UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE AGRONOMIA

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

AGRONOMIC PERFORMANCE IN *Paspalum* INTERSPECIFIC HYBRIDS SUBJECTED TO NITROGEN APPLICATION RATES OR IN MIXTURE WITH TEMPERATE LEGUMES

EDER ALEXANDRE MINSKI DA MOTTA

Zootecnista/UFSM
Mestre em Zootecnia/UFRGS

Tese apresentada como um dos requisitos à obtenção do grau de Doutor em Zootecnia
Área de Concentração Plantas Forrageiras

Porto Alegre (RS), Brasil
Março, 2018

CIP - Catalogação na Publicação

da Motta, Eder Alexandre Minski
Agronomic performance in Paspalum interspecific hybrids subjected to nitrogen application rates or in mixture with temperate legumes / Eder Alexandre Minski da Motta. -- 2018.
103 f.
Orientador: Miguel Dall'Agnol.

Coorientadora: Carine Simioni.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Programa de Pós-Graduação em Zootecnia, Porto Alegre, BR-RS, 2018.

1. Biomass yield. 2. Nitrogen use efficiency. 3. Nitrogen supply. 4. Nutritive value. 5. Plant selection. I. Dall'Agnol, Miguel, orient. II. Simioni, Carine, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Eder Alexandre Minski da Motta
Mestre em Zootecnia

TESE

Submetida como parte dos requisitos
para obtenção do Grau de

DOUTOR EM ZOOTECNIA

Programa de Pós-Graduação em Zootecnia
Faculdade de Agronomia
Universidade Federal do Rio Grande do Sul
Porto Alegre (RS), Brasil

Aprovada em: 27.03.2018
Pela Banca Examinadora

Homologado em: 20/06/18
Por

DANILO PEDRO STREIT JR.
Coordenador do Programa de
Pós-Graduação em Zootecnia

MIGUEL DALL'AGNOL
PPG Zootecnia/UFRGS
Orientador

André P. Brunes
UFRGS

Carine Simioni
UFRGS

Daniel P. Montardo
Embrapa

Esteban Fernando Rios
Universidade da Flórida

CARLOS ALBERTO BISSANI
Diretor da Faculdade de Agronomia

“*Remember to look up at the stars and not down at your feet. Try to make sense of what you see and wonder about what makes the universe exist. Be curious. And however difficult life may seem, there is always something you can do and succeed at. It matters that you don’t just give up*.”

Stephen William Hawking

ACKNOWLEDGEMENTS

Thank you so much to God for my life.

Many thanks to my parents Mariza and Antonio, my sister Janiele and my wife Paula, for her love, friendship, support and advice.

My thanks to my supervisor, Miguel Dall'Agnol for the opportunity, teaching, confidence, friendship and help during my PhD period.

My thanks to Forage Plants Breeding Group – UFRGS and its members for helping me during my PhD period. Special thanks to Cleber H. L. de Souza, Mariana R. de Ávila, Larissa A. Graminho and Karine C. Krycki.

Thanks to professors Carine Simioni, Roberto L. Weiler and Carlos Nabinger for helping me during my PhD period.

My thanks to my international supervisor, Esteban F. Rios, from University of Florida, FL - USA, for the opportunity, teaching, time, friendship and help during my PhD period in Florida. Thanks to members of the Forage Breeding and Genomics Lab.

My thanks to Marcelo O. Wallau for the opportunity, friendship and help during my PhD period in Florida.

My thanks to GOU – "Grupo de Oração da Capela do Bom".

Thanks to "Programa de Pós-Graduação em Zootecnia" – UFRGS. Special thanks to Paulo C. F. Carvalho and Ione Borcelli.

Thanks to "Estação Experimental Agronômica" – UFRGS. Special thanks to Carlos, Claudio and Roberto (Departamento de Plantas Forrageiras e Agrometeorologia).

My thanks to CAPES – "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" for the PhD scholarship.

My thanks to Sulpasto – "Associação Sul-brasileira para o Fomento de Pesquisa em Forrageiras" for the financial support provided for conducting this research.

Thanks to graduate student colleagues for the friendship and help during my PhD period. Special thanks to Jean V. Savian.

Agronomic performance in *Paspalum* interspecific hybrids subjected to nitrogen application rates or in mixture with temperate legumes[1]

Author: Eder Alexandre Minski da Motta
Advisor: Miguel Dall'Agnol

Abstract: Plicatula is a taxonomic group within of the genus *Paspalum* that contains interesting species considering their phenotypic diversity for forage traits, and some of these species have been improved through artificial interspecific hybridization. Nitrogen (N) is an important limiting factor to produce biomass. Forage legumes contribute with symbiotic N_2 fixation and can increase biomass yield and the nutritive value of the pastures. The objectives of this thesis were: (i) evaluate dry matter yield (DMY), N use efficiency (NUE), nutritive value, cold tolerance and plant persistence in hybrids of *Paspalum plicatulum* x *P*. *guenoarum* subjected to N application rates, (ii) compare biomass yield and nutritive value of the grass–legume system to a grass–N fertilizer system, and (iii) select the best hybrids for new steps within the breeding program. The study was conducted from September 2015 to May 2017. The experimental design was a randomized complete block in split-plot arrangement with three replicates. Treatments were five N rates (0, 60, 120, 240, and 480 kg N ha^{-1} N), and one grass-legumes mixture (*Trifolium repens* + *Lotus corniculatus*) as whole plots, and six genotypes (1020133, 102069, 103084, 103061, *P. guenoarum* ecotype Azulão and *Megathyrsus maximus* cv. Aruana used as a control) as subplots. Nitrogen rates of 240 and 480 kg N ha^{-1} increased Total-DMY, Leaf-DMY, cold tolerance and persistence but decreased NUE. Higher NUE was obtained with N rates between 60 and 120 kg N ha^{-1}. Total-DMY for grass-legume mixture was similar to the N rates of 60 and 120 kg N ha^{-1}. Hybrid 1020133 had Total-DMY similar to Azulão and Aruana, as well as Leaf-DMY greater than Aruana. Hybrid 1020133 showed greater cold tolerance and exhibited greater NUE at 60 kg N ha^{-1} than the other genotypes. At the N rate of 480 kg N ha^{-1} increased crude protein (27%) and digestibility (4%) and decreased neutral (6%) and acid (7%) detergent fiber compared to the 0 kg N ha^{-1}. Grass-legume mixture showed greater nutritive value compared to N-fertilized grass. Hybrid 103061 had greater crude protein and digestibility and lower neutral and acid detergent fiber than Azulão and Aruana. Therefore, there is opportunity to increase DMY, NUE, cold tolerance and plant persistence, and improve the nutritive value through genotype selection and N management. In addition, grass-legume mixture showed greater nutritive value than N-fertilized grass and can be an alternative practice to replace to the application of N fertilizer up to the rate of 120 kg N ha^{-1}. Hybrids 1020133 and 103061 should be indicated for new studies, such as seed production and animal performance.

Key words: biomass yield, nitrogen use efficiency, nitrogen supply, nutritive value, persistence, plant selection.

---

[1] Doctoral thesis in Animal Science – Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (103 p.), March, 2018.

TABLE OF CONTENTS

1. CHAPTER I 10
1.1. GENERAL INTRODUCTION 11
1.2. LITERATURE REVIEW 13
1.2.1. Genus *Paspalum*: importance of the Plicatula group 13
1.2.2. Improvement of species of the Plicatula group 14
1.2.3. Nitrogen: importance for forage performance of grasses 16
1.2.4. Nitrogen use efficiency 20
1.2.5. Nitrogen fertilizer and environmental problems 21
1.2.6. Grass-legume mixture in pastures 23
1.2.7. Legumes characteristics used in the research 25
1.2.8. Use of legumes x nitrogen fertilizer 29
1.3. HYPOTHESIS AND OBJECTIVES 32
2. CHAPTER II[1] 33
Agronomic Evaluation in Hybrids of *Paspalum* Subjected to Nitrogen Fertilization or in Mixture with Legumes 33
2.1. Introduction 36
2.2. Material and Methods 39
2.3. Results 43
2.4. Discussion 50
2.5. Conclusion 60
3. CHAPTER III[2] 67
Nutritive value and forage production in *Paspalum* interspecific hybrids fertilized with nitrogen or in mixture with temperate legumes 67
3.1. Introduction 69
3.2. Material and Methods 70
3.3. Results 73
3.4. Discussion 76
3.5. Conclusions 80
4. CHAPTER IV 91
4.1. FINAL CONSIDERATIONS 92
5. REFERENCES 92
6. AUTHOR BIOGRAPHY 103

LIST OF TABLES

CHAPTER II

Table 1. Average monthly minimum (min)–maximum (max) temperature (ºC) and rainfall (mm) during the experimental period (September 2015–May 2017) compared to 40–yr average (1970–2009). 39
Table 2. Chemical characteristics of soil before starting and during the accomplishment of the experiment….. 40

CHAPTER III

Table 1. Chemical characteristics of soil before starting and during the accomplishment of the experiment...……………..………..…………85
Table 2. Harvests carried out in four interspecific *Paspalum* hybrids, Azulão and Aruana during years 1 and 2………………………………………....85
Table 3. Genotype, N rate and Genotype x N rate effects on dry matter yield by season of four interspecific *Paspalum* hybrids, Azulão and Aruana in year 1...…………………………………………………………………....86
Table 4. Genotype, N rate and Genotype x N rate effects on dry matter yield by season of four interspecific *Paspalum* hybrids, Azulão and Aruana in year 2…………………………………………………………...…....87

LIST OF FIGURES

CHAPTER II

Figure 1. Genotype x N rate interaction effects on (a) total dry matter yield and (b) leaf dry matter yield of four interspecific *Paspalum* hybrids, *P. guenoarum* ecotype Azulão and *M. maximus* cv. Aruana in year 1…44
Figure 2. Genotype x N rate interaction effects on (a) total dry matter yield and (b) leaf dry matter yield of four interspecific *Paspalum* hybrids, *P. guenoarum* ecotype Azulão and *M. maximus* cv. Aruana in year 2…45
Figure 3. Genotype x N rate interaction effects on number of tillers in (a) year 1 and N rate effect on number of tillers in (b) year 2…………………….....46
Figure 4. Legume proportion on (a) Total-DMY and (b) DMY of birdsfoot trefoil–BT and white clover–WC in years 1 and 2……………..…………..…..47
Figure 5. Nitrogen rate effect on NUE in (a) year 1 and Genotype x N rate interaction effects on NUE in (b) year 2………..……………….…….48
Figure 6. Cold tolerance of four interspecific *Paspalum* hybrids, *P. guenoarum* ecotype Azulão and *M. maximus* cv. Aruana in response to (a) N rates or in mixture with legumes and (b) genotypes during the winter in 2016…..…………………………………………………………..…….49
Figure 7. Persistence of four interspecific *Paspalum* hybrids, *P. guenoarum* ecotype Azulão and *M. maximus* cv. Aruana in response to (a) N rates or in mixture with legumes and (b) genotypes after 2 years of harvesting.…………………………………………………..…….49

CHAPTER III

Figure 1. Maximum (Tmax), minimum (Tmin) and mean (Tmean) monthly temperature (ºC), rainfall and irrigation (mm) during the experimental period (September 2015–May 2017)..………………………………....88
Figure 2. The effect of genotypes on (a) CP = crude protein, (b) NDF = neutral detergent fiber, (c) ADF = acid detergent fiber and (d) DMD = dry matter digestibility of four interspecific hybrids *Paspalum*, Azulão and Aruana.………………………………………..…………………..89
Figure 3. The effect of N rates and mixture with legumes on (a) CP = crude protein, (b) NDF = neutral detergent fiber, (c) ADF = acid detergent fiber and (d) DMD = dry matter digestibility of four interspecific hybrids *Paspalum*, Azulão and Aruana. Leg = grass-legume mixture.………………………………………..…………………..90

1. CHAPTER I

1.1. GENERAL INTRODUCTION

The world population is expected to increase from the current 7 billion people to 9 billion in the year 2050. Thus, a significant increase in the production of meat and dairy products will be needed (Jank et al., 2014; Schröder, 2014). The FAO (Food and Agricultural Organization) estimates that to satisfy the population growth, food production will have to increase by 60% in the next decades (FAO, 2016).

Animal production in tropical and subtropical areas of the world is largely dependent on native or cultivated pastures, which requires higher production, quality and adaptation of forage plants (Jank et al. 2011). The use of proper management practices particularly water and nutrient management, as well as species selection, are some of the main options for improving forage yield (Adjesiwor et al., 2017).

After the drought stress, nitrogen (N) is often considered the most important limiting factor to produce biomass in natural ecosystems. In grasslands systems, N fertilization practices can provide N supply for plants to express the potential yield allowed by the climatic conditions (Lemaire et al., 2008). According to Pontes et al. (2016), forage yield and nutritive value of pastures are affected by the use of N fertilizer. Several studies report the positive effect of N fertilizer on forage yield and nutritional characteristics in different grasses species (Hennessy et al., 2008; Costa et al., 2010; Silveira et al., 2013; Obour et al., 2017). On the other hand, the industrial production of N fertilizer uses non-renewable reserves of fossil fuels (Schröder, 2014), and prices have increased 2.5 times in the last decade (Verhulst et al., 2015). Thus, producers are searching for alternative strategies to manage the requirements of N fertilizer for their grasslands (Biermacher et al., 2012).

The use of legume species may be an alternative to provide N to grasses and offer opportunities for sustainable grassland-based animal production (Schwinning and Parsons, 1996; Lüscher et al., 2014). Through the symbiotic fixation of N_2, legumes can replace the use of synthetic N fertilizer as well as contribute to increase yield and nutritive value of forage, raising the efficiency of conversion of forage to animal protein (Tekeli & Ates, 2005; Interrante et al., 2012; Lüscher et al., 2014). Therefore, forage legumes in mixtures with grasses can be considered a viable alternative to synthetic N fertilizer application to grass monocultures (Adjesiwor et al., 2017).

Species of the genus *Paspalum* are constituents of native grasslands in tropical and subtropical regions of the Americas (Strapasson et al., 2000; Sartor et al., 2011). The great diversity and area covered, and wide adaptability support the importance of the genus for animal production in these regions (Novo et al., 2016). Previous studies have shown that N fertilizer influences on biomass yield (Towsend, 2008; Machado, 2014), and nutritive value (Silveira et al., 2013; Pontes et al., 2016) of *Paspalum* species.

There is a taxonomic group within genus *Paspalum* named Plicatula (Chase, 1929), with approximately 30 species (Ortiz et al., 2013). Among the species of the group are *P. plicatulum*, *P. lepton*, *P. atratum* and *P. guenoarum*, which were cultivated as pastures in Australia, Argentina and United States (Aguilera et al., 2011).

The collection of a sexual diploid ecotype of *P. plicatulum*, and its artificial chromosome duplication resulted in the generation of a sexual tetraploid plant (Sartor et al., 2009). In recent years, this sexual plant has been used as a female parent in artificial hybridizations for improvement of apomictic tetraploid species of the Plicatula group (Aguilera et al., 2011; Pereira, 2013; Novo et al., 2016; Novo et al., 2017). Agronomic studies have shown the genetic potential of interspecific hybrids generated for some characteristics: forage production, cold tolerance (Motta et al., 2016, 2017) and cattle preference (Novo et al., 2017).

However, biomass yield and the nutritive value of these new hybrids are unknown when subjected to N fertilization rates, as well as mixed with legumes. Thus, the evaluation of these hybrids under N fertilizer can contribute to selection of genotypes with higher forage yield and quality, persistence and N use efficiency. In addition, testing these hybrids in mixtures with legumes may provide greater knowledge about its production potential when competing with other species.

1.2. LITERATURE REVIEW

1.2.1. Genus *Paspalum*: importance of the Plicatula group

Paspalum species are distributed naturally in all tropical and subtropical regions of the Americas, where are the major constituents of the native pastures of those regions (Sartor et al., 2009). There is great diversity among species, with different morphological characteristics, habit of growth and areas of adaptation, as well as mode of reproduction and cytological composition (Burson, 1997). More than 300 species are found in different ecosystems, and some are cultivated as perennial warm-season grasslands (Novo et al., 2017).

In the United States, Argentina and Brazil several species of *Paspalum* are being improved with the main of generating new forage cultivars for subtropical regions (Acuna et al., 2009; Aguilera et al., 2011; Novo et al., 2016; Weiler et al., 2018). Among the characteristics researched are: cold tolerance and cool-season growth; seed yield; grazing tolerance; nutritive value; and tolerance to biotic stresses (Ortiz et al., 2013).

There is an informal taxonomic group within the genus *Paspalum* named Plicatula (Chase, 1929), which contains many species considered important due to its diverse ecotypes, and forage characteristics (Novo et al., 2017). The center of variation for this group is central-western Brazil, eastern Bolivia, eastern Paraguay, and northeastern Argentina (Sartor et al., 2009). The Plicatula group has approximately 30 species and most of them are tetraploid and apomictic (Ortiz et al., 2013). According to Batista and Godoy (1998) Plicatula group presents variability to produce viable seeds, mainly for the flowering season, seed germination, tolerance to pathogens and vigor of the seedlings in the field establishment. These characteristics can be used in breeding programs for seed propagation.

Plicatula group may be considered a large agamic complex with considerable forage potential. At least three species of this group have been introduced to cultivation: *P. plicatulum*, *P. guenoarum* and *P. atratum* (Espinoza et al., 2001). In Central Brazil, the species *P. guenoarum* and *P. plicatulum* stand out for the forage potential (Strapasson et al., 2000). Both are also found in the Rio Grande do Sul State (Barreto, 1974). These species have been used in interspecific hybridizations (Aguilera et al., 2011; Novo et al., 2017), as well as evaluated in agronomic performance studies (Pereira et al., 2012; Motta et al., 2016; Huber et al., 2016).

P. guenoarum is a perennial species which presents summer growth, but with cold tolerance. The plant can reach 1.5-m tall or more in the flowering. In general, ecotypes have stems, nodes and glabrous sheaths, and may have pilosity on the leaf blade (Nabinger and Dall'Agnol, 2008).

Several researches were conducted to evaluate forage production and the nutritive value of this species. Mella (1980) studied the effect of grazing in a mixture between *P. guenoraum* and *Desmodium intortum* and observed greater grass proportion with higher interval of harvest (0, 14, 28, 42, 56 and 70 day) and lower herbage allowance (0, 2, 3.5, 5, 6.5 and 8 kg DM per 100 kg LW ha^{-1} day^{-1}). Moreover, the author described that grass had 58% of *in vitro* organic matter digestibility (IVOMD) and 10% of crude protein (CP) content, regardless of grazing system. In a study with two *P. guenoarum* ecotypes named Azulão and Baio, Paim and Nabinger (1982) found approximate values for CP (8.6–8.7%) and IVOMD (56–53%) in whole plant respectively.

