genotypes 30N, 36N, 70N, 83N, and 95N, respectively. Although the maximum TDMP was not reached, the 70N genotype exhibited the best conversion to TDMP with the tested N levels.

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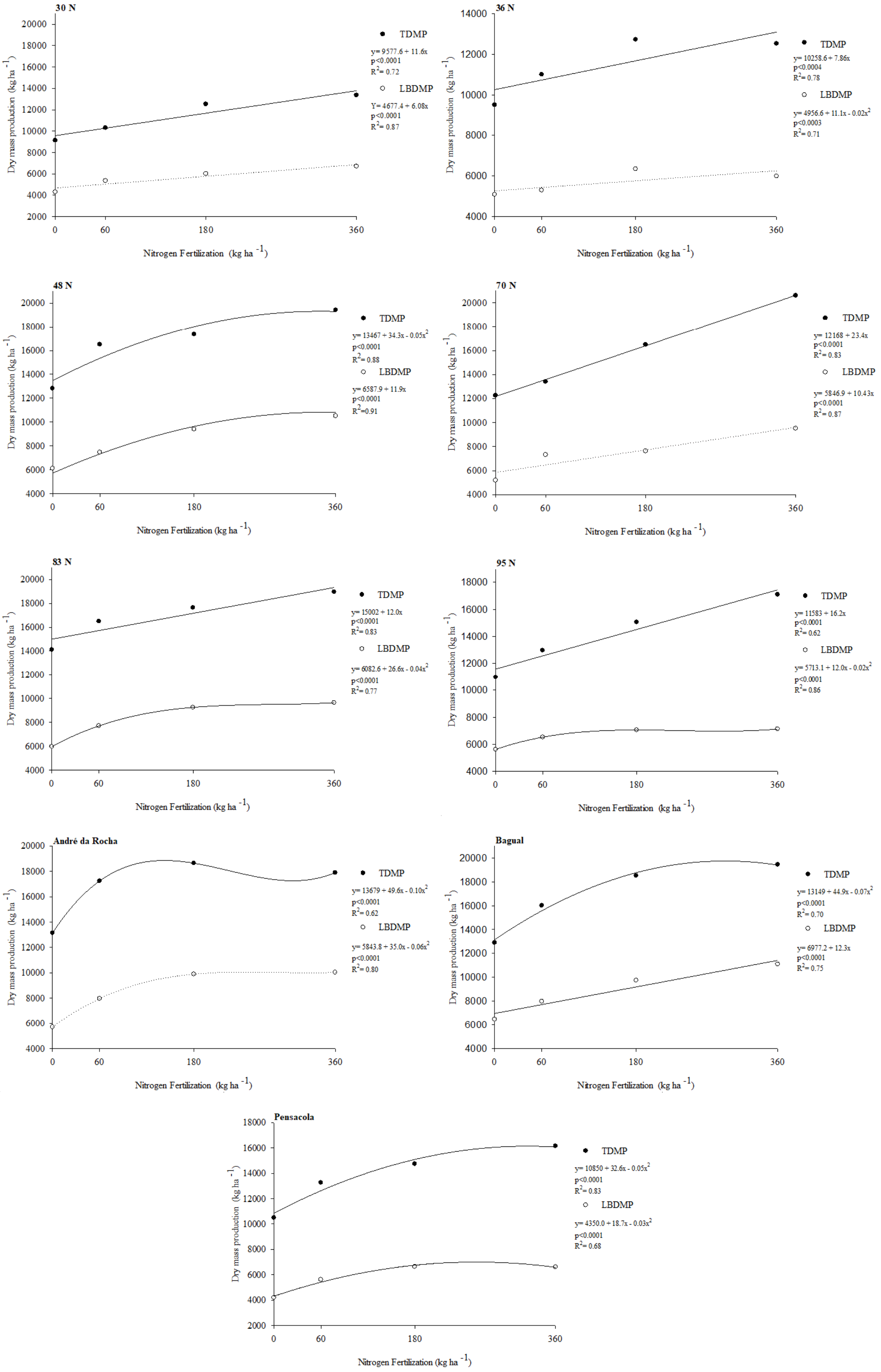
30 N
Dry mass production (kg ha -1)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
60
180
360
Nitrogen Fertilization (kg ha -1)
● TDMP
y= 9577.6 + 11.6x
p<0.0001
R2= 0.72
○ LBDMP
Y= 4677.4 + 6.08x
p<0.0001
R2= 0.87
36 N
14000
12000
10000
8000
6000
4000
● TDMP
y= 10258.6 + 7.86x
p<0.0004
R2= 0.78
○ LBDMP
y= 4956.6 + 11.1x - 0.02x2
p<0.0003
R2= 0.71
48 N
● TDMP
y= 13467 + 34.3x - 0.05x2
p<0.0001
R2= 0.88
○ LBDMP
y= 6587.9 + 11.9x
p<0.0001
R2=0.91
70 N
● TDMP
y= 12168 + 23.4x
p<0.0001
R2= 0.83
○ LBDMP
y= 5846.9 + 10.43x
p<0.0001
R2= 0.87
83 N
● TDMP
y= 15002 + 12.0x
p<0.0001
R2= 0.83
○ LBDMP
y= 6082.6 + 26.6x - 0.04x2
p<0.0001
R2= 0.77
95 N
● TDMP
y= 11583 + 16.2x
p<0.0001
R2= 0.62
○ LBDMP
y= 5713.1 + 12.0x - 0.02x2
p<0.0001
R2= 0.86
André da Rocha
● TDMP
y= 13679 + 49.6x - 0.10x2
p<0.0001
R2= 0.62
○ LBDMP
y= 5843.8 + 35.0x - 0.06x2
p<0.0001
R2= 0.80
Bagual
● TDMP
y= 13149 + 44.9x - 0.07x2
p<0.0001
R2= 0.70
○ LBDMP
y= 6977.2 + 12.3x
p<0.0001
R2= 0.75
Pensacola
● TDMP
y= 10850 + 32.6x - 0.05x2
p<0.0001
R2= 0.83
○ LBDMP
y= 4350.0 + 18.7x - 0.03x2
p<0.0001
R2= 0.68