In greenhouse, Mota (1980) studied the effects of age at the first cut (64, 113 and 134 days old) and stubble height (4 and 8 cm) on dry matter yield (DMY), CP and total nonstructural carbohydrates (TNC) in *P. guenoarum* and observed that plants with 134 days old, cut at 8 cm showed highest DMY and CP, and this result was associated with weight of TNC of roots and stem. Costa and Saibro (1984) evaluated the effects of N rates (0, 100, 200 and 400 kg ha^{-1}), growth stages (vegetative, boot and flower) and stubble height (5 and 10 cm) on DMY and CP of *P. guenoarum*. These authors reported that N application linearly increased DMY and CP, while forage harvested at later maturity stages yielded higher DMY and reduction on CP content. In addition, plants at the vegetative stage when clipped at 10 cm height, showed the highest DMY.

Costa and Saibro (1985) evaluated the influence of sowing methods in alfalfa (*Medicago sativa*) and *P. guenoarum* alone and in mixtures, with different stubble height (5 and 10 cm). These authors described that mixtures were more efficient in DMY and provided better weed control as compared to the grass or legume species seeded alone. In addition, there was a tendency for the 10 cm stubble height to increase the percentage of grasses.

Recently, Steiner et al. (2017) evaluated the forage performance and chemical composition (leaf blades) of Azulão and Baio. Azulão showed a yield of 18,560 kg DM ha^{-1}, and a content of 14.7% CP, 68.8% of neutral detergent fiber (NDF) and 40.3% of acid detergent fiber (ADF), while Baio showed a yield of 18,243 kg DM ha^{-1} and a content of 14.3% CP, 70.5% of NDF and 43.2% of ADF. Lopes et al. (2016) studied the production and seed quality (2 yr.) of Azulão subjected to different frequency of harvests (0, 1, 2 and 3), after a cleaning harvest at the beginning of spring of each year. In this study, seed production was higher 700 kg ha^{-1}, with values of 98.4% of purity, 72.2% of germination and 4.9% dormant seeds.

P. plicatulum is a perennial species which presents summer growth and can reach 1.2-m tall. It's characterized by spikelets with transversely wrinkled lemma and shining dark brown anthecium (Espinoza et al., 2001). It presents contents between 11.7–22.3% CP, 55–66.8% of NDF and 39.5–45.9% of ADF, with good tolerance to drought, and maintains forage production during fall. It has high number of tillers and grows up from of basal sprouting, since this feature is very important to guarantee the persistence of forages under grazing (Scheffer-Basso and Gallo, 2008). Currently, few agronomic studies have been carried out with this species, reducing the availability of data in the literature.

1.2.2. Improvement of species of the Plicatula group

Most of *Paspalum* species reproduce asexually by apomixis (Quarin, 1992), which results in the production of progeny clones of the mother plant and allows perpetuation of fixed genotypes (Acuña et al., 2009). In plant breeding, apomixis provides a unique mechanism for developing superior cultivars and preserving those genotypes indefinitely. In general, the three fundamental prerequisites of any successful plant breeding programme are: (i) availability of a diverse germplasm collection; (ii) adequate knowledge of the biology, cytology and reproductive system of the available material; and (iii) explicit and achievable objectives (Ortiz et al., 2013).

Specific breeding techniques must be used to improve *Paspalum* species. Ecotype selection is the oldest among the techniques used, which involves germplasm collection, evaluation and selection, multiplication of the best ecotypes, and the release of superior genotypes as new apomictic cultivars, that is, clones propagated by seeds (Ortiz et al., 2013). An example of the application of this technique has been described by Aguilera et al. (2011), in which *P. atratum*, *P. plicatulum* and *P. guenoarum* species, all tetraploids and apomictic were introduced in Australia, Argentina and United States, and the released cultivars were selected from natural ecotypes collected mainly in South America and without any genetic improvement through breeding.

Apomixis can be considered an obstacle to plant breeding because it impedes gene recombination, and consequently the generation of variability. In addition, it prevents the protection of cultivars in the Ministério da Agricultura Pecuária e Abastecimento (MAPA-Brazil), because the primary attribute for a cultivar to be considered eligible for protection is to be the result of a plant breeding process (Brasil, 2011). Due to MAPA guidelines, cultivar protection can provide *royalties* for breeding programs, and benefit producers by obtaining higher yielding cultivars as well as producing quality and purity seeds.

Species of the genus *Paspalum* are characterized by presenting a complex genetic system, with variation in ploidy levels and reproductive modes (Quarin, 1992). Approximately 60% of the species that have been characterized are apomictic, and these are all polyploid (Brugnoli et al., 2013). Apomixis and polyploidy is most common combination for most *Paspalum* species. Among ploidy levels, tetraploidy is the most frequent condition (Sartor et al., 2011).

The existence of fully sexual plants at the 4x level is an important prerequisite for any breeding program of polyploid apomictic grasses. In this case, sexual tetraploid individuals can be used as female parent in crosses with 4x apomictic plants, allowing the study of agronomic traits (Sartor et al., 2009). The collection of a sexual diploid plant of *P. plicatulum* and its chromosomal duplication resulted in the generation of a tetraploid sexual plant (Sartor et al., 2009). This sexual plant of *P. plicatulum* has been used as a female parent in crosses with other apomictic species, which allowed initiating the improvement of species of the Plicatula group through interspecific hybridizations.

Aguilera et al. (2011) carried out a cross between *P. plicatulum* 4PT and *P. guenoarum* Rojas apomictic cultivar, which resulted in 23 interspecific hybrids (14 sexual and 9 apomictic). Among the apomictic hybrids obtained, seven produced seeds and were evaluated in rows in the field by Pereira et al. (2015), that due to the higher forage performance selected the hybrids named H12, H13, H20 and H22. Motta et al. (2017) evaluated these hybrids in plots under different edafoclimatic conditions and found a dry matter yield greater than 21,000 kg ha^{-1} in some hybrids. In addition, greater cold tolerance was observed in relation to the male parent Rojas and *Megathyrsus maximus* cv. Aruana (used as controls), which demonstrated the existence of hybrid vigor obtained in the artificial hybridization between different species of the Plicatula group.

Recently another hybridization was performed by Novo et al. (2017) using *P. plicatulum* 4PT as a female parent and 11 apomictic species as male parents: *P. chaseanum*, *P. compressifolium*, *P. guenoarum*, *P. lenticulare, P. macedoi*, *P. modestum*, *P. nicorae*, *P. oteroi*, *P. palustre*, *P. rojasii* e *P. wrigthii*, with the objective of analyzing the occurrence of heterosis for agronomic traits in apomictic hybrids. In this research it was observed that some hybrids presented heterosis for cold tolerance and cattle preference, with high levels of seed fertility and growth capacity. In addition, the authors reported that the efficiency of hybridization and fertility of the generated progeny were dependent on the male parent involved, and that apomictic hybrids exhibiting heterosis for cold tolerance and cattle preference were obtained at crosses between *P. plicatulum* and *P guenoarum*.

Currently, there is no cultivar of the Plicatula group originated by artificial hybridization technique available for commercialization. However, an example that demonstrates the improvement of *Paspalum* species through hybridizations as a tool to make new cultivars available to farms is the release of *P. notatum* cv. Boyero UNNE in 2012. This cultivar originated by the hybridization of a facultative apomictic tetraploid access with a high rate sexual reproduction, pollinated with a highly apomictic tetraploid access, followed by selection. Thus, Boyero UNNE is the first registered cultivar of tetraploid apomictic *P. notatum* that was developed by breeding through a sexual x apomictic hybridization scheme exploring the apomixis and plant selection in the F_1 progeny (Urbani et al., 2017).

1.2.3. Nitrogen: importance for forage performance of grasses

Nitrogen (N) was discovered in the late 18th century through the work of several early chemists–Scheele (1742–1786, Sweden), Rutherford (1749–1819, Scotland), Lavoisier (1743–1794, France). N importance in crop production was recognized in the mid-19th century, initially by Boussingault (1802–1887) and then more thoroughly by von Liebig (1803–1873), who developed the theory of nutrient limitation in plant productivity. Near the end of the 19th century, Hellriegel (1831–1895) and Wilfarth (1853–1904) discovered that microbial communities could extract nonreactive N_2 from the atmosphere and convert it into a useable form, process known as biological nitrogen fixation (Galloway and Cowling, 2002).

Nitrogen gas (N_2) represents about 80% of the total mass of the Earth's atmosphere. To most organisms, this huge N reservoir is not biologically available (Galloway and Cowling, 2002). However, some types of organisms, especially bacteria can fix the N_2 of the atmosphere and make it available to plants (Martinelli, 2007).

Nitrogen cycling in the soil–plant system is very dynamic and complex due to the interaction between several factors such as climate, soil, plant and microorganisms. The addition, transformation, utilization and possible losses of N from soil–plant systems are the main components of N cycling. In commercial crops, most of the N is added to the soil through inorganic fertilizers. However, there are other sources of N addition to the soil, such as biological fixation, gas adsorption and organic fertilizers (Fageria and Baligar, 2005).

Plant demand for N is met by supplying N fertilizer and N mineralization from soil organic matter. N fertilizer is generally applied in a form easily available for plant uptake, whereas N from soil organic matter must be mineralized before it becomes available for plant uptake. The rate of mineralization is largely controlled by the availability of water, and by temperature and aeration (Ladha et al., 2005). In general, N uptake of plants is by the uptake of nitrate (NO_3^-) and ammonium (NH_4^+), although soil organic N can be absorbed and represent a significant proportion of total N absorption under particular situations such as acidic soils and low temperature environments (Gastal and Lemaire, 2002).

Nitrogen is an important part of the nucleic acids that determine the genetic character of all living things and of the enzymatic proteins that drive the metabolic machinery of every living cell (Galloway and Cowling, 2002). It is also constituent of compounds such as chlorophyll and alkaloids (Fageria and Baligar, 2005). Thus, N influences the rates of cell division and cell expansion of growing leaves, as well as the response of leaf photosynthesis to radiation (Gastal and Lemaire, 2002). According to Costa et al. (2013) N is essential for maintenance of the productivity and persistence of forage grasses, since this nutrient constitutes the proteins that actively participate in the synthesis of organic compounds that make up the structure of the plant, and also maximizing the morphogenic (leaf appearance, expansion and senescence rates) and structural (leaf size, tiller density and number of live leaves per tiller) characteristics of the grasses.

The availability of N in amounts lower than those required by the plants compromises the expression of the production potential. However, it is necessary to know the adequate level of this nutrient, capable of economically maximizing the biomass yield, avoiding losses and increasing the efficiency of this nutrient in the productivity of grasses, and consequently in animal performance (Lugão et al., 2003).

Herbage accumulation

Several researches with different forage species, sources and rates of N fertilization have been carried out to evaluate the effect of N on the grass biomass yield. In a study with *Digitaria ciliaris* subjected to different N rates (0, 56, 112, 168, 224, 280 and 336 kg ha^{-1}) and sources (ammonium nitrate and broiler litter), Teutsch et al. (2005) reported an increased in forage production with higher application N rate and observed a maximum yield when more than 300 kg of N was applied. Hennessy et al. (2008) evaluated the influence of four N rates (0, 50, 150 and 250 kg ha^{-1} yr^{-1}) on perennial ryegrass (*Lolium perenne*) for two years and observed an increased dry matter yield with the additional N rates. Castagnara et al. (2011) evaluated the species *Panicum maximum* cv. Tanzania and Mombaça and *Brachiaria* sp. cv. Mulato with increasing N rates (0, 40, 80 and 160 kg ha^{-1}), and reported that N applied increased the dry matter accumulation rate, canopy height and number of tillers. For each 40 kg N ha^{-1} applied there was an increase of 4,600 kg ha^{-1} on biomass yield, and 809 kg ha^{-1} on dry matter yield.

Pitman (2012) evaluated *P. notatum* cv. Pensacola subjected to three rates of N (336, 168 and 112 kg ha^{-1} year^{-1}) and three harvest frequencies (each 4, 8 and 12 weeks), and reported that forage yield, except for the first year was always higher due to the higher N rate and shorter harvest interval. In addition, the cumulative effects of N from the second year may have contributed to benefit production with the higher N rate. Also evaluating *P. notatum* subjected to different sources (six) and N rates (0, 60 and 120 kg N ha^{-1}), Silveira et al. (2013) observed that regardless of the year or N source, dry matter yield increased linearly as N rates increased. In addition, dry matter yield increased by 25 and 50% relative to the control treatment when N was applied at 60–120 kg ha^{-1} respectively. Seepaul et al. (2016) studied the biomass yield of *Panicum virgatum* in response to four N rates (0, 80, 160 and 240 kg ha^{-1} yr^{-1}) for two years. These authors reported that the biomass yield increased by 29% (2008) and 69% (2009) at 80 kg N ha^{-1} compared to the control, but there was no further increases with the additional N rates.

Some studies have evaluated native ecotypes of the genus *Paspalum* and its response to N fertilization. Towsend (2008) evaluated *P. guenoarum*, *P. notatum* and *P. lividum* ecotypes subjected to four N rates (0, 60, 180 and 360 kg ha^{-1} yr^{-1}). This author observed a linear response to the application of N for biomass production, which shows the ability of these ecotypes to respond at rates greater than 360 kg N ha^{-1} and express the maximum yield potential in response to N applied. In another work with *P. notatum* ecotypes also under rates of 0, 60, 180 and 360 kg N ha^{-1} year^{-1}, Machado (2014) observed that dry matter yield doubled when the N rate increased from 60 to 180 kg N ha^{-1}, with a response up to the rate of 360 kg N ha^{-1}. However, the increase in yield was not as effective when compared to the rate of 180 kg N ha^{-1}.

Through economic analysis Cuomo et al. (2005) indicated two general approaches to pasture management; one is a minimal cost (no N fertilization or legumes) and minimal return (low forage production, low stocking rates), and the other is a higher cost approach (inclusion of N fertilization or legumes) with those costs reduced by higher forage production and higher potential stocking rates. Adequacy of an approach depends on an individual producer’s goals and their ability to effectively manage and use additional forage produced with N or inclusion of legumes. In this context, Santos et al. (2008) evaluated the effect of N application (100 and 200 kg ha^{-1}) on forage production, live weight and on the efficiency of conversion of N to animal product in native pasture. These authors reported that fertilization investment is biologically and economically viable, regardless of the N rate up to 200 kg ha^{-1}, as well as the application of 200 kg N ha^{-1} increases the production of live weight and the conversion efficiency of N in animal product.

Evaluating several species and cultivars of grasses, Bartl et al. (2009) reported that N fertilization provided increased dry matter yield and productive differences between species, i.e. when N was not applied the species showed similar performances. Generally, when plants are under unfavorable conditions for their development, for example, such as water or nutrient deficit, the expression of variability between genotypes decreases because they cannot express its yield potential and only try to survive. Mostly plant breeding is conducted in the presence of sufficient nutrients (as N), allowing the new cultivars to express their maximum yield potential.

Nutritive value

Nutritional value may not be considered as important as dry matter yield in many forage systems, but when forage constitutes the majority of the diet of a growing or lactation ruminant, or when the farms chooses not to supplement with concentrate, it assumes substantially greater importance (Brink et al., 2015). The study of chemical parameters such as CP, NDF and ADF is important in analysis of the quality of grasses, since these parameters can influence in animal performance (Van Soest, 1994). According to Costa et al. (2010) high contents of NDF in forage plant limits intake capacity of the animals, while high contents of ADF decreases dry matter digestibility.

Researches with different forage grasses have been conducted to verify the N influence on nutritional value. Hennessy et al. (2008) reported the effect of N (0, 50, 150 and 250 kg ha^{-1} yr^{-1}) on CP content of perennial ryegrass (*Lolium perenne*), especially when there is an increase in applied N rates. Costa et al. (2010) evaluated the biomass yield of *Brachiaria brizantha* cv. Marandu with different N sources (urea and ammonium sulfate) and rates (0, 100, 200 and 300 kg ha^{-1}), and observed a linear increase on CP content with the increasing N rates. In addition, NDF and ADF contents presented a linear decrease with the additional N rates, reducing 8.9 and 26%, respectively compared to 0 kg N ha^{-1}.

In a study with *P. notatum* cv. Pensacola, Pitman (2012) evaluated its chemical composition and digestibility when subjected to three N rates (336, 168 and 112 kg ha^{-1}) combined with three harvest frequencies (4, 8 and 12-week intervals) and observed that CP and digestibility increased, while NDF and ADF decreased when the N rates increased, and the harvest interval decreased. Paulino et al. (2015) studied the effect of increasing N rates (0, 150, 300 and 450 kg ha^{-1}) on the chemical composition and digestibility of *Megathyrsus maximus* cv. Aruana and observed that N application decreased the NDF and FDA contents, and increased the CP content, but had no effect on the digestibility of the grass. These authors reported that applying 450 kg N ha^{-1} increased CP from 15.8% to 24.6% in leaf blades.

Pontes et al. (2016) evaluated six perennial grasses subjected to two N rates (0 and 300 kg ha^{-1} yr^{-1}) and two cutting intensities (removal of 50 and 70% of the canopy height) and verified that N application and the lower cutting intensity resulted in an increase in the nutritional value of the species. In a study conducted with *Panicum virgatum*, Obour et al. (2017) reported that the additional N rates increased digestibility and CP content from 614 and 53 g kg^{-1} (zero) to 664 and 90 g kg^{-1} with 180 kg N ha^{-1} applied respectively.

1.2.4. Nitrogen use efficiency

World population growth, coupled with increased demand for protein, fiber and biofuels, makes it necessary to increase world food production (Snyder, 2009). Nitrogen application can be an option to increase productivity, and to alleviate the ever-increasing food insecurity caused by the worldwide increase in people numbers, as well as by the inequality in food access because of limited land, limited purchasing power, and/or inefficient distribution (Ladha et al., 2005). In the last 50 years, use N fertilizers have increased about 20 times, with an expected increase about 180 million tons by 2030. However, prices have risen more than 2.5 times in the last decade (Verhulst et al., 2015).

There is a greater need to increase the N use efficiency (NUE) in production systems, especially in regions with potential for food production. Plant breeders can contribute to optimizing the NUE of agricultural systems by producing cultivars with high NUE, i.e. cultivars that produce a large amount of biomass per N unit supplied via fertilizers. However, breeding for NUE has not yet been incorporated to a large extent in practical breeding programs of many crops, because NUE is not only a complex trait but also one that is largely influenced by soil conditions that are difficult to measure or control (Van Bueren and Struik, 2017).

NUE may be defined as the yield obtained per unit of N available in the soil (supplied by soil + N fertilizer), which is the product of the absorption efficiency (amount absorbed N/amount of N available) and the utilization efficiency (yield/absorbed N) (Cassman et al., 2002; Hirel et al., 2011). Put very simply, plants can either use the N they take up more efficiently, doing more with less (increased utilization efficiency), or they can increase the amount of N they acquire from the soil (increased uptake efficiency) (Garnett et al., 2009).

The determination of NUE in plants is an important approach to evaluate the fate of applied chemical fertilizers and their role in improving crop yields (Fageria and Baligar, 2005). There is a need to compare the performance of genotypes at various N levels, since genotypes that showing good performance and are efficient under high N, are not always the same that showing the highest performances and efficiency under low N input (Van Bueren and Struik, 2017).

Improving NUE is more difficult than for any other fertilizer nutrient. This is because N mobility in soil–plant systems is high and variable. In addition, many sources of addition and loss pathways of N in soil–plant systems occur, which complicates N balances and N use by plants. Nitrogen sources and methods of application significantly influence N uptake efficiency in crop plants. Generally, urea and ammonium sulfate are the principal sources of N fertilizers (Fageria and Baligar, 2005).

The development and preferential planting of cultivars that have greater NUE are essential, since they will result in lower N–input requirements. Although plant breeders concentrated in the past mainly on improving potential yield, there is increased emphasis on aspects such as the nutritional value of foods and biotic and abiotic stress tolerance. Thus, improvement of cultivars to increase production stability or reduce nutrient losses may contribute to increase NUE, improving economic results (Ladha et al., 2005). NUE is controlled by a complex array of physiological and environmental interactions that are specific to the genotype of a given species. It is therefore essential that an extensive survey of a wide range of genotypes covering the genetic diversity of a crop should be performed (Hirel et al., 2011).

Studies with several species, cultivars and rates of N fertilization have been performed to quantify the NUE. In a study with *Panicum maximum* subjected to different N rates (0, 150, 300 and 400 kg ha^{-1}) Lugão et al. (2003) found a decrease on NUE with the increase of N rates applied. Costa et al. (2010) evaluated the application of 50, 100 and 200 kg N ha^{-1} in *Axonopus aureus*, and observed a NUE of 29.8, 19.9 and 10.7 kg DM kg^{-1} N applied respectively. Castagnara et al. (2011) evaluated the species *Panicum maximum* cv. Tanzania and Mombaça and *Brachiaria* sp. cv. Mulato subjected to four N rates (0, 40, 80 and 160 kg ha^{-1}), and reported that the maximum NUE was obtained with the rate of 106 kg N ha^{-1}, which showed in this study that forages evaluated did not showed potential to use efficiently additional N rates.

In a study with *Brachiaria plantaginea*, Sartor et al. (2011) tested two N rates (200 and 400 kg ha^{-1}) and two grazing intensities, and observed that NUE with application of 200 kg N ha^{-1} (30.9 kg DM kg^{-1} N) was three times higher when compared to 400 kg N ha^{-1} applied, which showed a NUE of 10.4 kg DM kg^{-1} N. Obour et al. (2017) studied the application of different N rates (0, 45, 90, 135, and 180 kg ha^{-1}) in *Panicum virgatum*, and verified that the NUE decreased linearly with the increase of N rate. These authors reported that the NUE decreased from 46 kg kg^{-1} N applied at 45 kg N ha^{-1} to 22 kg kg^{-1} N applied when fertilized with 180 kg N ha^{-1}.

Towsend (2008) evaluated NUE in *P. guenoarum*, *P. notatum* and *P. lividum* species subjected to different N rates (0, 60, 180 and 360 kg ha^{-1}), and observed that NUE ranged from 47 to 11 kg DM kg^{-1} N for *P. notatum* Bagual, with 60 kg of N and *P. guenoarum* Baio, with 360 kg of N applied, respectively. In addition, this author reported that the maximum NUE was observed with N rates between 60 and 180 kg ha^{-1} yr^{-1}. Therefore, to provide sufficient N to supply the growing demand for food and maintain a safe environment, the N fertilizer should be used with care while seeks to improve the NUE of the plants.

1.2.5. Nitrogen fertilizer and environmental problems

Some underprivileged populations in the world have difficulties to food produce because there is not enough N to supply to the crops. On the other hand, in certain regions of our planet, this nutrient is supplied to crops in a much larger quantity than is necessary. In these situations, the N becomes a pollutant, because its presence in excess causes a series of reactions and processes extremely harmful to the environment, and to the health of the populations (Martinelli, 2007).

When N fertilizer is added in the field, part of N is uptake by the plant and eventually reaches our table in the form of food. Unfortunately, plants are not able to uptake all the N that is applied to the soil, and part of this nutrient will be lost to water bodies and/or atmosphere (Martinelli, 2007). Lost N can have negative impacts on the quality of air and water, as it is associated with the formation of atmospheric pollution, global warming effects and depletion of stratospheric ozone, and negatively affects the quality of groundwater and surface water (Schröder, 2014).

Nitrogen undergoes various transformation processes in soil. The main loss pathways are (i) leaching, predominantly nitrate (NO_3^-) but also occasionally ammonium and soluble organic N; (ii) denitrification, resulting in emissions of nitrous oxide (N_2O), nitric oxide (NO), and dinitrogen (N_2) gases; and (iii) volatilization with ammonia (NH_3) losses (Ladha et al., 2005; Schröder, 2014; Verhulst et al., 2015).

Leaching of NO_3^- to the surface waters causes the phenomenon of eutrophication, which promotes a high algae production (Verhulst et al., 2015). After death of these algae, the decomposition process begins. Decomposing organisms use oxygen (O_2) dissolved in water to obtain the energy required by the decomposition process. The lack of dissolved O_2 causes a series of chemical and biological changes. Most known is fish mortality that regularly occurs in rivers, bays and estuaries (Martinelli, 2007).

The magnitude of the loss of N by leaching (NO_3^-) to groundwater depends on soil characteristics, management practices, and climatic conditions. The time taken by NO_3^- to move from the root zone to the water table may vary considerably. In sandy soils characterized by high percolation rates and shallow water tables and with high rates of fertilizer application, NO_3^- may reach the water table in a matter of days with irrigation or rainwater. Conversely, in heavy soils with deep water, low rainfall and low rates of N application create conditions whereby NO_3^- may take more time to reach the groundwater (Ladha et al., 2005).

Water contamination by the NO_3^- is slow and gradual, and the increase in NO_3^- concentration is usually only perceived when it reaches critical levels that are detrimental to human health (Carvalho and Zabot, 2012). Concentrations of NO_3^- in water for human consumption may not exceed the potability values of 10 mg L^{-1}, established by Ordinance No. 2,914 of the Ministério da Saúde (Brasil, 2011). High concentrations of NO_3^- in water for the human consumption may cause thyroid gland dysfunction, carcinogenic nitrosamines and nitrosamides production, and in babies a decrease in the ability of the blood to carry O_2 known as methemoglobinemia (Fernandes et al., 2017).

In a study conducted in the Médio Alto Uruguai region of southern Brazil, Fernandes et al. (2017) evaluated the relationship between total N rates applied annually via organic fertilizers (pig manure and poultry litter) and chemicals in perennial pastures, and the concentrations of mineral N found in soil, pasture, and leached water. Positive correlation (r = 0.49) was observed between the amount of N applied via fertilizer and the NO_3^- content in the leached water. For the authors, this correlation demonstrates the great polluting potential of the excess application of fertilizers in perennial pastures of the Médio Alto Uruguai region, which may be compromising the quality of the water resources.

Also, about environmental aspects, use N fertilizers contribute to the production of greenhouse gases during its manufacture, when natural gas is combined with atmospheric N2 to produce ammonia and carbon dioxide (CO_2). Following application, N fertilizer also stimulates soil bacteria to emit nitrous oxide (N_2O), which favors the emission of this greenhouse gas, which affects global warming (McSwiney et al., 2010). N_2O is emitted in less quantity than the other gases of greenhouse effect, nevertheless, it has a global warming potential 296 times higher than the CO_2 molecule (Costa et al., 2009).

Another important aspect about N fertilizers is the loss of N in the form of ammonia (NH_3), known as volatilization. The main loss occurs through N ureic and can reach 70% of the applied fertilizer. The higher inefficiency of urea applied to the surface arises from the volatilization of NH_3 due to the hydrolysis of urea by the enzyme urease (Costa et al., 2009; Doe, 2013). NH_3 may cause a risk to human health when concentrations reach critical levels, because when large quantities are released into the atmosphere, they interfere with air quality and can cause respiratory problems (Rotz et al., 2014).

According to Martinelli (2007), this N duality – extreme need versus pollutant effect is one of the biggest challenges that humanity will have in the next decades. Especially if N is considered a limiting factor in agriculture and based on the law of supply and demand, N becomes a commodity.

1.2.6. Grass-legume mixture in pastures

Biological N fixation (BNF) by legumes is a spectacular biological phenomenon in nature and its importance for adding N to agricultural systems is enormous. Similarly, the contribution of biological N fixation to reducing costs of crop production and maintaining soil quality is of paramount importance (Fageria and Baligar, 2005). Use of N_2 fixing legumes species to replace fossil fuel–based N fertilizers exemplifies a convergence of agricultural and energy sustainability (Ashworth et al., 2015).

There are several advantages to cultivating grass–legume mixtures in pastures. Legumes have the capacity of BNF, which can be used by the grass. This may compensate for the need annual of N application in the grass. The mixtures are also more productive and have higher nutritive value than grass alone. In general, grass–legume mixtures will usually result in better forage production and animal performance than a single species grown alone (Tekeli and Ates, 2005; Barcellos et al., 2008; Lüscher et al., 2014).

In research conducted with several species of grasses and legumes mixture, Sanderson et al. (2010) reported that CP content was positively correlated with proportion of legumes and negatively correlated with proportion of grasses when evaluating the two types of species mixed. The NDF content was positively correlated with the proportion of grasses in the mixture. These relationships between the nutritive value and the proportion of species in the mixture follow the trend that legumes have a higher CP and lower NDF content than grasses. Ergon et al. (2016, 2017) evaluated grasses (*Lolium perennial* and *Festuca arundinacea*) and legumes (*Trifolium repens* and *T*. *pratense*) in pure and mixture stand and observed that the mixture provided higher intra-annual yield stability and nutritive value compared to the pure stand. For the authors, this result can be because grasses and legumes utilize growth conditions differently during the consecutive growths, as they have different seasonal patterns of stem formation and reproductive development.

There are some problems with legume cultivation, which limits its use by producers. Mostly market demand is often weak, at least initially, so that seed companies are reluctant to invest in a new crop. Even when there is demand, it is often for such small quantities that retailers cannot justify carrying inventory. Legumes typically require greater amounts of phosphorus (P) and potassium (K) fertilizer, the costs of which have increased drastically over the past years. Thus, the reduction in N fertilizer cost can be nullified by increased cost with limestone and P and K fertilizer (Butler and Muir, 2012). In addition, legumes seed prices it tends to trend in stride with the price of N; that is, when the price of N increases, so does the price of legume seed (Biermacher et al. 2012).

Mixture management is more complex than pure pasture since it includes effects such as competition between species and animal selectivity over components in the community (Barcellos et al., 2008). In general, failure to adopt and use mixtures is attributed to the low legumes persistence, which in many cases is associated with lack of specific or efficient management techniques and inadequate fertilization for these pastures (Aroeira et al., 2005). Thus, establishing grass-legume mixtures, not only requires selection of adapted species that are compatible, but also optimum proportions between different species in the mixture (Adjesiwor et al., 2017), determining the persistence and contribution of the legumes to the soil-plant-animal system.

According to Biermacher et al. (2012) it is important to reemphasize that currently, managing synthetic N sources is relatively simple for most producers. In fact, this activity usually only requires producers to request that a local fertilizer dealer apply the N in their pastures, or the producers themselves perform such activity. Legume systems require additional management by producers, such as which legume species are ideal for their locality and mixture management strategies (Butler and Muir, 2012). Tekeli and Ates (2005) reported that the choice of what species to grow on a mixture should be based on (i) species adaptation to the site, (ii) species response to the grazing system, (iii) potential forage yield and seasonal distribution, (iv) nutritional value, and (v) persistence.

The amount of N fixed by the legume varies with species and environmental conditions. Soil acidity, salinity, deficiency or excess of minerals, water stress, variations in temperature, amount of inorganic N in the soil, pests and diseases influence in the BNF. The lower N in the soil will resulted in greater proportion of N for plant derived of BNF, which will be above 85% (Barcellos et al., 2008). The amount of N available from legumes depends on the species of legume grown, the total biomass produced, and the percentage of N in the plant tissue. Cultural and environmental conditions that limit legume growth, such as a delayed planting date, poor stand establishment, and drought will reduce the amount of N produced. Conditions that encourage good N production include getting a good stand, optimum soil nutrient levels and soil pH, good nodulation, and adequate soil moisture (Hirel et al., 2011).

Nitrogen transfers will occur below and above of soil surface through by the N excretion in the rhizosphere of the legume, roots and nodules decomposition, mycorrhizal connection of the grass roots with those of legume, and/or by the action of fauna of the soil on roots and nodules of the legume. In the soil surface will occur by the litter decomposition of surface leaves (Barcellos et al., 2008).

Legumes have roots that, especially in the case of perennials, allow them to penetrate deeper into soil profiles in search of moisture and nutrients. Generally, their disadvantages include slow recovery from herbivory, less seed production and poor seedling vigor compared with most grasses. Grasses have fibrous root systems that give them an advantage when competing for shallow moisture and soil nutrients. They also tend to establish more easily, grow more rapidly and recover from grazing more quickly. Only when soil N is low are grasses at a disadvantage relative to legumes (Muir et al., 2011).

According to Reis et al. (2006) some points should receive a greater focus of the research with the objective of using more BFN in grasses. Among them are: (i) genotype selection, due to the existence of variability between them; (ii) knowledge of which bacterium or group should be the best combination with the most promising genotype; (iii) environmental factors related to process efficiency, such as temperature, humidity, luminosity, N availability in the soil, association with other microorganisms and interaction with native microflora; (iv) modifications in both the plant and bacterium, for the improvement of this association.

Barcellos et al. (2008) reported that even with an important contribution for cattle production in pasture and studies already carried out by different teaching and research institutions, use of legumes in pastures in the Brazil is still limited. Thus, to change this situation the use grass-legume mixture should generate the perception of low risk and low-cost technology. Therefore, adoption should be based on knowledge of the potentials and limitations of the cultivars and the detection of the best opportunities for inclusion in production systems, to maximize the use of this technological option.

1.2.7. Legumes characteristics used in the research

White clover (*Trifolium repens* L.)

White clover is a widely distributed legume species in the world. It is originating in the countries of the Eastern Mediterranean or Asia Minor. Its dispersion to other continents was associated with colonization and presence of grazing domestic animals. It is a constituent of the flora of all the continents, which confirms its wide distribution (Carvalho et al., 2010). It is characterized as a glabrous, creeping and stoloniferous plant. It grows close to the soil, expanding through vigorous stolons, which lengthen and form roots at nodes when the soil has moisture (Dall'Agnol and Scheffer-Basso, 2004).

It's a plant of temperate climate, does not tolerate high temperatures. It develops well in neutral soils and in those that contain high level of organic matter. It's tolerant to frost and shading (Fontaneli et al., 2009). Temperature suitable for growth is between 20 and 25 °C. In general, it shows a slower growth than temperate grasses at temperatures below 10 °C, and faster at temperatures above 20 °C (Carvalho et al., 2010). It presents low tolerance to acid soils preferring clayey and corrected soils or with pH around 6.0 with good fertility, and humidity to enable its establishment and persistence (Dall'Agnol and Scheffer-Basso, 2004; Scheffer-Basso et al., 2005). Phosphorus and K applications are required annually for legume maintenance (Carvalho et al., 2010). The sowing season of the white clover is from March to June. It can be sown in pure stand, mixture with grasses or overgrown in native pastures (Carvalho et al., 2010). It can be sown with ryegrass, red clover, birdsfoot trefoil and tall fescue (Fontaneli et al., 2009).

White clover has gems in its stolons that can develop into ramifications or floral buds. Therefore, even with intense grazing or frequent cuts, in the season of intense flowering there is seed production, because the stolons are at soil level, and for maturation of the seeds are necessary only 20 to 30 days in hot periods (Paim and Riboldi, 1994). According to Brink et al. (1999) greater proportion of stolon nodes that produce branches and become rooted are examples of mechanisms that improve vegetative persistence of ecotypes in heat and drought stressed environments subject to frequent defoliation. In addition, the ability to produce an abundance of seeds under continuous stocking can be an additional persistence mechanism. The importance of the stolon is related to the persistence of the plant, since the permanence of the clover in the pasture results of the continuous stolons or seeds production (Bortolini et al., 2006).

White clover develops stolons near soil surface which provide tolerant to intense defoliation, since its growth points are protected of the grazing. Thus, this legume under intense grazing in association with other species such as ryegrass or birdsfoot trefoil prevails in the mixture. In this case, when it becomes the main component of diet of the animals, its high content of degradable proteins may be providing conditions for ruminal bloat (timpany) occurrence (Carvalho et al., 2010).

White clover is one of the most important forage legumes of winter production, mainly for direct grazing in humid temperate and subtropical regions (Paim and Riboldi, 1994). It presents good forage yield and high nutritional value (Dall’Agnol and Scheffer-Basso, 2004), and is used under continuous stocking, since it is adapted to produce under conditions of intense defoliation, increasing protein content of the forage harvested by the animals (Carvalho et al., 2010).

According to Brink et al. (1999) white clover is a vital component of permanent pasture systems in New Zealand and Great Britain, contributing to greater animal performance and reduced reliance on N fertilizer. In Rio Grande do Sul (RS) State, is most used legume species in the improvement of natural pastures (Scheffer-Basso et al., 2005), as well as in pastures mixture for the use grazing during winter and spring.

In years with regular rainfall distribution, the white clover behaves as a perennial species. However, in years with water deficiency during the hot period, many plants die and the survival of stolons is reduced and can behave as an annual species. High temperatures restrict its growth and production, which requires a good water supply (moisture) for normal growth (Paim and Riboldi, 1994; Dall’Agnol and Scheffer-Basso, 2004).

In subtropical conditions this species has its persistence compromised by the occurrence of hot and dry summers. This promotes high mortality of stolons, which delays growing in fall (Scheffer-Basso et al., 2002). In study with white clover accesses in two RS physiographic regions, Schneider et al. (2011) reported that it is possible to select more productive and persistent genotypes in summer conditions. For these authors, plants selection able to survive in unfavorable conditions in hot season could improve the yield and increase the period of use of this forage species, specifically in hottest regions. White clover has great capacity of fixing of atmospheric N_2 and the amount of N_2 fixed can reach more than 500 kg ha^{-1} yr^{-1} (Assmann et al., 2007). Enriquez-Hidalgo et al. (2015) evaluated white clover in a mixture with perennial ryegrass (*Lolium perenne*) subjected to different N rates (0, 60, 120, 200 and 240 kg N ha^{-1}) and observed a variation in N_2 fixation from 52 to 291 kg N ha^{-1}, which decreased when N rates increased.

In a study carried out in Depressão Central region of RS, Rocha et al. (2007) obtained a production for the white clover in a pure stand of 3,054 kg DM ha^{-1}. On the other hand, in Planalto Médio region of RS, when the species was sown in a mixture with tall fescue (*Festuca arundinacea*) and ryegrass (*Lolium multiflorum*), Scheffer-Basso et al. (2002) verified a production of 6,000 kg DM ha^{-1} for cv. Yi. According to the authors, the rainfall that occurred during spring-summer contributed for performance of the species (stolons expansion), and consequently provided a reduction in weeds presence. In well-balanced mixture of white clover and grasses, the average daily gains of growing cattle may be greater than 1,200 g $animal^{-1}$ (Carvalho et al., 2010). According to Fontaneli et al. (2009), when this legume constitutes perennial winter and summer pastures, estimates of animal performance range from 300 to over 760 kg ha^{-1} of live weight gain.