Figure 1 Total accumulated dry mass production and leaf blades of *P. notatum* genotypes treated with different levels of nitrogen fertilization.

Genotypes 48N, André da Rocha, Bagual, and cultivar Pensacola fit the TDMP quadratic regression model (Figure 1), reaching the maxima with the N levels tested. The highest and lowest TDMP corresponded to the Bagual (20.349 kg DM ha^{-1}) and 48N (19.347 kg DM ha^{-1}) genotypes when 320 and 350 kg N ha^{-1}were applied, respectively.

In this context, the André da Rocha genotype, which had a TDMP of 19.829 kg DM ha^{-1} using 250 kg N ha^{-1}, was remarkable. On the other hand, Pensacola only reached the maximum TDMP (16.163 kg DM ha^{-1}) when 330 kg N ha^{-1}was applied, and this TDMP was lower than the maximum achieved by genotypes 48N, André da Rocha, and Bagual (Table 2).

Table 2 Maximum yield efficiency of *Paspalum notatum* genotypes according to nitrogen fertilization

Genotypes	Maximum productive efficiency (kg ha^{-1})			
	TDMP	Nitrogen levels	LBDMP	Nitrogen levels
30N	13.354*	360	6.698*	360
36N	12.520*	360	6.496	270
48N	19.347	350	10.520*	360
70N	20.602*	360	9.512*	360
83N	18.964*	360	10.505	330
95N	17.098*	360	7.513	300
André da Rocha	19.829	250	10.948	290
Bagual	20.349	320	11.100*	360
Pensacola	16.163	330	7.264	310

TDMP = accumulated total dry mass production. LBDMP = accumulated dry leaf blade mass production. *TDMP and LBDMP can be overcome by applying N doses greater than 360kg N ha^{-1}.

Studies have shown that the cultivar Pensacola has lower performance than native (Fachinetto et al., 2012; Graminho et al., 2017) and *P. notatum* hybrids (Weiler et al., 2018). The results here suggested that the 48N, André da Rocha, and Bagual genotypes may be used for future hybridization for development of superior genotypes. In addition, notably, the 70N and 83N genotypes did not reach the maximum TDMP with the N doses tested but showed high TDMP, suggesting the need for further studies with these genotypes, including with different N sources and edaphoclimatic conditions.

When superior genotypes are obtained with respect to the traits of interest of the breeding program, such as TDMP and LBDMP, they can be launched as new cultivars; as *P. notatum* are an apomitic species, the characteristics of the obtained hybrids will be fixed in F1 (Acuna et al., 2009).In a study conducted with *P. notatum*, Silveira et al. (2013) observed increases in forage production of 25% to 50% when 60 and 120 kg N ha^{-1} were applied, respectively. Scheffer-Basso et al. (2010), who evaluated *P.pauciliatum* exposed to different levels of N, observed positive responses of variable forage dry mass production to the

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application. According to Santos et al. (2008), the application of N in natural pastures belonging to the Pampa Biome is economically viable up to 200 kg of N ha^{-1} from both a biological and economic point of view.

For the LBDMP variable, the 30N, 48N, 70N and Bagual genotypes fit the linear regression model. For each kg of N applied, there was a conversion of 6.1, 11.9, 10.4 and 12.3 kg of LBDMP, respectively. Genotypes 36N, 83N, 95N, André da Rocha, and cultivar Pensacola fit the LBDMP quadratic regression model (Figure 1), reaching the maxima within the N levels tested. The highest and lowest LBDMP corresponded to the André da Rocha (10.948 kg DM ha^{-1}) and 36N (6.496 kg DM ha^{-1}) genotypes when 290 and 270 N ha^{-1} were applied, respectively.

As the genotype André da Rocha and cultivar Pensacola required adjustments to the quadratic regression model for the TDMP and LBDMP variables, it was possible to calculate the percentage of LBDMP present in the TDMP. LBDMP values of 55.2% and 44.9% in the TDMP were obtained for André da Rocha and cultivar Pensacola, respectively. The highest nutritional quality of forage plants is in the leaf blades, which is the structure preferred by grazing animals (Bratti et al., 2009).Thus, the highest proportion of leaf blades is one of the main criterion for genotype selection in forage breeding programs.

There was a positive correlation between TDMP and LBDMP ($r = 0.99$; $p < 0.0001$), confirming the positive increments obtained between the variables. The high correlation between the variables described above was also observed in *Brachiaria ruziziensis* S. genotypes (Borges et al., 2011) and *P. notatum* genotypes and hybrids (Weiler et al., 2018).This result is particularly important, because selecting genotypes for high TDMP will result in selection of genotypes with high LBDMP. Pereira et al. (2012), who evaluated genetic variability in the genus *Paspalum*, emphasized that TDMP and LBDMP were the most effective traits for identifying genotypes with higher forage traits. Thus, the selection of genotypes with higher TDMP remains, in general, the main focus in forage breeding programs. Moreover, the high correlation between TDMP and LBDMP suggested that the laborious task of separating morphological components, at least in this species, could be avoided, saving time and resources.

The applied N levels had the lowest influence on the LSR variable. Most genotypes did not show increased LSR with increasing N fertilization levels, except for genotypes 83N and André da Rocha, which presented responses to the level equivalent to 60 kg N ha^{-1} (Table 3).Selection for reduced stem production and increased leaf production has been strongly advocated for forage breeding (Pereira et al., 2011). In the present study, notably, the LSR of cultivar Pensacola remained at 0.9 without N fertilization. A high LSR is desired in forage plants as it confers better grazing adaptation or tolerance to mowing because it presents a phenological moment when apical meristems are closer to the ground and therefore less vulnerable to grazing elimination (Silva et al., 2013).

Table 3 Leaf: stem ratio *Paspalum notatum* genotypes in response to different of nitrogen fertilization

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Genotypes	Nitrogen levels (kg ha^{-1})			
	0	60	180	360
	LSR			
30N	1.25 Ab	1.21 Aa	1.38 Aa	1.26 Ab
36N	1.34 Aa	1.36 Aa	1.29 Ab	1.19 Ab
48N	1.29 Ab	1.27 Aa	1.43 Aa	1.45 Aa
70N	1.17 Ab	1.35 Aa	1.18 Ab	1.38 Aa
83N	1.15 Bb	1.46 Aa	1.47 Aa	1.35 Aa
95N	1.53 Aa	1.45 Aa	1.45 Aa	1.27 Ab
André da Rocha	1.02 Bb	1.39 Aa	1.59 Aa	1.48 Aa
Bagual	1.56 Aa	1.57 Aa	1.55 Aa	1.56 Aa
Pensacola	0.92 Ab	1.19 Aa	1.17 Ab	1.08 Ab

Means followed by different uppercase letters are significantly diferente within a row, and means followed by different lowercase letters are significantly diferente within a column based on the Scott-Knott 5% mean test.
LSR = leaf: stem ratio.