Birdsfoot trefoil (*Lotus corniculatus* L.)

Birdsfoot trefoil has a natural distribution in Western Europe and North Africa, and secondary distribution in the Northeast and Midwest of the United States, Southeast Canada, Southern Latin America, Eastern and Central Europe, and parts of Asia (Carvalho et al., 2010). In Brazil, species is cultivated in the South region, where the subtropical climate is more appropriate for its development and the production cycle is concentrated in the spring-summer season (Scheffer-Basso et al., 2011). It is characterized as a perennial and glabrous plant with pinnate leaves composed of three apical leaflets and two distal basal leaves. It has branching and pivoting root system (Fontaneli et al., 2009).

It is a forage legume that presents versatility (grazing, hay), acidity tolerance and lower requirement of soil fertility when compared to other temperate legumes (Soster et al., 2004; Scheffer-Basso et al., 2005). Contain secondary metabolites named “condensed tannins” that appear to prevent ruminal bloat by acting as protein precipitants in the rumen (Dalrymple et al., 1984). Thus, cases of ruminal bloat are rarely reported, even in pastures dominated by the birdsfoot trefoil. Although it is not very demanding with respect to soils, it responds to the fertility correction, especially P. It is tolerant to pH below 6.0 up to 4.8 (Carvalho et al., 2010).

Birdsfoot trefoil has cold tolerance and prefers climates from cold temperate to medium temperate with frost resistance. However, it presents better adaptation in temperatures around 24ºC. It is very well adapted to most of the soils and RS regions, especially in the most drought regions. For this reason, it is one of the preferred legumes for the Campanha region of RS (Scheffer-Basso et al., 2002). Its tolerance to water deficiency derives from its pivoting roots system that deepens in the soil seeking water in deeper layers (Carvalho et al., 2010).

It is a species of erect growth. Thus, their management should be done with care to maintain a larger area of leaves and avoid the removal of growth points, which are mostly well above the soil surface. Its height can vary between 50 and 80 cm not grazing (Carvalho et al., 2010). It is widely used for hay production, demanding 7–10 cm stubble height to not harm regrowth. The most intense growth of the birdsfoot trefoil is from the middle of July to November. It has low shading tolerant and is harmed in mixture with tall species and with high biomass production (Fontaneli et al., 2009). In the grazing situation, canopy persistence depends on the management to ensure the natural sowing and/or establishment of new plants (Scheffer-Basso et al., 2011).

The sowing season of the birdsfoot trefoil is from April to June and can be established by broadcast or in rows 20 cm apart (Fontaneli et al., 2009). It presents a slow establishment and reaches its maximum production only after one year (Carvalho et al., 2010). In this context, Silveira et al. (2015) evaluated different sowing densities: 0, 50, 100, 150 and 200% more than recommended amount of seed (6 kg ha^{-1}), in a mixture with ryegrass (*Lolium multiflorum*), both overgrown in pasture African star (*Cynodon nlemfuensis*). These authors observed that use of 150 and 200% more than recommended sowing density of the birdsfoot trefoil increases its forage production but does not affect the total forage production of the mixture.

According to Fontanelli et al. (2009) birdsfoot trefoil can produce from 15,000 to 19,000 kg ha^{-1} of green fodder, which corresponding to about 4,000 to 6,000 kg DM ha^{-1}. In New Zealand, Ramírez-Restrepo et al. (2006) reported in a dryland commercial farming system characterized by soil moisture stress in summer that birdsfoot trefoil cv. 'Grasslands Goldie' growing in a moderately fertile and acidic soil exhibited similar levels of seasonal and total dry matter production to that of perennial ryegrass/white clover over 3 years, with both forages averaging 8,000 DM ha^{-1} yr^{-1}. The annual production of birdsfoot trefoil ranged from 5,300 to 8,500 DM ha^{-1}. However, field observations suggested that the agronomic performance of birdsfoot trefoil in the last year decreased due to reduced plant population because of the grazing frequencies and/or grazing intensities used.

Scheffer-Basso et al. (2002) evaluated the birdsfoot trefoil in a mixture with tall fescue (*Festuca arundinacea*) and observed that legume is a good option for forage production, since it accumulated in 91 days, about 3,500 kg DM ha^{-1}, and by the lower weeds amount in summer and fall season. Rocha et al. (2007) reported a production of 6,627 kg DM ha^{-1} in the Depressão Central region of RS.

Alison and Hoveland (1989) evaluated the dry matter yield and digestibility of the birdsfoot trefoil subjected to harvest intervals of 21, 28 and 42 days. These authors verified that the digestibility was not influenced by the harvest intervals and ranged from 70.3 to 76.7%, whereas the dry matter production presented was 5,420, 7,880 and 9,850 kg ha^{-1} for the harvest intervals of 21, 28 and 42 days respectively. In a study with different birdsfoot trefoil genotypes, Soster et al. (2004) reported that the legume presented content of 11.2% to 25.5% of CP, 51.8 to 65.5% of NDF and 17.1 to 41.8% of FDA, which vary due to physiological stage during the productive cycle.

1.2.8. Use of legumes x nitrogen fertilizer

The shared ambition of societies all over the world is to produce more food, fiber, biofuel and other raw materials without a proportional increase of inputs such as N and land. Currently, if the use of mineral fertilizer N had to be replaced by N fixing legumes, up to twice as much arable land would be needed to grow these N fixers. On the other hand, industrial N fixation hardly consumes scarce land but depletes fossil fuel reserves by using, yet, around one cubic meter of natural gas per kilogram N (Schröder, 2014). According to Lüscher et al. (2014) industrial production of each kg of inorganic N emits 2.25 kg of CO_2. Legumes offer a big advantage because the entire C needed for symbiotic N_2 fixation comes directly from the atmosphere via photosynthesis and, thus, they are considered to be “greenhouse gas neutral”.

Over the past several years, prices synthetic sources of N have trended increasingly higher. With these higher prices, producers are searching for alternative strategies for managing N fertilizer requirements for their pastures (Biermacher et al., 2012). In this case, biological N fixation by legumes incorporated in to grass pastures could be a good alternative to replace the use of synthetic N fertilizers, and to provide a sustainable production of forage with high nutritional value (Interrante et al., 2012).

According to Brennan and Evans (2001) the economic benefit of a legume-derived N compared to synthetic N fertilizers depends on the legume of choice and the cost of the N fertilizer during time of assessment. Some researches have been conducted with the purpose of evaluating the agronomic performance and economic cost of different species of grasses in mixtures with legumes or fertilized with N synthetic.

Assmann et al. (2004) evaluated the effect of N rates on a ryegrass (*Lolium multiflorium*) and oat (*Avena strigosa*) pasture with presence and absence of white clover (*Trifolium repens*) in a crop-livestock integration system, and observed that the accumulation daily dry matter, dry matter yield, stocking rate and live weight gain per hectare were positively influenced by the increase in applied N rates. In addition, the authors reported that the mixture between grasses with white clover allows the increase of the grazing period and consequently results in better animal performance.

Enriquez-Hidalgo et al. (2015) evaluated perennial ryegrass (*Lolium perenne*) in pure stand or mixture with white clover (*Trifolium repens*) subjected to different N rates (0, 60, 120, 200 and 240 kg ha^{-1}) for three years. These authors observed that the presence of white clover in the swards increased total herbage harvested across all N application rates. An increase of 1% in white clover content represented a 174 kg DM ha^{-1} increase in herbage harvested. However, the application of N fertilizer reduced the potential of white clover to compete with the mixture grass. Every 1 kg of fertilizer N applied reduced the white clover yields by 9.9, 8.2 and 6.3 kg DM ha^{-1} in 2011, 2012 and 2013, respectively.

In a study evaluating the nutritive value of forage and the animal production in mixture of *Coastcross* and forage peanut, with or without N, Barbero et al. (2010) reported that the nutritive value of mixture pastures without the addition of N is lower than that pasture fertilized but is satisfactory for good animal performance. According to the authors, the production by area and animal is impaired in pastures that do not receive N through mineral fertilization; however, mixture is an alternative for the beef production, for minimize the use of inputs and decrease the production costs.

Martuscello et al. (2011) evaluated *Brachiaria decumbens* with N (50 and 100 kg ha^{-1}) or in mixture with legumes (*Stylosanthes guianensis* cv. Mineirão and *Calopogonium mucunoides*) and concluded that the use of *S. guianensis* in mixture with *B. decumbens* resulted in forage production similar than that N fertilizer. According to the authors, these results show that the use of *S. guianensis* can be an alternative for to replace the N fertilizer, which represents economy with fertilization, as well as improve forage quality for the animals, since the legumes have better nutritional value than grasses.

Adjesiwor et al. (2017) evaluated the nutritive value of mixture between one grass (*Bromus biebersteinii*) with legumes (*Medicago sativa*, *Onobrychis viciifolia* and *Lotus corniculatus*) compared to the use of N (0, 56 and 112 kg ha^{-1}) in grass. These authors suggested that mixture containing legumes species will be more viable strategies for increasing CP concentration than N application to grass monocultures. In addition, the mixture showed lower concentrations of NDF and ADF than grass with or without N, and greater digestibility when N was not applied.

According to Sanderson et al. (2009) using grass-legume mixtures for grazing dairy cattle was more economical on a whole-farm basis and less risky than grass + N pastures. The increased forage production from the mixtures reduced purchased feed inputs. In all the pasture scenarios modeled, increasing stand life of the pastures increased economic returns. Even with a shorter stand life, the grass-legume mixture was more profitable compared with a long-lived and lower yielding grass + N pasture.

Biermacher et al. (2012) evaluated the animal performance in three N supply systems: bermudagrass (*Cynodon dactylon*) plus 112 kg N ha^{-1}, bermudagrass interseeded with white clover (*Trifolium repens*) and hairy vetch (*Vicia villosa*), and bermudagrass interseeded with alfalfa (*Medicago sativa*). Under the conditions of this research, the authors observed a similar average daily gain among the evaluated systems. However, the total gain per hectare was higher in the bermudagrass + N system compared to the legume systems, which was attributed to the greater number of grazing days performed in this system.

Interrante et al. (2012) compared the agronomic performance of a tall fescue–legume (*V. villosa*, *Pisum sativum* and *T. vesiculosum*) system to a tall fescue–N fertilizer (112 kg ha^{-1}) system for cattle production and observed that tall fescue–N system had 28% more total grazing days compared to the tall fescue–legume (429 and 334 steer grazing d ha^{-1}, respectively). The greater number of grazing days in the tall fescue–N system was attributed to limited production of the legumes due to competition with tall fescue during establishment. On the other hand, average daily gain was similar between the systems (0.69 and 0.71 kg per head d^{-1}, respectively). For the authors, the greater forage quality associated with the legumes in the tall fescue–legume system may have lessened the effect of less forage allowance, possibly resulting in similar average daily gain between systems.

According to Crews and Peoples (2004) the energetic basis of N_2 fixation in legume versus fertilizer-based systems is the greatest factor that differentiates the sustainability of these two N sources. Nitrogen biologically fixed by legumes is ultimately derived from solar energy, while fertilizer N requires the use of non-renewable fossil fuels. As the uses of known commercial energy resources are constrained in the next century due to global warming or resource exhaustion, many countries may not be able to count on an uninterrupted supply of energy or N imports due to poverty or political conflicts.

1.3. HYPOTHESIS AND OBJECTIVES

We hypothesized that *Paspalum* interspecific hybrids (*Paspalum plicatulum* 4PT x *P*. *guenoarum* Azulão and Baio) shows differences in agronomic and nutritional characteristics when subjected to variable N application rates or in mixture with temperate legumes.

The objectives of this thesis were: (i) to evaluate biomass yield (total and leaf dry matter), N use efficiency, nutritive value (crude protein, neutral and acid detergent fiber, and digestibility), cold tolerance and plant persistence in hybrids of *Paspalum plicatulum* x *P*. *guenoarum* subjected to N application rates and (ii) to compare biomass yield and nutritive value of the grass–legume system to a grass–N fertilizer system. In addition, (iii) to select the best hybrids for further steps within the breeding program.

2. CHAPTER II[1]

Agronomic Evaluation in Hybrids of *Paspalum* Subjected to Nitrogen Fertilization or in Mixture with Legumes

[1]Prepared in accordance with the standards of the Agronomy Journal.

Agronomic Evaluation in Hybrids of *Paspalum* Subjected to Nitrogen Fertilization or in Mixture with Legumes

Core Ideas

- Nitrogen supply may affect agronomic performance in *Paspalum* interspecific hybrids.
- The effect of N fertilizer on biomass yield, NUE, cold tolerance and persistence was evaluated.
- Total-DMY, Leaf-DMY, cold tolerance and persistence were higher when increasing N rates, while NUE was lower.
- Total-DMY for grass-legume mixture was similar to the N rates of 60 and 120 kg N ha^{-1}.
- Hybrid 1020133 had Total-DMY similar to Azulão and Aruana, while Leaf-DMY was greater than Aruana.

ABSTRACT

Studies on factors that may affect agronomic performance in hybrids of *Paspalum* are needed to develop information about management decisions and cultivar selection. The goals of this study were: determine dry matter yield (DMY), cold tolerance, persistence and N use efficiency (NUE) in four hybrids of *P*. *plicatulum* x *P*. *guenoarum* subjected to N rates, compare agronomic performance of the grass–legume system to a grass–N fertilizer system and select the best hybrid for further evaluations. Treatments consisted of a split-plot arrangement of five N rates (0, 60, 120, 240, and 480 kg ha^{-1} N), and one grass-legume mixture (*Trifolium repens* + *Lotus corniculatus*) as whole plots, and six genotypes (1020133, 102069, 103084, 103061, *P. guenoarum* ecotype Azulão and *Megathyrsus maximus* cv. Aruana used as controls) as subplots. The experimental design was a randomized complete block, with three replications. Higher N rates increased Total-DMY, Leaf-DMY, cold tolerance and persistence but decreased NUE. Higher NUE is obtained between 60 and

120 kg N ha^{-1}. Total-DMY for grass-legume mixture was similar to the N rates of 60 and 120 kg N ha^{-1}. Hybrid 1020133 had Total-DMY similar to the controls, as well as Leaf-DMY greater than Aruana. Hybrid 1020133 showed greater cold tolerance and exhibited greater NUE at 60 kg N ha^{-1}. Hybrid 1020133 should be selected for new studies within our breeding program. There is opportunity to increase grasses agronomic performance through N management and genotype selection. Grass-legume mixture can be an alternative to replace the application of N fertilizer.

Abbreviations: Total-DMY, total dry matter yield; Leaf-DMY, leaf dry matter yield; NUE, nitrogen use efficiency.

2.1. INTRODUCTION

Livestock production depends largely on grasslands, which are the main feeding system in tropical and subtropical regions (Pontes et al., 2016). Soil fertility is an important factor in production and longevity of pastures in grazing systems. Thus, when nutrient deficiency is a limiting factor in the soil, application of synthetic nitrogen fertilizers or biological nitrogen fixation, in addition to increasing yield may have a greater impact on persistence of forage plants.

Nitrogen (N) is an important nutrient for plant growth, and therefore for pasture productivity and persistence. It is a constituent of proteins that actively participate in the synthesis of organic compounds, which make up the structure of the plant in addition to maximizing the morphogenetic and structural characteristics of the grass (Lavres Jr et al., 2004; Costa et al., 2013). Pasture management techniques and breeding approaches to improve N use efficiency (NUE) are required under both conditions that favor over-fertilization, for example subsidized economies with high fertility, and systems where shortage of N is chronic, for example semiarid and arid regions in developing countries (Lemaire and Gastal, 2009). Several studies reported the positive effect of N fertilizer on forage yield of different perennial warm-season grass (Towsend, 2008; Seepaul et al., 2016; Pontes et al., 2016). However, prices for synthetic N sources have increased over the past several years, and these higher prices may have influence profitability of forage/livestock producers (Interrante et al., 2012). Industrial production of N fertilizer uses non-renewable reserves of fossil fuels. Moreover, N losses can have negative impacts on the environment, as it is associated to global warming effects and contamination of groundwater and surface water (Schröder, 2014).

Considering the high N costs, an alternative approach for increasing production without additional N inputs and attendant to environmental regulations is desirable. Grass-legume mixtures can be considered a viable alternative to synthetic N fertilizer application to grass monocultures (Cox et al., 2017). The use of N-fixing legumes in pastures to replace commercial N has been reported by Interrante et al. (2012) with hairy vetch (*Vicia villosa*), field pea (*Pisum sativum*) and arrowleaf clover (*Trifolium vesiculosum*), and by Biermacher et al. (2012) with white clover (*Trifolium repens*), hairy vetch and alfalfa (*Medicago sativa*). Forage legumes have the potential to transfer N fixed to grasses and contribute to increase biomass yield and nutritive value of the pasture (Lüscher et al., 2014; Cox et al., 2017).

The grass genus *Paspalum* L. is an important forage constituent in natural grasslands in South America (Chase, 1929), where presents a great diversity of species and large area covered, which support the importance of the genus for livestock production (Novo et al., 2016). *Paspalum* species have been genetically improved in the United States, Brazil, and Argentina with the aim of generating new forage cultivars for subtropical regions (Acuña et al., 2009; Aguilera et al., 2011; Weiler et al., 2018). Plicatula group is a taxonomic informal category within of the genus *Paspalum*, described by Chase (1929). Particularly, *P. plicatulum* and *P. guenoarum* species were introduced to cultivation and sown in other areas of the world. Because these species are primarily tetraploid and apomictic, the released cultivars were selected from natural ecotypes and without any genetic improvement through breeding (Aguilera et al., 2011).

The collection of a sexual diploid ecotype of *P. plicatulum*, and its chromosome duplication resulted in the generation of a sexual tetraploid plant, allowing new genetic studies of agronomic traits (Sartor et al., 2009). Hybridization is one of the most prominent methods applied in forage grass breeding (Fernandes et al., 2014), and has been the primary tool used for improvement of Plicatula group species (Aguilera et al., 2011; Novo et al., 2017). Previous studies reported that hybrids obtained have genetic potential for some forage traits, such as dry matter production, cold tolerance (Motta et al., 2016, 2017) and cattle preference (Novo et al., 2017).

Development of new cultivars with higher biomass yield and adapted subtropical conditions can contribute to improve animal performance in these regions. In addition, evaluating the response of these novel interspecific hybrids under different N rates or in mixtures with legumes can provide information about agronomic management decisions and can aid in cultivar selection. However, quantitative information required for optimizing forage production in interspecific hybrids of *Paspalum* subjected to N fertilization rates or in mixture with legumes is incomplete and inadequate. The goals of this study were: (i) determine total dry matter yield (Total-DMY), Leaf-DMY, cold tolerance, plant persistence and N use efficiency (NUE) in four hybrids of *P*. *plicatulum* x *P*. *guenoarum* subjected to N rates, (ii) compare agronomic performance of the grass–legume system to a grass–N fertilizer system in subtropical conditions, and (iii) select the best hybrid for new steps within the breeding program.

2.2. MATERIAL AND METHODS

Experimental design

The study was conducted in at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul (UFRGS), Rio Grande do Sul State, Brazil (30°05´S, 51°40´W, 46 m asl). The experiment was planted in December 2014, and treatment imposition and data collection were conducted from September 2015 to May 2017. The climate in the region is classified as subtropical humid (Cfa according to Köppen classification). The minimum and maximum monthly temperature for the last 40 years varied between 8.5ºC (July) to 30.2ºC (January) respectively. The average annual precipitation was approximately 1450mm (Bergamaschi et al., 2013). Irrigation was applied until the soil was saturated after planting and as needed afterwards to maintain plant growth. The average monthly minimum and maximum temperature and rainfall during the conduction of the study are presented in Table 1.

Table 1. Average monthly minimum (min)–maximum (max) temperature (ºC) and rainfall (mm) during the experimental period (September 2015–May 2017) compared to 40–yr average (1970–2009).

	Temperature (°C, min–max)				Rainfall (mm)			
Month	2015	2016	2017	40-yr avg.	2015	2016	2017	40-yr avg.
Jan.		21.3–32.0	21.3–31.3	19.3–30.2		109	197	106
Feb.		21.8–31.8	22.4–32.2	19.0–29.4		139	51	106
Mar.		19.5–27.8	19.4–29.1	18.0–28.2		302	166	102
April		19.3–27.1	17.0–25.9	14.5–25.2		216	115	110
May		12.3–19.6	15.6–22.8	11.2–21.8		73	180	108
June		8.3–16.4		8.7–18.6		7		154
July		10.4–20.1		8.5–18.7		151		144
Aug.		12.1–22.0		9.7–20.2		99		134
Sept.	13.8–22.1	12.4–22.0		11.2–21.4	184	91		142
Oct.	15.4–23.6	15.5–24.8		13.8–24.0	307	192		129
Nov.	17.5–25.7	15.9–27.5		15.4–26.6	124	104		109
Dec.	20.2–29.0	20.0–30.9		17.5–29.0	100	128		111

The soil at the experimental site was classified as Dystrophic Red Argisol. Soil analyses were conducted before starting and during the completion of the experiment (Table 2). Soil samples were collected at 0 to 15-cm depth across all the treatments and then bulked and sent to Soil Analysis Lab–UFRGS.

Table 2. Chemical characteristics of soil before starting and during the accomplishment of the experiment.

Year	Clay	pH H_2O	OM	P	K	H+Al	Al	Ca	Mg	CEC	Base sat.	Al sat.
	$g\ kg^{-1}$		$g\ kg^{-1}$	$mg\ dm^{-3}$		$cmol_c\ dm^{-3}$					%	
*	220	5,5	15,0	8,9	105	2,5	0,0	2,4	1,0	6,2	60	0,0
1	260	5,3	14,0	15,0	72	2,8	0,1	2,8	1,1	6,8	59	2,4
2	190	5,2	17,0	15,0	108	3,9	0,2	2,5	0,8	7,5	48	5,3

*Soil analysis before starting experiment (2014); [1]Soil analysis during the first year of the experiment (2015/16); [2]Soil analysis during the second year of the experiment (2016/17).

The experiment consisted of 108 plots arranged in a split-plot under a randomized complete block design with three replications. Treatments were five N rates (0, 60, 120, 240, and 480 kg ha^{-1} N), and one grass-legumes mixture (no N application) as whole plots, and six genotypes as subplots. For the grass-legume mixture, two legumes species were sown: white clover (*Trifolium repens* L.) cv. BRSURS Entrevero and birdsfoot trefoil (*Lotus corniculatus* L.) cv. URSBRS Posteiro at a rate of 8 and 20 kg seed ha^{-1}, respectively.

The germplasm evaluated included four *Paspalum* apomictic interspecific hybrids (“1020133”, “102069”, “103084” and “103061”), *Paspalum guenoarum* ecotype “Azulão” and *Megathyrsus maximus* cv. “Aruana”, used as controls. Hybrids 1020133 and 102069 resulted from a cross between *P. plicatulum* ecotype “4PT” and *P. guenoarum* ecotype “Azulão”, while hybrids 103084 and 103061 resulted from a cross between *P. plicatulum* ecotype “4PT” and *P. guenoarum* ecotype “Baio”. Hybrids 1020133, 102069, 103084 and

103061 were selected because presented good forage performance in different edaphoclimatic conditions (Saraiva, 2015). Ecotype Azulão is native in the subtropical and temperate regions of southern Brazil, Argentina and Paraguay (Steiner et al., 2017), and was indicated as a male parent in hybridization with sexual plants due to good forage performance (Pereira et al., 2012). Aruana is a perennial grass used in intensive beef production systems in Brazil (Fernandes et al., 2014). The seedlings of the genotypes were transplanted in pure stands in six rows (10 seedlings row^{-1}) with 20 cm apart (2.4 m^2 plot size). Replanting was performed for dead seedlings. Weeds were manually removed from the plots (once) to reduce competition.

Crop management and data collection

In September 2014, plots were fertilized with 80 kg ha^{-1} of phosphorus (P) and 60 kg ha^{-1} of potassium (K). In 2015, plots were fertilized with 60 kg ha^{-1} of P and 60 kg ha^{-1} of K. In 2016, plots were fertilized with 210 kg ha^{-1} of P and 210 kg ha^{-1} of K. The N rates (as ammonium sulfate) were supplied during the spring and summer divided into four applications per year. Liming, as well as P and K doses were performed according to the soil analysis for warm-season grasses following the recommendations of the Soil Chemistry and Fertility Commission–RS/SC, Brazil (CQFS, 2004). In January 2015, the experimental site received N fertilization at 60 kg N ha^{-1} to guarantee the establishment of the seedlings. In April 2015, plots were harvested to sow the legumes species, which had their seeds inoculated and pelleted. The legumes were established by broadcast on April 17, 2015 and March 30, 2016 between the rows of grass.

Two different forage management practices related to stubble height were considered for the experiment: the first began in September 2015 and ended in April 2016 (subsequently called year 1); the second began in September 2016 and ended May 2017 (subsequently called year 2). In year 1, plots were harvested at 10-cm stubble height when the higher N rates (240 and 480 kg ha^{-1}) reached 35-cm. In year 2, plots were harvested leaving a 15-cm stubble height when genotypes reached 30-cm in each treatment. The harvest criterion was modified because 10-cm stubble height was impairing regrowth. With this change, most of the genotypes reached 30-cm tall with a light interception of 95%. In both years, biomass yield was evaluated from two representative samples per subplot (square 0.25 m^2).

Forage samples were manually separated into leaf blade, stem + leaf sheath, inflorescence and dead material fractions. The grass-legume treatment was separated by the same components previously mentioned, in addition each legume species (birdsfoot trefoil and white clover). Samples were dried at 60°C in a forced-air oven for 72 h and then weighed. Total-DMY, Leaf-DMY and Legume-DMY were calculated as the sum of all harvests per plot in each year of the experiment. The number of tillers (tillers m^{-2}) was evaluated from two representative samples per experimental unit (square 0.0625 m^2) and was always counted at a point representative of the harvest area.

Nitrogen use efficiency (NUE, Kg DM kg^{-1} N applied) was calculated according to the following equation NUE = (yield fertilized plot – yield unfertilized plot)/ (applied N rate). Cold tolerance was visually estimated on 14 June and 22 July 2016 after frost events, with temperatures of 2°C, using a 1 to

5 scale, where 1 = the least cold tolerance, and 5 = the greatest cold tolerance. In addition, a scoring system from 1 (few plants present) to 5 (fully covered with plants) to was used to assess the persistence of the genotypes in the plots after 2 years of harvesting.

Data analysis

Data were analyzed using linear models on R statistical software (R Core Team, 2015). Analysis of variance (ANOVA) was performed for all variables to test the statistical significance of the main factors and their interactions: block, N rate, and genotype. The graphs were created using the package *ggplot2* in R (Vu, 2011). A Tukey-HSD test was used to separate treatment means at P = 0.05 using the *lsmeans* package (Lenth, 2016) in R.

2.3. RESULTS

Total-DMY and Leaf-DMY

Significant genotype x N rate interaction (P < 0.001) effect was observed for Total-DMY in year 1. There was not difference among genotypes at 0, 60 and 120 kg N ha^{-1} (Figure 1a). In the N rates of 240 and 480 kg N ha^{-1}, hybrids 1020133 and 102069 had greater Total-DMY than hybrid 103061 but were not statistically different from hybrid 103084, Azulão and Aruana. In the grass-legume mixture, hybrid 1020133 showed greater Total-DMY than Azulão; however, was not different than the other genotypes.

There was a genotype x N rate interaction (P < 0.05) effect on Leaf-DMY in year 1. The genotypes showed similar Leaf-DMY when fertilized at 0, 60 and 120 kg N ha^{-1} or in mixture with legumes (Figure 1b). At the N rate of

240 kg N ha^{-1}, hybrid 102069 showed higher Leaf-DMY than hybrid 103061 and Aruana but did not differ from hybrids 1020133 and 103084 and Azulão. Hybrids1020133 and 102069 when fertilized with 480 kg N ha^{-1} had greater Leaf-DMY than hybrid 103061 but were not different from hybrid 103084, Azulão and Aruana.

The genotypes showed the greatest increased on Total-DMY and Leaf-DMY following additional N rates, especially at 240 and 480 kg N ha^{-1} in year 1 (Figure 1a and 1b). Total-DMY of genotypes in the grass-legume mixture was similar than N rate of 60 kg N ha^{-1}, except for Azulão. On the other than, all genotypes in the grass-legume mixture showed Leaf-DMY similar to the control (0 kg N ha^{-1}) and lower than that additional N rates.

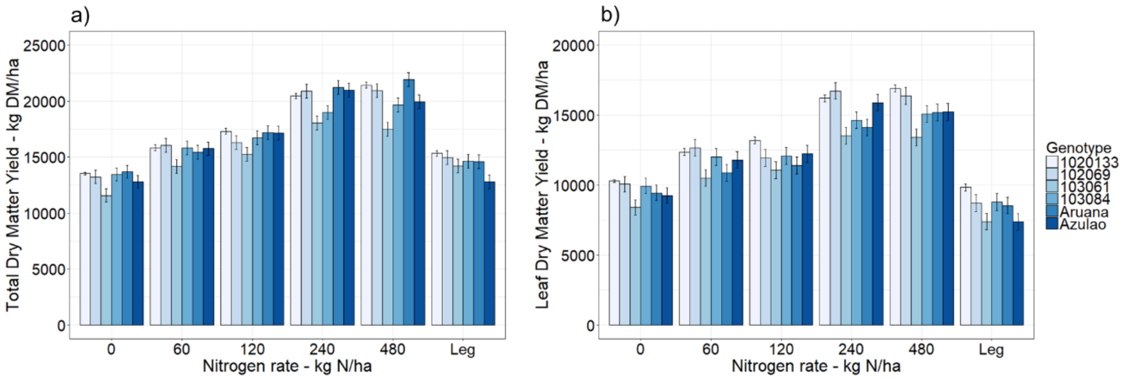

Figure 1. Genotype x N rate interaction effects on (a) total dry matter yield and (b) leaf dry matter yield of four interspecific *Paspalum* hybrids, *P. guenoarum* ecotype Azulão and *M. maximus* cv. Aruana in year 1.

Significant genotype x N rate interaction (P < 0.001) effect was observed for Total-DMY in year 2. There was not difference among genotypes at 0, 60 and 120 kg N ha^{-1} or in mixture with legumes (Figure 2a). However, significant differences were found for Total-DMY among genotypes with application of 240 and 480 kg N ha^{-1}. Hybrid 1020133 had greater Total-DMY compared to the hybrids 103061 and 103084 but was not statistically different from hybrid 102069, Azulão and Aruana.

Genotype x N rate interaction ($P < 0.01$) effect was observed on Leaf-DMY in year 2. The genotypes showed similar Leaf-DMY at 0 and 60 kg N ha^{-1} or in mixture with legumes (Figure 2b). At the N rate of 120 kg N ha^{-1}, hybrid 1020133 and Azulão had greater Leaf-DMY compared to the hybrid 103084 but were not difference than the other genotypes. In the N rate of 240 kg N ha^{-1}, hybrid 1020133 exhibited a greater Leaf-DMY than hybrids 103061 and 103084 and Aruana but did not differ from hybrid 102069 and Azulão. Hybrid 1020133 had greater Leaf-DMY than hybrids 103061 and 103084 when fertilized with 480 kg N ha^{-1}; however, was not different than other genotypes.

In year 2, the genotypes had the greatest increased on Total-DMY and Leaf-DMY when additional N rates of 120, 240 and 480 kg N ha^{-1} were applied (Figure 2a and 2b). In this year, Total-DMY for all genotypes in the grass-legume mixture was similar to the N rate of 120 kg N ha^{-1}. However, Leaf-DMY for all genotypes in the grass-legume mixture had Leaf-DMY similar to the N rate of 0 and 60 kg N ha^{-1}, except for hybrid 1020133.

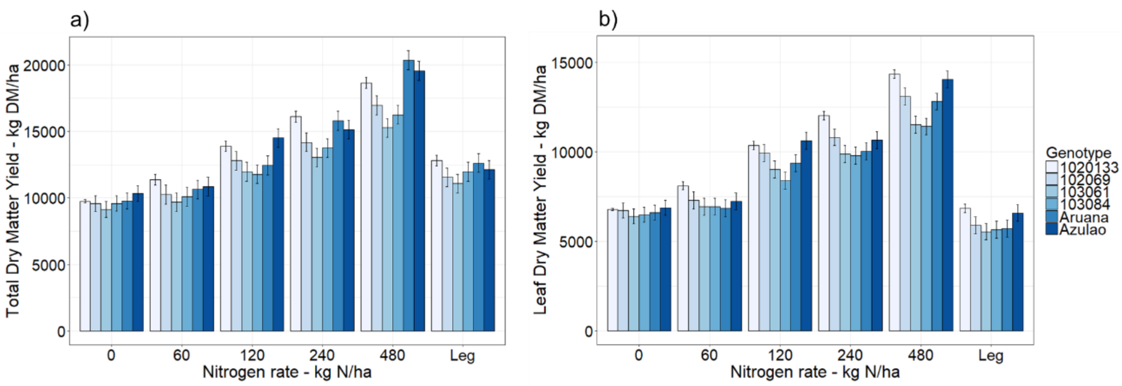

Figure 2. Genotype x N rate interaction effects on (a) total dry matter yield and (b) leaf dry matter yield of four interspecific *Paspalum* hybrids, *P. guenoarum* ecotype Azulão and *M. maximus* cv. Aruana in year 2.

Number of Tillers

There was significant interaction (*P* < 0.05) between genotype x N rate for number of tillers in year 1. In the N rates of 0, 60 and 120 kg N ha^{-1}, hybrid 103061 had greater number of tillers than Azulão but was not statistically different than the other genotypes (Figure 3a). On the other than, when fertilized with 240 and 480 kg N ha^{-1}, hybrid 103061 showed higher number of tillers than hybrid 103084 and Azulão but did not differ than the other genotypes.

The N rate (*P* < 0.001) had an effect on number of tillers in year 2. There was greater number of tillers with application of 480 kg N ha^{-1} compared to the other treatments, except for N rate of 240 kg N ha^{-1} (Figure 3b).

In years 1 and 2, the higher N rates provided larger number of tillers, especially N rates of 240 and 480 kg N ha^{-1} (Figure 3a and 3b). For all genotypes, the number of tillers in the grass-legume mixture was similar to the N rate of 120 kg N ha^{-1} in year 1, whereas it was similar to the control (0 kg N ha^{-1}) and lower than other treatments in year 2.

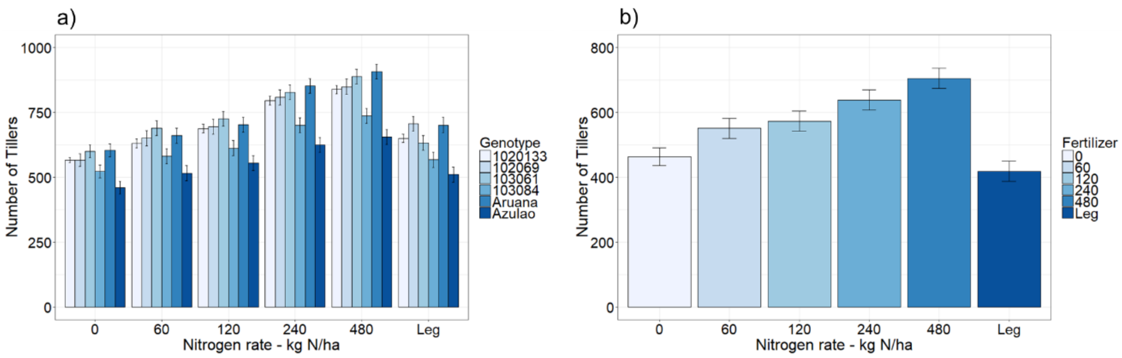

Figure 3. Genotype x N rate interaction effects on number of tillers in (a) year 1 and N rate effect on number of tillers in (b) year 2.

Legume proportion on Total-DMY

Significant genotype (*P* < 0.05) effects was observed for legume proportion on Total-DMY of the grass-legume mixture. In year 1, hybrid 103061

(34%) had the greatest legume proportion on Total-DMY, and hybrid 102069 (25%) had greater legume proportion than hybrid 1020133 (16%) and Aruana (11%) but was not different from hybrid 103084 (18%) and Azulão (23%) (Figure 4a). In year 2, hybrid 103084 (52%) had greater legume proportion on Total-DMY than hybrid 1020133 (42%) and Azulão (36%) but was not different from hybrids 103061 (49%) and 102069 (45%), and Aruana (44%).

There was significant legume species (*P* < 0.001) effects for Legume-DMY. In year 1, white clover (1874 kg DM ha^{-1}) had greater DMY compared to the birdsfoot trefoil (576 kg DM ha^{-1}) (Figure 4b). In year 2, there was an increase in DMY of white clover (3318 kg DM ha^{-1}), while birdsfoot trefoil (608 kg DM ha^{-1}) showed similar production to year 1.