For the NUE variable, the highest value observed at the level equivalent to 60 kg of N ha-1, decreasing with increasing N levels applied (Table 4).

Table 4 Nitrogen utilization efficiency of different *Paspalum notatum* genotypes

Genotypes	NUE (kg of DM kg^{-1} de N applied)		
	Nitrogen levels (kg ha^{-1})		
	60	180	360
30N	9.8 Ac	9.4 Bb	5.8 Cc
36N	69.0 Aa	27.8 Ba	13.6 Ca
48N	30.6 Ab	12.6 Bb	9.1 Cb
70N	16.6 Ac	11.7 Bb	11.5 Bb
83N	19.8 Ac	13.4 Bb	6.7 Cc
95N	16.7 Ac	11.3 Bb	8.5 Cc
André da Rocha	34.1 Ab	15.2 Bb	6.6 Cc
Bagual	36.2 Ab	15.5 Bb	9.1 Cb
Pensacola	23.0 Ab	11.7 Bb	7.8 Cc

Means followed by different uppercase letters are significantly diferente within a row, and means followed by different lowercase letters are significantly diferente within a column based on the Scott-Knott 5% mean test.
NUE: nitrogen utilization efficiency.

The 36N genotype presented the highest NUE when N was applied at 60 kg N ha^{-1}, but 60% and 80% reductions were observed for the levels equivalent to 180 and 360 kg N ha^{-1}, respectively. Obour et al. (2017), who evaluated *Panicum virgatum* L. at increasing levels of N fertilization (0, 45, 90, 135, and 180 kg N ha^{-1}), observed a linear reduction in NUE. The reduction in efficiency can be explained by the reduced capacity of the plant to absorb and use the nutrient for production, as well as possible soil leaching.

N is one of the most difficult nutrients to manage effectively. In many agroecological systems, a substantial portion of applied N is lost from soil to groundwater, rivers, and oceans (Glass, 2003), because plants convert only 30% to 40% of the applied N into useful products. Thus, the variability between the studied genotypes allows the selection of plants with higher genetic potential for the NUE trait. According to Seepaul et al. (2016), several factors contribute to the amount of N removed by plant biomass, including the genotype and amount of N applied.NUE has been used to describe a plant's ability to acquire and use N to produce biomass, and is expressed as the yield of biomass produced per unit of N applied (Seepaul et al., 2016).In this sense, including this information as a tool for selecting genotypes of this species in forage breeding programs is important.

Indirect calculation of NUE has been widely used owing to its practicality and low cost (Silveira et al., 2013; Obour et al., 2017). The most commonly used source of N in Brazil is urea; however, research data indicate volatilization losses of up to 30% of N. Measures that increase the NUE should be implemented in order to promote the management of an economically sustainable, forage quality production system with minimal negative environmental impact.

The results of this study showed that N fertilization influences the agronomic traits of different genotypes, increasing biomass production. The use of NUE as a selection criterion may help the breeder obtain plants with higher forage yields with lower N levels. This may favor more profitable and sustainable livestock farming owing to reduced spending on N fertilizer and reduced impacts on the environment, such as the use of non-renewable fossil fuel reserves, effects on global warming, and water contamination.

The results of this work indicated that the evaluation and selection of native genotypes may produce new cultivars that are more productive and use N more efficiently than currently available genotypes on the market, such as *P. notatum* ‘Pensacola’. Thus, based on the variability observed in the evaluated agronomic traits, the 48N, André da Rocha, and Bagual genotypes were indicated to be promising for continued use in the breeding program because they reached the maximum productivity efficiencies according to the N rates tested. The 70N and 83N genotypes are also indicated for new evaluations, because they showed ability to express forage yields with higher N rates than those tested in the present study, which may indicate their applicability in intensive livestock systems. The 36N genotype had the highest N use efficiency, proving to be biologically viable with the equivalent of 60 kg N ha^{-1}.

These genotypes could be introduced in rural areas to recover degraded natural pastures or directly as cultivated pastures, with higher forage production due to positive responses to N application. In addition, this introduction could reduce the use of exotic species and preserve the natural ecosystem, as *P. notatum*is native to this environment and better studied; thus, more information on *P. notatum* management practices is available.

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