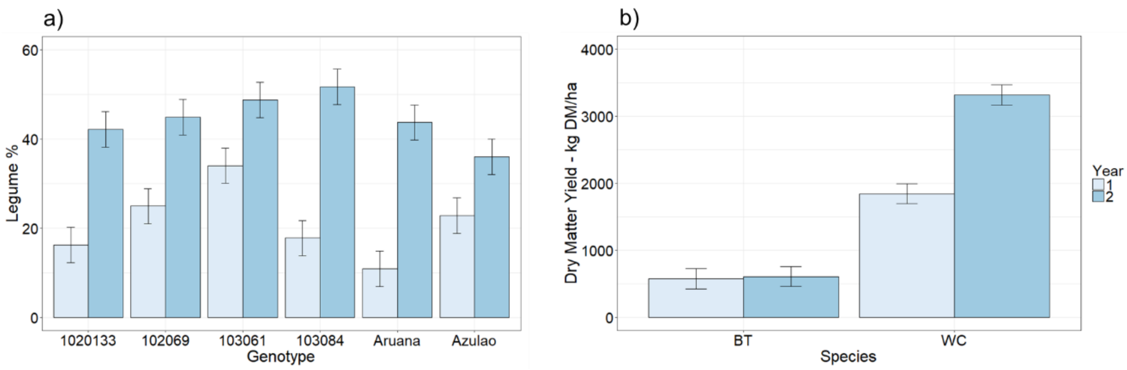

Figure 4. Legume proportion on (a) Total-DMY and (b) DMY of birdsfoot trefoil–BT and white clover–WC in years 1 and 2.

Nitrogen Use Efficiency

The N rate (*P* < 0.001) had an effect on NUE in year 1. Higher NUE was observed at 60 kg N ha^{-1} compared to the other N rates (Figure 5a). There was not difference between N rates of 120 and 240 kg N ha^{-1} and these were greatest than 480 kg N ha^{-1}. NUE decreased from 32 kg kg^{-1} N applied at 60 kg N ha^{-1} to 5 kg kg^{-1} N applied when fertilized with 480 kg N ha^{-1}.

In year 2, there was a two-way interaction (N x genotype, $P < 0.05$) for NUE. In the rate of 60 kg N ha^{-1}, hybrid 1020133 (27 kg kg^{-1} N) showed greatest NUE than the other genotypes (Figure 5b). At the rate of 120 kg N ha^{-1}, hybrid 1020133 (34 kg kg^{-1} N) and Azulão (35 kg kg^{-1} N) had greater NUE than hybrid 103084 (18 kg kg^{-1} N) but were not different from Aruana (22 kg kg^{-1} N). At the rate of 240 kg N ha^{-1}, NUE ranged from 16 kg kg^{-1} N for hybrid 103061, to 26 kg kg^{-1} N (hybrid 1020133). In the rate of 480 kg N ha^{-1}, NUE ranged from 22 kg kg^{-1} N (cv. Aruana) to 12 kg kg^{-1} N for hybrid 103061. In general, NUE increased from N rate of 60 kg N ha^{-1} to 120 kg N ha^{-1}, and then decreased with higher (240 and 480 kg N ha^{-1}) N rates, except for Aruana (Figure 5b).

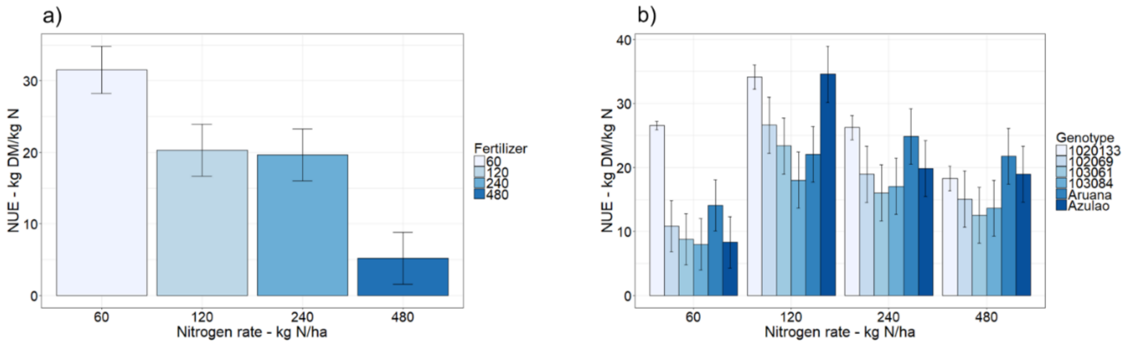

Figure 5. Nitrogen rate effect on NUE in (a) year 1 and Genotype x N rate interaction effects on NUE in (b) year 2.

Cold Tolerance

Main factors N rate and genotype had significant ($P < 0.001$) effect on cold tolerance. Estimated visual scores ranged from 2.5 at 0 kg N ha^{-1} to 3.2 when fertilized at 480 kg N ha^{-1} (Figure 6a). Greater value of cold tolerance was observed for N rate of 480 kg ha^{-1} when compared to the 0 and 60 kg N ha^{-1}, and grass-legume mixture; however, was not different than 120 and 240 kg N ha^{-1}. Average across genotypes, hybrid 1020133 had higher cold tolerance than the other genotypes, except from hybrid 102069 (Figure 6b).

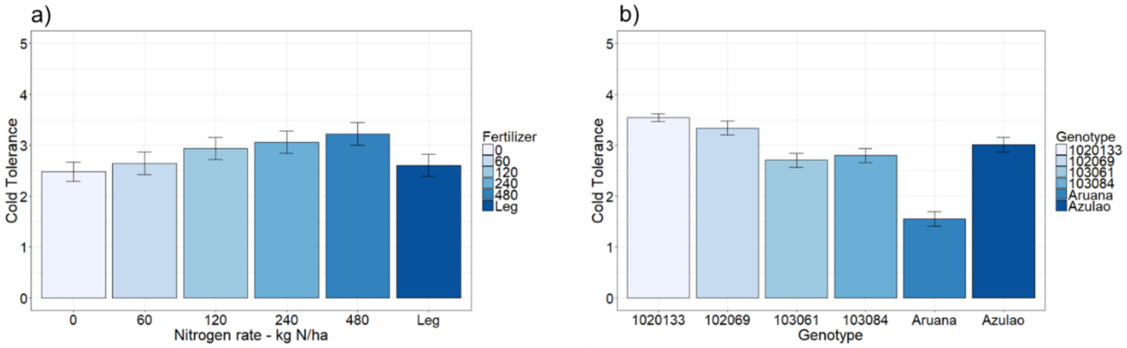

Figure 6. Cold tolerance of four interspecific *Paspalum* hybrids, *P. guenoarum* ecotype Azulão and *M. maximus* cv. Aruana in response to (a) N rates or in mixture with legumes and (b) genotypes during the winter in 2016.

Persistence

The N rate and genotype ($P < 0.001$) had an effect on persistence. Estimated visual scores ranged from 3.4 at 0 kg N ha^{-1} to 4.9 when fertilized at 480 kg N ha^{-1} (Figure 7a). The higher scores of persistence were observed for N rates of 240 and 480 kg ha^{-1} when compared to the control and 60 kg ha^{-1} but was not different than that 120 kg ha^{-1}. Grass-legume mixture had persistence similar to the other treatments, except for 480 kg ha^{-1}. Average across genotypes, hybrids 1020133 and 102069 showed higher persistence than hybrids 103084 and 103061 but were not statistically different from Azulão and Aruana (Figure 7b).

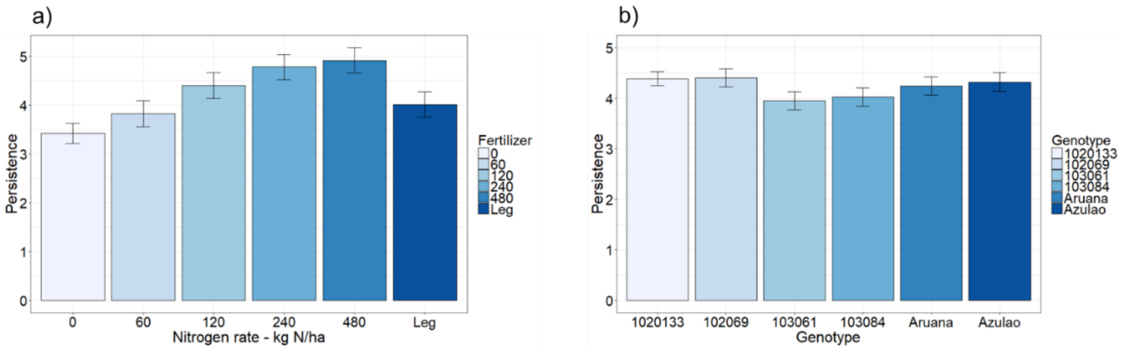

Figure 7. Persistence of four interspecific *Paspalum* hybrids, *P. guenoarum* ecotype Azulão and *M. maximus* cv. Aruana in response to (a) N rates or in mixture with legumes and (b) genotypes after 2 years of harvesting.

2.4. DISCUSSION

Interspecific hybridization has been the primary tool used for genetic improvement in the Plicatula group of the genus *Paspalum* (Aguilera et al., 2011; Novo et al., 2017). Crossing artificially-induced sexual tetraploid germplasm with locally-adapted tetraploid apomicts can generate highly-productive F_1 hybrids that exploit heterosis. However, plant breeders should also consider current socio-economic and environmental conditions at the time of conducting agronomic performance studies for such novel germplasm. Besides increasing biomass yield, cultivar development should also be focused on abiotic stress resistance, such as drought tolerance, water use efficiency, NUE and heat/cold tolerance (Parsons et al., 2011). In this study, biomass yield, cold tolerance, persistence and NUE were determined for four interspecific *Paspalum* hybrids, Azulão and Aruana as controls, grown under five N rates and in a grass-legume mixture with birdsfoot trefoil and white clover. Significant phenotypic variation was observed among genotypes and N rates for the traits measured.

Total-DMY and Leaf-DMY

Phenotypic variation was observed among genotypes for Total-DMY and Leaf-DMY; however, genotypic performance was dependent upon N rates. In years 1 and 2, hybrid 1020133 produced similar Total-DMY than Azulão and Aruana, regardless N rates applied (Figure 1a and 2a) and greater Total-DMY in the grass-legume mixture than that Azulão in year 1. The hybrid 1020133 resulted from hybridization between 4PT sexual tetraploid and the apomictic Azulão. Ecotype Azulão is a locally-adapted plant that produces high biomass yields (Pereira et al., 2012; Steiner et al., 2017), possibly explaining the similar Total-DMY and the response to N fertilization between these two related genotypes. Aruana has high regrowth capacity after each grazing/cutting cycle (Lavres Jr et al., 2004), as well as known forage potential in the South region of Brazil (Motta et al., 2017). A study focused on evaluating several genotypes of *M. maximus* reported DMY of 12,900 kg ha^{-1} for cv. Aruana at 250 kg N ha^{-1} applied (Fernandes et al., 2014). In this study, Aruana showed a Total-DMY of 21,206 kg ha^{-1} (year 1) and 15,802 kg ha^{-1} (year 2) when fertilized at 240 kg N ha^{-1} (Figure 1a and 2a), confirming its ability to respond to high N rates. Thus, similar Total-DMY among hybrid 1020133, Azulão and Aruana suggests that interspecific hybridization techniques used in *Paspalum* species may provide germplasm with excellent forage potential, similar to those found in currently cultivated pastures.

According to Seepaul et al. (2016) N has an effect on plant growth, development, and physiological processes and determines crop productivity. Our findings revealed that N fertilization provided significant Total-DMY benefits. As shown in Figure 1a and 2a, Total-DMY for hybrids 1020133 and 102069, Azulão and Aruana increased dramatically with higher N rates. At the 480 kg N ha^{-1}, hybrids 1020133 and 102069, Azulão and Aruana increased Total-DMY by 58%, 58%, 56% and 60% in first year, while in year 2 increased by 91%, 77%, 89% and 108% when compared to the control (0 kg N ha^{-1}) respectively. These results are consistent with other studies that also showed an increase on forage production of *Digitaria ciliaris* (Teutsch et al., 2005), *P. guenoarum*, *P. notatum* (Towsend, 2008), *Brachiaria brizantha* (Costa et al.,

2010) as N fertilization rates increased. Large genotypic differences on Total-DMY were observed at higher N rates, while lower Total-DMY and less variability among genotypes were observed at 0, 60 and 120 kg N ha^{-1}. Similar results were observed by Bartl et al. (2009), which reported that species with a potential for a high relative growth rate under conditions of high nutrient availability responded with a larger decline in this trait under nutrient deficiency. Generally, when plants are under stress, as N deficiency, extreme phenotypic variability among genotypes decreases because they cannot express their yield potential.

Currently, high N fertilizer prices and increasing concerns/regulations regarding the use of inorganic N fertilizers, guided researcher towards alternative approaches for increasing forage production without additional inorganic N inputs. Grass-legume proved to be a viable alternative to synthetic N fertilizer application to grass monocultures (Cox et al., 2017; Adjesiwor et al., 2017). Several positive benefits have been reported for grass-legume mixtures, including N transfer from legumes to grasses and improvements in forage nutritive value of the pasture (Lüscher et al., 2014; Cox et al., 2017). In this study, grass monoculture fertilized with 240 and 480 kg N ha^{-1} rates produced higher Total-DMY compared with grass-legume mixture (Figure 1a and 2a). On the other hand, grass-legume mixture had similar Total-DMY when compared to the N rates of 60 and 120 kg N ha^{-1} in year 1 and 2 respectively. These results agree with other studies that contrasted synthetic N fertilizer application with grass-legumes mixture. Adjesiwor et al. (2017) reported that meadow bromegrass (*Bromus biebersteinii*) mixed with alfalfa (*Medicago sativa*) and birdsfoot trefoil showed similar Total-DMY than meadow bromegrass fertilized at 56 and 112 kg N ha^{-1}. Cox et al. (2017) observed that tall fescue, meadow bromegrass, orchardgrass (*Dactylis glomerata*), and perennial ryegrass (*Lolium perenne*) growing in mixtures with alfalfa, birdsfoot trefoil, and cicer milkvetch (*Astragalus cicer*) showed higher biomass production than any respective grass monoculture fertilized with 67 kg N ha^{-1} and equal to or higher production than 134 kg N ha^{-1}.

Therefore, our findings suggest that mixture with legumes can be an alternative to replace N fertilizer application up to the rate of 120 kg N ha^{-1}. Legumes offer important opportunities for sustainable grassland-based animal production because they can contribute to the pasture by substituting inorganic N-fertilizer inputs with symbiotic N_2 fixation and increasing forage yield and the nutritive value of herbage (Lüscher et al., 2014). However, the additional management effort that is required to incorporate legumes into the forage system is generally greater than the labor required to have N fertilizer applications in pastures and should be taken into account the decision process. Legumes typically require higher limestone, and P and K fertilization (Butler & Muir, 2012) and increasing seed prices may affect the economics of the grass-legume systems (Adjesiwor et al., 2017).

In both years, hybrid 1020133 had similar or greater Leaf-DMY than Azulão and Aruana (Figure 1b and 2b). In general, across years and N rates the lower genetic distance between hybrid 1020133 and Azulão may possibly be explaining why there was no difference in Leaf-DMY response to N application rates among these two related genotypes, as was the case for Total-DMY.

Previous studies highlighted Leaf-DMY higher than 10,000 kg ha^{-1} for Azulão (Motta et al., 2017, Steiner et al., 2017) which shown its genetic potential for this plant component. Hybrid 1020133 showed higher Leaf-DMY than that Aruana at 240 kg N ha^{-1} in year 2. Fernandes et al. (2014) observed that Aruana produced approximately half the leaf in the Total-DMY. The authors attributed the result due to the influence by its continuous flowering, favoring its stem production. Usually, a relative amount of plant components such as percentage of leaves and/or stems impacts on ingestive behavior of cattle grazing (Fernandes et al., 2014). According to Benvenutti et al. (2015) when animals grazing upper grazing strata (higher proportion of leaves), they access a diet better quality, whereas when animals have access to the lower grazing strata the diet quality decline due to a greater proportion of stems. So, the selection of productive species with a higher proportion of leaves, a better-quality plant component than stem is beneficial for better animal performance in pasture-based production systems.

The findings also reveal that the N application rates provided significant Leaf-DMY benefit, as was the case for Total-DMY. Leaf proportion has a large participation in Total-DMY of interspecific hybrids *Paspalum* (Motta et al., 2016, 2017), which may explain response similar to observed on Total-DMY. As shown in Figure 1b and 2b, Leaf-DMY for hybrids 1020133 and 102069, Azulão and Aruana increased with higher N rates. At the 480 kg N ha^{-1}, hybrids 1020133 and 102069, Azulão and Aruana increased Total-DMY by 64%, 62%, 65% and 61% in year 1, while in second year increased by 111%, 95%, 104% and 94% when compared at 0 kg N ha^{-1} respectively. According to

Lavres Jr et al (2004) the N application have effect on the increase of the numbers of leaves and green leaves per tiller and is also associated with the reduced senescence of this component, contributing to a longer life of the leaf, i.e. greater photosynthetic capacity for longer periods due to limited N redistribution toward younger tissues. In general, Leaf-DMY in the mixture with legumes was similar at 0 kg N ha^{-1} in the first year and similar at 0 and 60 kg N ha^{-1} in the second year (Figure 1b and 2b). This result can be attributed to competition between species by interception of light, which may have impaired the development of leaves in the grass. Martuscello et al. (2011) found a Leaf-DMY similar between *Brachiaria decumbens* cv. Basilisk fertilized at 50 and 100 kg N ha^{-1} or in mixture with *Stylosanthes guianensis* cv. Mineirão; however, reported a similar Leaf-DMY between the mixture with *Calopogonium mucunoides* and N rate of 0 kg ha^{-1}, and lower than the other treatments.

Number of Tillers

The contribution of tillers to pasture production can be considered from two aspects: tiller density (number of tillers per area) and tiller weight (Premavezzi et al., 2003). In year 1, hybrid 103061 exhibited a larger number of tillers than Azulão (Figure 3a); however, Azulão had greater Total-DMY under N fertilization. It is possible that a lower tiller density observed for Azulão in response to N fertilization was compensated for a greater growing or weight of the tillers of this genotype resulting greater Total-DMY. Phenotypic plasticity of the grasses may have an inverse relationship between tiller density and weight, referred to as compensation between tiller size and density (Pontes et al., 2016). According to Pinto et al. (1994) a greater number of growing tillers, which compete among themselves for assimilates produced by the plant, results in smaller individual tiller weight whereas a lower number of tillers occurs in reverse.

In years 1 and 2, high N rates, which provided greater Total-DMY and Leaf-DMY, also promoted larger number of tillers, especially when fertilized with 240 and 480 Kg N ha^{-1} (Figure 3a and 3b). Fagundes et al. (2006) observed similar effects of N application on forage yield and tillering in *Brachiaria decumbens* cv. Basilisk when fertilized with 50, 100, 150, 200, 250, 300 and 350 kg N ha^{-1}. In year 1, grass-legume mixture had number of tillers similar to N rate of 120 kg N ha^{-1}; however, the tillering was lower when compared to the additional N rates and similar to 0 kg N ha^{-1} in year 2. It is possible that the competition for water, nutrients and light may have reduced tillering in the grass. In a study with *Brachiaria decumbens* cv. Basilisk, subjected to N rates (0, 50 and 100 kg ha^{-1}) or in mixture with *Stylosanthes guianensis* cv. Mineirão or *Calopogonium mucunoides*, Martuscello et al. (2011) reported greater number of tillers with N rates applied and mixture with *S. guianensis* cv. Mineirão. On the other than, the mixture with *C. mucunoides* showed tillering similar to 0 kg ha^{-1}.

Legume proportion on Total-DMY

Considerable variability was observed among genotypes for legume proportion on Total-DMY. In year 1, legume proportion ranged from 11% (Aruana) to 34% for the hybrid 103061 (Figure 4a). This result may be attributed to the establishment grass stand and different competition capacity of each genotype. In year 2, legume proportion for all genotypes increased and showed values between 30–50%, except for the hybrid 103084 (52%). According to Lüscher et al. (2014) the advantages presented by the grass–legumes mixture such as increased forage production, higher nutritive value, 'energy neutral' N input into grasslands via symbiotic N_2 fixation and support of non-N_2-fixing plants in the grassland through transfer of symbiotically fixed N are most pronounced in mixed swards with 30–50% of legumes.

In years 1 and 2, white clover showed greater DMY (3.3 and 5.5 times respectively) than that birdsfoot trefoil (Figure 4b). White clover showed an increase of 78% in DMY during year 2 compared to the previous year. Thus, the value observed suggests a higher competition capacity and DMY contribution of white clover in the mixture, especially when compared to the birdsfoot trefoil. Legume proportion was lower in year 1, possibly due to increased competitiveness of grass resulting from well-established stand, since it was planting before than legume species. In addition, the grass may have used soil mineral N and fixed N by the legume, which may have benefited its growing. According to (Schwinning and Parsons, 1996) grass exploits the legume by taking advantage of the additional N which the legume brings into the system, while suppressing legume growth through competition for light. In year 2, increased observed in the legume percentage, especially for the white clover can be explained by the trade-off between grass and white clover, i.e. when soil mineral N is low, white clover has a greater relative growth rate than grass, since it can supplement mineral N uptake with N fixation (Schwinning and Parsons, 1996). In addition, frost occurrence (June and July 2016) and no N application may have decreased competitiveness of grass in year 2.

Nitrogen Use Efficiency

According to Seepaul et al. (2016) NUE has been used to describe a plant's capacity to acquire and utilize N for producing biomass and is expressed as the biomass yield produced per unit N applied. In general, for each kilogram of applied N in year 1, the greatest NUE was observed at 60 kg N ha^{-1} and lower NUE at 480 kg N ha^{-1} (Figure 5a). The results observed in the first-year support previous findings in *Axonopus aureus* (Costa et al., 2010) and *Panicum virgatum* (Obour et al., 2017) that showed a decrease in NUE with increased N application rate. In year 2, there was a change in results, NUE increased with N rate up to 120 kg N ha^{-1} (Figure 5b). Most genotypes showed NUE similar with N application rates of 240 and 480 kg ha^{-1}. In a study with *P. guenoarum*, *P. notatum* and *P. lividum* species subjected to N rates of 0, 60, 180 and 360 kg ha^{-1} Towsend (2008) reported that NUE ranged from 47 to 11 kg DM kg^{-1} N for *P. notatum*, fertilized with 60 kg of N ha^{-1} and *P. guenoarum* at 360 kg of N ha^{-1} respectively. In addition, highlighted that maximum NUE was observed with N rates between 60 and 180 kg N ha^{-1}. The present results shown that maximum NUE was obtained with N rates between 60 (year 1) and 120 kg N ha^{-1} (year 2). Our results also reveal that hybrid 1020133 showed efficient N utilization and converting to biomass production and had greater or similar NUE than that Azulão and Aruana in year 2. According to Van Bueren & Struik (2017) it is important to compare the performance of genotypes at various N rates, since genotypes that showing highest performance and are efficient under high N input, are not always the same that showing the highest performance and efficiency under low N input.

Cold Tolerance

In this study, cold tolerance was accessed through an evaluation of the damage caused in the leaves of the plants after frost occurrence, i.e. genotypes with lower damages (canopy with green leaves) were classified with greater cold tolerance, while genotypes with greater damages (canopy with dead leaves) were classified with lower cold tolerance.

According to Jacobsen et al. (2005) a certain degree and duration of frost can be lethal to most organisms, due to dehydration of the intracellular environment and physical damage by ice crystals. In this experiment hybrid 1020133 showed higher cold tolerance compared to the Azulão and Aruana, which suggest an adaptation to climatic condition to the South region of Brazil. Azulão it is known for exhibiting cold tolerance in subtropical conditions (Motta et al., 2016) while Aruana is one of the cultivars of *Megathyrsus maximus* that shows higher cold tolerance (Corrêa, 2002). Therefore, our results indicate that interspecific crosses can provided hybrid vigor for this trait, resulting in the superiority of hybrids compared to the controls considered cold tolerant.

Persistence

Soil fertility is an important factor in production and longevity of pastures in grazing systems. Thus, when nutrient deficiency is a limiting factor in the soil, fertilizer application such as N, in addition to increasing yield may have a greater impact on persistence of forage plants.

After 2 years of data collection, our findings show that N rates higher than 120 kg ha^{-1} provided a benefit for persistence compared to the control and 60 kg N ha^{-1}. Possibly, the cumulative effect of high N rates for 2 years provided favorable growing conditions, reducing the competition between plants for nutrient and increasing their capacity for growing, through the development of tillers. The higher persistence observed with high N rates can be related to the higher number of tillers obtained with increasing N rates as shown in Figure 3. McKenzie et al. (2002) observed that application of 75, 150 and 225 kg N ha^{-1} resulted in increased tiller densities and forage yield of perennial ryegrass compared to the 0 kg N ha^{-1}. However, the authors reported that N fertilizer alone had not would enhance the persistence of pasture, and that persistence is likely to be influenced by a combination of factors including grazing management and climatic effects, rather than N fertilizer alone.

After 2 years of harvesting, hybrids 1020133 and 102069 had higher persistence than hybrids 103084 and 103061 and similar to the controls. According to Nie et al. (2004) one of the key attributes of plants is their ability to capture and use resources (light, water, and nutrients) to successfully occupy and colonize space. Identification of an improved hybrids of *Paspalum* with higher DMY and incorporating persistence may have a significant positive impact in pasture-based production systems.

2.5. CONCLUSION

Nitrogen application is necessary to increase Total-DMY, Leaf-DMY, number of tillers, cold tolerance and plant persistence in *Paspalum* interspecific hybrids. Highest NUE is obtained with application of N rates between 60 and 120 kg N ha^{-1}. The grass-legume mixture can be an alternative practice of agronomic management to replace to the application of 60 and 120 kg N ha^{-1}.

Hybrid 1020133 showed high response to N supply and had Total-DMY similar to Azulão and Aruana, as well as Leaf-DMY similar to Azulão and greater than Aruana. Hybrid 1020133 exhibited greater NUE at 60 kg N ha^{-1} than the other genotypes. In addition, cold tolerance for hybrid 1020133 was greater than Azulão and Aruana. Therefore, hybrid 1020133 should be selected for follow up studies within our breeding program, such as seed production and animal performance.

We suggest that replication of the study at different sites and additional years would provide more information, since it is expected that in the long term, the results will vary under different soil types and environmental conditions. In addition, research would be needed for testing the impact of animal grazing in the response of the most promising genotypes when subjected to N fertilizer or mixed with legumes, especially due to the return of nutrients by the animals.

ACKNOWLEDGMENTS

We acknowledge for the financial support provided by Sulpasto (Associação Sul-brasileira para o Fomento de Pesquisa em Forrageiras) and CNPq for conducting this research.

REFERENCES

Acuña, C. A., A. R. Blount, K. H. Quesenberry, K. E. Kenworthy, and W. W. Hanna. 2009. Bahiagrass tetraploid germplasm: reproductive and agronomic characterization of segregating progeny. Crop Sci. 49: 581–588. doi: 10.2135/cropsci2008.07.0402

Adjesiwor, A. T., M. A. Islam, V. D. Zheljazkov, J. P. Ritten, and A. Garcia y Garcia. 2017. Grass-legume seed mass ratios and nitrogen rates affect forage accumulation, nutritive value, and profitability. Crop Sci. 57: 1–13. doi: 10.2135/cropsci2016.09.0776

Aguilera, P. M., M. E. Sartor, F. Galdeano, F. Espinoza, and C. L. Quarin. 2011. Interspecific tetraploid hybrids between two forage grass species: sexual *Paspalum plicatulum* and apomictic *P. guenoarum*. Crop Sci. 51:1544–1550. doi: 10.2135/cropsci2010.10.0610

Bartl, K. J., Gamarra, C. A. Gomez, H. R. Wettstein, M. Kreuzer, and H. D. Hess. 2009. Agronomic performance and nutritive value of common and alternative grass and legume species in the Peruvian highlands. Grass Forage Sci. 64:109–121. doi: 10.1111/j.1365-2494.2008.00675.x

Benvenutti, M. A., D. R. Pavetti, D. P. Poppi, I. J. Gordon, and C. A. Cangiano. 2015. Defoliation patterns and their implications for the management of vegetative tropical pastures to control intake and diet quality by cattle. Grass Forage Sci. 71: 424–436. doi: 10.1111/gfs.12186

Bergamaschi, H., R. W., M. R. Guadagnin, L. S. Cardoso, M. I. G, Silva, F. Comiran, F. Dalsin, M. L. Tessari, and P. C. Brauner. 2013. Boletins agrometereológicos da estação experimental agronômica da UFRGS. Departamento de Plantas Forrageiras e Agrometeorologia, Universidade Federal do Rio Grande do Sul, Porto Alegre-RS, Brasil.

Biermacher, J. T., R. Reuter, M. K. Kering, J. K. Rogers, J. Blanton, Jr., J. A. Guretzky, and T. J. Butler. 2012. Expected economic potential of substituting legumes for nitrogen in bermudagrass pastures. Crop Sci. 52: 1923–1930. doi: 10.2135/cropsci2011.08.0455

Butler, T. J., and J. P. Muir. 2012. Perspective on forage legume systems for the tallgrass and mixed-grass prairies of the southern Great Plains of Texas and Oklahoma. Crop Sci. 52: 1971–1979. doi: 10.2135/cropsci2011.12.0674

Chase, A. 1929. The North American species of *Paspalum*. Contributions US Natl Herb 28:1–310

Comissão de Química e Fertilidade do Solo (CQFS) - RS/SC. 2004. Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina. 10 ed. 400. Print, Porto Alegre-RS, Brasil.

Corrêa, L. A. 2002. Características agronômicas das principais plantas forrageiras tropicais. 1. ed. São Carlos: Embrapa, 5 p. (Comunicado Técnico, 35).

Costa, K. A. P., V. Faquin, and I. P. Oliveira. 2010. Doses e fontes de nitrogênio na recuperação de pastagens do capim-marandu. (In Portuguese, with English abstract.) Arq. Bras. Med. Vet. Zootec. 62: 192–199

Costa, N. L., A. de Moraes, A. L. G. Monteiro, A. C. V. Motta, R. A. de Oliveira, and A. N. A. Rodrigues. 2013. Forage productivity and morphogenesis of *Axonopus aureus* under different nitrogen fertilization rates. R. Bras. Zootec. 42: 541–548

Cox, S., M. Peel, J. E. Creech, B. L. Waldron, J. Ean, D. R. Zobell, R. L. Miller, and D. L. Snyder. 2017. Forage production of grass-legume binary mixtures on Intermountain Western USA irrigated pastures. Crop Sci. 57:1742–1753. doi: 10.2135/cropsci2016.04.0235

Fagundes, J. L., D. M. Fonseca, R. V. Morais, C. Mistura, C. M. T Vitor, J. A. Gomide, D. Nascimento Jr., M. E. R. Santos, and D. M. Lambertucci. 2006. Evaluation of structural characteristics of the signalgrass in a nitrogen fertilized pasture over the seasons of the year. R. Bras. Zootec. 35: 30–37. doi:10.1590/S1516-35982006000100004

Fernandes, F. D., A. K. B. Ramos, L. Jank, M. A. Carvalho, G. B. Martha Jr., and G. J. Braga. 2014. Forage yield and nutritive value of *Panicum maximum* genotypes in the Brazilian savannah. Sci. Agric. 71: 23–29. doi: 10.1590/S0103-90162014000100003

Interrante, S. M., J. T. Biermacher, M. K. Kering, and T. J. Butler. Production and economics of steers grazing tall fescue with annual legumes or fertilized with nitrogen. Crop Sci. 52:1940–1948. doi: 10.2135/cropsci2011.11.0610

Jacobsen, S. E., C. Monteros, J. L. Christiansen, L. A. Bravo, L. J. Corcuera, and A. Mujica. 2005. Plant responses of quinoa (*Chenopodium quinoa* Willd.) to frost at various phenological stages. Europ. J. Agronomy. 22: 131–139. doi:10.1016/j.eja.2004.01.003

Lavres Jr., J., M. D. C. Ferragine, L. Gerdes, R. W. C. Raposo, M. N. X. da Costa, and F. A. Monteiro. 2004. Yield components and morphogenesis of Aruana grass in response to nitrogen supply. Sci. Agric. 61: 632–639

Lemaire, G., and F. Gastal. 2009. Quantifying crop responses to nitrogen deficiency and avenues to improve nitrogen use efficiency. In: Sadras, V. O., and D. F. Calderini, editors, Crop Physiology: Applications for genetic improvement and agronomy. Academic Press, London. p.171–199

Lenth, R. V. 2016. Least-squares means: the R package lsmeans. J Stat Softw. 69: 1–33

Lüscher, A., I. Mueller-Harvey, J. F. Soussana, R. M. Rees, and J. L. Peyraud. 2014. Potential of legume-based grassland–livestock systems in Europe: a review. Grass Forage Sci. 69: 206–228. doi: 10.1111/gfs.12124

Martuscello, J. A., A. B. de Oliveira, D. N. F. V. da Cunha, P. L. de Amorim, P. A. L. Dantas, and D. A. Lima. 2011. Produção de biomassa e morfogênese do capim-Braquiária cultivado sob doses de nitrogênio ou consorciado com leguminosas. (In Portuguese, with English abstract.) Rev. Bras. Saúde Prod. An. 12: 923–934

McKenzie, F. R., J. L. Jacobs, and G. Keamey. 2002. The long-term impact of nitrogen fertilizer on perennial ryegrass tiller densities and white clover growing points in grazed dairy pastures in south western Victoria. Aust. J. Agric. Res. 53:1203-1209. doi:10.1071/AR02020

Motta, E. A. M., M. Dall'Agnol, E. A. Pereira, J. M. Machado, and C. Simioni. 2017. Valor forrageiro de híbridos interespecíficos superiores de *Paspalum*. (In Portuguese, with English abstract.) Revista Ciência Agronômica. 48: 191–198. doi: 10.5935/1806-6690.20170022

Motta, E. A. M., M. Dall'Agnol, F. L. Nascimento, E. A. Pereira, J. M. Machado, M. R. Barbosa, C. Simioni, and P. B. Ferreira. 2016. Forage performance of *Paspalum* hybrids from an interspecific cross. Ciência Rural. 46: 1025–1031. doi: 10.1590/0103-8478cr20150232

Munshaw, C. C., E. H. Ervin, C. Shang, S. D. Askew, X. Zhang, and R. W. Lemus. 2006. Influence of late-season iron, nitrogen and seaweed extract on fall colour retention and cold tolerance of four Bermudagrass cultivars. Crop Sci. 46: 273–283. doi:10.2135/cropsci2005.0078

Nie, Z. N., D. F. Chapman, J. Tharmaraj, and R. Clements. 2004. Effects of pasture species mixture, management, and environment on the productivity and persistence of dairy pastures in south-west Victoria. 2. Plant population density and persistence. Aust. J. Agric. Res. 55: 637–643. doi:10.1071/AR03175

Novo, P. E., C. A. Acuña, C. L. Quarin., M. H. Urbani, F. Marcón, and F. Espinoza. 2017. Hybridization and heterosis in the Plicatula group of *Paspalum*. Euphytica. 213: 198–210. doi: 10.1007/s10681-017-1983-4

Novo, P. E., F. Galdeano, J. F. Valls, A. I. Honfi, F. Espinoza, and C. L. Quarin. 2016. Interspecific hybrids between *Paspalum plicatulum* and *P. oteroi*: a key tool for forage breeding. Sci. Agric. 73:356–362. doi: 10.1590/0103-9016-2015-0218

Obour, A. K., K. Harmoney, and J. D. Holman. 2017. Nitrogen fertilizer application effects on switchgrass herbage mass, nutritive value and nutrient removal. Crop Sci. 57: 1754–1763. doi: 10.2135/cropsci2016.07.0582

Parsons, A. J., G. R. Edwards, P. C. D Newton, D. F Chapman, J. R. Caradus, S. Rasmussen, and J. S. Rowarth. 2011. Past lessons and future prospects: plant breeding for yield and persistence in cool-temperate pastures. Grass Forage Sci. 66: 153–172. doi: 10.1111/j.1365-2494.2011.00785.x

Pereira, E. A., T. Barros, G. K. Volkmann, G. K. Battisti, J. A. G. da Silva, C. Simioni, and M. Dall'Agnol. 2012. Variabilidade genética de caracteres forrageiros em *Paspalum.* (In Portuguese, with English abstract.) Pesq. Agropec. Bras. 47: 1533–1540. doi: 10.1590/S0100- 204X2012001000017

Pinto, J. C., J. A. Gomide, and M. Maestri. 1994. Produção de matéria seca e relação folha/caule de gramíneas forrageiras tropicais, cultivadas em vasos, com duas doses de nitrogênio. (In Portuguese, with English abstract.) R. Bras. Zootec. 23: 313–326

Pontes, L. S., T. C. Baldissera, A. F. Giostri, G. Stafin, B. R. C. dos Santos, and P. C. F. Carvalho. 2016. Effects of nitrogen fertilization and cutting intensity on the agronomic performance of warm-season grasses. Grass Forage Sci. 1: 1–13. doi: 10.1111/gfs.12267

Premazzi, L. M., F. A. Monteiro, and J. E. Corrente. 2003. Tillering of Tifton 85 bermudagrass in response to nitrogen rates and time of application after cutting. Sci. Agric. 60: 565–571

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Saraiva, K. M. 2015. Caracterização agronômica e qualidade de forragem de híbridos interespecíficos do gênero *Paspalum*. Ph.D. diss., Universidade Federal do Rio Grande do Sul, Porto Alegre-RS, Brasil.

Sartor, M. E., C. L. Quarin, and F. Espinoza. 2009. Mode of reproduction of colchicine-induced *Paspalum plicatulum* tetraploids. Crop Sci. 49:1270–1276. doi: 10.2135/cropsci2008.05.0270

Schroder, J. J. 2014. The position of mineral nitrogen fertilizer in efficient use of nitrogen and land: a review. Natural Resources 5: 936–948. doi: 10.4236/nr.2014.515080

Schwinning, S., and A. J. Parsons. 1996. Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. Journal of Ecology 84: 799–813

Seepaul, R., B. Macoon, K. R. Reddy, W. B. Evans. 2016. Nitrogen application rate and genotype effects on switchgrass production and chemical characteristics. American Journal of Plant Sciences 7: 533–546. doi: 10.4236/ajps.2016.73047

Steiner, M. G., M. Dall'agnol, C. Nabinger, S. M. Scheffer-Basso, R. L. Weiler, C. Simioni, M. T. Schifino-Wittmann, and E. A. M. da Motta. 2017. Forage potential of native ecotypes of *Paspalum notatum* and *P. guenoarum*. An. Acad. Bras. Cienc. 89: 1753–1760. doi: 10.1590/0001-3765201720160662

Teutsch, C. D., J. H. Fike, and W. M. Tilson. 2005. Yield, digestibility, and nutritive value of crabgrass as impacted by nitrogen fertilization rate and source. Agron. J. 97:1640–1646. doi:10.2134/agronj2005.0047

Towsend, C. 2008. Características produtivas de gramíneas nativas do gênero *Paspalum*, em resposta a disponibilidade de nitrogênio. Ph.D. diss., Universidade Federal do Rio Grande do Sul, Porto Alegre-RS, Brasil.

Van Bueren, E. T. L., and P. C. Struik. 2017. Diverse concepts of breeding for nitrogen use efficiency. A review. Agron. Sustain. Dev. 37: 1–24. doi: 10.1007/s13593-017-0457-3

Vu, V. Q. 2011. ggbiplot: A ggplot2 based biplot. R package version 0.55. Retrieved from: http://github.com/vqv/ggbiplot

Weiler, R. L, M. Dall'Agnol, C. Simioni, K. C. Krycki, E. A. Pereira, J. M. Machado, E. A. M. da Motta. 2018. Intraspecific tetraploid hybrids of *Paspalum notatum*: agronomic evaluation of segregating progeny. Sci. Agric. 75: 36–42. doi: http://dx.doi.org/10.1590/1678-992X-2016-0354

3. CHAPTER III[2]

Nutritive value and forage production in *Paspalum* interspecific hybrids fertilized with nitrogen or in mixture with temperate legumes

[2]Prepared in accordance with the standards of the Chilean Journal of Agricultural Research.

Nutritive value and forage production in *Paspalum* interspecific hybrids fertilized with nitrogen or in mixture with temperate legumes

ABSTRACT

The improvement *Paspalum* species has made available novel genetic resources for livestock systems; however, it is unknown the forage potential of these new hybrids when subjected to N fertilizer or in mixture with legumes. Our goal was to evaluate dry matter yield (DMY) by season and nutritive value in hybrids of *P*. *plicatulum* x *P*. *guenoarum* subjected to N rates or in mixture with legumes. The experiment was a randomized complete block design in split-plot arrangement with three replications. Treatments were five N rates (0, 60, 120, 240, and 480 kg ha^{-1} N), and one grass-legume mixture (*Trifolium repens* + *Lotus corniculatus*) as whole plots, and six genotypes (1020133, 102069, 103084, 103061, *P*. *guenoarum* Azulão and *Megathyrsus maximus* Aruana used as a controls) as subplots. DMY, crude protein (CP), neutral and acid detergent fiber (NDF, ADF) and dry matter digestibility (DMD) were determined. Higher N rates enhanced DMY, regardless growth season, increased CP and DMD, decreased NDF and ADF than control treatment ($P < 0.05$). Grass-legume mixture promoted greater DMY in spring and fall than control treatment and had greater nutritive value compared to N-fertilized grass ($P < 0.05$). Hybrid 1020133 had DMY similar to controls. Hybrid 103061 had greater CP and DMD and lower NDF and ADF than to controls ($P < 0.05$). Nitrogen application and mixture with legumes to enhance DMY and nutritive value in hybrids of *Paspalum* is recommended. Hybrids 1020133 and 103061 should be indicated for new steps of evaluation within the breeding program.

Key words: breeding forage, biomass yield, crude protein, digestibility, nitrogen fertilization.

3.1. INTRODUCTION

Animal production in tropical and subtropical areas of the world is largely dependent on native or cultivated pastures, which requires higher production, quality and adaptation of forage plants (Jank et al., 2011). Agronomic practices such as nutrient management and species selection, or the combination of these two factors are some of the options for increasing biomass yield and the nutritive value of pastures.

Proper soil fertility management is required for efficient grass forage production. Nitrogen (N) has an effect on crop growth, development, and physiological processes (Seepaul et al., 2016), and supply of N fertilizer tends to improve yields and nutritive value in warm-season grasses (Silveira et al., 2013; Pontes et al., 2016; Obour et al., 2017). However, prices for synthetic N sources have increased over the past several years, and these higher prices may have influence profitability of forage and livestock producers (Interrante et al., 2012). Industrial production of N fertilizer uses non-renewable reserves of fossil fuels. Moreover, lost N when high rates are applied can have negative impacts on the environmental, as it is associated to global warming effects and contamination of the quality of groundwater and surface water (Schröder, 2014).

Grass-legume mixture can be considered a viable alternative to synthetic N fertilizer application to grass monocultures (Adjesiwor et al., 2017). Legumes offer important opportunities for sustainable grassland-based animal production because they can contribute to increasing forage yield, substituting inorganic N-fertilizer inputs with symbiotic N_2 fixation, increasing forage yield and the nutritive value, and raising the efficiency of conversion of herbage to animal protein (Lüscher et al., 2014). Generally, legume intake is higher than grasses due to greater crude protein (CP) content, lower cell wall content, and faster rate of particle size reduction and passage rate of the rumen (Elgersma and Søegaard, 2016).

The grass genus *Paspalum* L. is an important forage constituent in natural grasslands in South America (Chase, 1929), where shows a great diversity of species and has a large area covered, which support the importance of the genus for livestock production (Novo et al., 2016). There is an informal taxonomic group within *Paspalum,* named Plicatula (Chase 1929) and at least three species of this group have been introduced to cultivation: *P. plicatulum*, *P. guenoarum* and *P. atratum*. However, these species are primarily tetraploid and apomictic, and released cultivars were based on selection from natural ecotypes (Aguilera et al., 2011). The collection of a sexual diploid ecotype of *P. plicatulum*, and its chromosome duplication resulted in the generation of a sexual tetraploid plant, allowing new genetic studies of agronomic traits (Sartor et al., 2009). Particularly, *P. plicatulum* and *P. guenoarum* have been used in interspecific hybridizations (Aguilera et al., 2011; Novo et al., 2017) and the hybrids obtained showed genetic potential for biomass yield (Motta et al., 2017) and cattle preference (Novo et al., 2017).

Evaluating the response of these novel interspecific hybrids under N supply or in mixtures with legumes can provide information about agronomic management decisions and aid in cultivar selection. However, there are limited data for biomass yield and nutritive value of these hybrids in subtropical conditions when subjected to different N fertilizer rates or in mixture with legumes. The goal of this study was to evaluate dry matter yield by season and the nutritive value in hybrids of *Paspalum plicatulum* x *P. guenoarum* in response to different rates of N fertilizer or in mixture with temperate legumes.

3.2. MATERIAL AND METHODS

Experimental design and treatments

The study was conducted in at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul (UFRGS), Rio Grande do Sul State, Brazil (30°05´S, 51°40´W, 46 m asl). The experiment was planted in December 2014, and treatment imposition and data collection were conducted from September 2015 to May 2017. The climate in the region is classified as subtropical humid (Cfa according to Köppen classification). The monthly temperature for the last 40 years varied between 8.5ºC (July, minimum temperature) to 30.2ºC (January, maximum temperature) while annual precipitation was approximately 1450 mm (Bergamaschi et al., 2013). Irrigation was applied until the soil was saturated after planting and as needed afterwards to maintain plant growth. The monthly temperature, rainfall and irrigation during the conduction of the study are presented in Figure 1. The soil at the experimental site was classified as Dystrophic Red Argisol. Soil analyses were conducted before starting and during the completion of the experiment (Table 1). Soil samples were collected at 0 to 15 cm depth across all the treatments and then bulked and sent to lab.

The experiment was a split-plot arrangement of a randomized complete block design with three replications. Treatments were five N rates (0, 60, 120, 240, and 480 kg ha^{-1} N), and one grass-legumes mixture (no N application) as whole plots, and six genotypes as subplots. In the grass-legume mixture were sown white clover (*Trifolium repens* L.) cv. BRSURS Entrevero and birdsfoot trefoil (*Lotus corniculatus* L.) cv. URSBRS Posteiro at a rate of 8 and 20 kg seed ha^{-1}, respectively.

In this study were evaluated the hybrids 1020133 and 102069 resulted from an artificial hybridization between *P. plicatulum* 4PT x *P. guenoarum* Azulão, and hybrids 103084 and 103061 resulted from an artificial hybridization between *P. plicatulum* 4PT x *P. guenoarum* Baio. The crosses were performed in a greenhouse at the Forage Plants Breeding Group - UFRGS. In addition, were included *P*. *guenoarum* ecotype Azulão and *Megathyrsus maximus* cv. Aruana, used as controls. Hybrids 1020133, 102069, 103084 and 103061 were selected because presented good forage performance in different edaphoclimatic conditions (Saraiva, 2015). *P*. *guenoarum* Azulão is native in the subtropical and temperate regions of southern Brazil, Argentina and Paraguay (Steiner et al., 2017), and has been indicated as a male parent in hybridization with sexual plants due to good forage performance (Pereira et al., 2012). *M*. *maximus* cv. Aruana is a perennial grass used in intensive beef production systems in Brazil (Fernandes et al., 2014). The genotypes were planted in pure stands in six rows (10 seedlings row^{-1}) with 20 cm apart (2.4 m^2 plot size). Replanting was performed for dead plants as needed. Weeds were manually removed of the plots (once) to reduce competition.

Crop management

In January 2015, the experimental plots received N supply at 60 kg ha^{-1} to guarantee the establishment of the genotypes in the field. In April 2015, plots were harvested to sown of the legumes species, which had their seeds inoculated and pelleted. The legumes were established by broadcast on April 17, 2015 and March 30, 2016 between the rows of grass. The N fertilizer rates (as ammonium sulfate) were supplied during the spring and summer divided into four applications per year. Limestone, phosphorus and potassium application were performed according to the soil analysis (see Table 1) for warm-season grasses following the recommendations of the Soil Chemistry and Fertility Commission–RS/SC, Brazil (CQFS, 2004).

Two different forage management practices related to stubble height were used for the experiment: the first began in September 2015 and ended in April 2016 (called year 1); the second began in September 2016 and ended May 2017 (called year 2). In year 1, plots were harvested leaving a 10-cm stubble height when the higher N rates (240 and 480 kg ha^{-1}) reached 35 cm tall. In year 2, plots were harvested leaving a 15 cm stubble height when genotypes reached 30-cm tall in each treatment. The criterion was modified because 10 cm stubble height was impairing plants regrowth. With this change, most of the genotypes reached 30 cm tall with a light interception of 95%. In both years, biomass yield was evaluated from two representative samples per subplot (square 0.25 m^2).

Forage samples were manually separated into leaf blade, stem + leaf sheath, inflorescence and dead material fractions. Samples were dried at 60°C in a forced-air oven for 72 h and then weighed. Dry matter yield (DMY) was calculated as the sum of all harvests per plot in each season within each year of the experiment. Harvests carried out from September to December were considered as spring; January to March as summer; and April and May as fall. In both years, there was not harvest during winter. The number of harvest in each month are listed in Table 2.

The nutritive value of the harvested forage was assessed using the leaf blades of the samples collected for DMY during the harvests in the spring and summer of year 1. Legumes species were included in the samples together with the leaf blades of grass. The samples were ground and passed through a 1-mm mesh screen in a Wiley mill. Each sample was analysed using nearinfrared reflectance spectroscopy (NIRS, FOSS-NIRSystems 5000 Silver Spring, MD, USA, which scans the spectral range of 1100–2500 nm; CEPA laboratory, Passo Fundo-RS, Brazil) to determine the CP, dry matter digestibility (DMD), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents.

Statistical analysis

Data were analyzed using linear models on R statistical software (R Core Team, 2015). Analysis of variance (ANOVA) was performed for all variables to test the statistical significance of the main factors and their interactions: block, N rate, and genotype. The graphs were created using the package *ggplot2* in R (Vu, 2011). A Tukey-HSD test was used to separate treatment means at P = 0.05 using the *lsmeans* package (Lenth, 2016) in R. Data were analyzed to verify their normality by Shapiro-Wilk test and when necessary were transformed.

3.3. RESULTS

Dry matter yield

In the spring of the first year, significant N rate x genotype interaction (P < 0.001) effect was observed for DMY. There was greater variability between genotypes with higher N application rates. In all N rates, hybrids 1020133 and 102069 showed similar DMY than Aruana (Table 3). In addition, hybrid 1020133 had (P < 0.05) greater DMY than Azulão when fertilized with 480 kg ha^{-1} or in mixture with legumes. In general, greater DMY were observed with N application rates of 240 and 480 kg N ha^{-1}. In this season, DMY of the grass-legume mixture for the hybrids 1020133, 102069, and 103084 were similar to 120 kg N ha^{-1}, while for the hybrid 103084, Aruana and Azulão were similar to 480, 60 and 0 kg N ha^{-1} respectively.

In the summer of year 1, significant N rate (P < 0.001) and genotype (P < 0.05) effects were observed for DMY. Hybrid 102069 had greater (P <

0.05) DMY than Aruana but was not different from the other genotypes (Table 3). Among N rates, there was (P < 0.05) greater DMY when genotypes were fertilized with 240 and 480 kg N ha^{-1}. Grass-legume mixture showed greater DMY than that 0 kg N ha^{-1} and similar to 60 kg N ha^{-1}.

On the other hand, in the fall of year 1, significant N rate x genotype interaction (P < 0.001) effect was observed for DMY. There was not difference (P > 0.05) for DMY between genotypes in the N rates of 0, 60 and 120 kg N ha^{-1} (Table 3). However, Aruana had (P < 0.05) greater DMY than the other genotypes in the N rates of 240 and 480 kg N ha^{-1}. Hybrids 1020133, 102069 and 103061 had similar DMY than Aruana and Azulão in grass-legume mixture. Among N rates, greater DMY were observed with N application rates of 240 and 480 kg N ha^{-1}, except for hybrids 103061 and 103084. In addition, DMY of the grass-legume mixture for the hybrids 1020133 and 103084 and Aruana were similar to 120 kg N ha^{-1} while for hybrid 103061, hybrid 102069 and Azulão was similar to 240, 60 and 0 kg N ha^{-1} respectively.

There were significant N rate and genotype (P < 0.001) main effects on DMY, in the spring of year 2. The average across genotypes, showed that the hybrid 1020133 had (P < 0.05) greater DMY than hybrids 102069, 103061 and 103084 but was not different from Aruana and Azulão (Table 4). Among N rates, grass-legume mixture had similar DMY than 240 and 480 kg N ha^{-1}, and these N rates showed (P < 0.05) greater DMY compared to the other N rates.

In the summer of year 2, there was a significant N rate x genotype interaction (P < 0.05) on DMY. Hybrid 1020133 had similar DMY than hybrid 102069, Aruana and Azulão in all N rates, except for Aruana fertilized with 480 kg N ha^{-1} (Table 4). Higher (P < 0.05) DMY were observed for N rates of 120, 240 and 480 kg N ha^{-1} compared to the other N rates, except for Aruana and Azulão.

Finally, in the fall of year 2, there was a significant N rate x genotype interaction (P < 0.01) for DMY. There was not harvest for the N rate of 0 kg N ha^{-1} (Table 4). There was not difference for DMY among genotypes in N rate of 60 kg N ha^{-1}. Hybrid 1020133 showed similar DMY than Aruana and Azulão in all N rates, except when fertilized with 240 kg N ha^{-1}. Among N rates, there was greater (P < 0.05) DMY when genotypes were fertilized with 480 kg N ha^{-1}. In addition, DMY of the grass-legume mixture for all genotypes were similar to 240 kg N ha^{-1}, except for Azulão, which was similar to 120 kg N ha^{-1}.

Nutritive value

There was significant genotype (P < 0.001) effect for CP, NDF and ADF content and DMD concentration. Higher (P < 0.05) CP content was observed for hybrid 103061 (163 g kg^{-1}) compared to the other genotypes, except for the hybrid 103084 (156 g kg^{-1}), which showed greater CP content than hybrids 1020133 and 102069 and Azulão (Figure 1a). Lowest (P < 0.05) NDF contents were observed in hybrids 103061 (463 g kg^{-1}) and 103084 (467 g kg^{-1}) compared to the other genotypes; however, there was no difference between hybrid 103084 and Azulão (478 g kg^{-1}) (Figure 1b). Hybrids 103084 (288 g kg^{-1}) and 103061 (292 g kg^{-1}) showed the lowest (P < 0.05) ADF contents than the other genotypes (Figure 1c). Highest (P < 0.05) DMD concentrations were observed for hybrids 103084 (673 g kg^{-1}) and 103061 (665 g kg^{-1}) compared to the other genotypes (Figure 1d).

The N rate (P < 0.001) had an effect on CP, NDF and ADF content and DMD concentration. The average across N rates, CP content increased from 124 g kg^{-1} without N applied to 157 g kg^{-1} when fertilized with 480 kg N ha^{-1} (Figure 2a). The grass-legume mixture (192 g kg^{-1}) showed a greater (P < 0.05) CP content compared to the other treatments. The application of N rates decreased NDF content from 503 g kg^{-1} (0 kg N ha^{-1}) to 473 g kg^{-1} when 480 kg N ha^{-1} was applied (Figure 2b). The grass-legume mixture (448 g kg^{-1}) had an NDF content (P < 0.05) lower than N rates of 120, 60 and 0 kg N ha^{-1}. The average across N application rates showed a decrease on ADF content, which ranged from 321 g kg^{-1} at 0 kg N ha^{-1} to 301 g kg^{-1} when fertilized with 480 kg N ha^{-1} (Figure 2c). The grass-legume mixture (280 g kg^{-1}) showed lower (P < 0.05) ADF content than the other treatments. The DMD concentration ranged from 637 g kg^{-1} (0 kg N ha^{-1}) to 661 g kg^{-1} with application of 480 kg N ha^{-1} (Figure 2d). The grass-legume mixture (697 g kg^{-1}) had a greater (P < 0.05) DMD concentration than the other treatments.

3.4. DISCUSSION

In order to increase its success, plant breeding has focused on increasing the annual and seasonal pattern of pasture production, and on parameters that lead to improved forage quality, intake and feed conversion (Parsons et al., 2011). In this context, nutritive value assumes substantially importance when forage constitutes most of the diet of a ruminant animal, or when a producer chooses not to supplement the diet with grain concentrates (Brink et al., 2015). In this research, DMY in different season of the year, and the CP, NDF and ADF contents and DMD concentration were determined for four hybrids of *P. plicatulum* x *P. guenoarum*, Aruana and Azulão as controls, grown under five N application rates and in a grass-legume mixture with birdsfoot trefoil and white clover. Significant phenotypic variation was observed among genotypes and N rates or mixture with temperate legumes for the traits measured.

Dry matter yield

In general, greater variability among genotypes was observed when N rates of 240 and 480 kg N ha^{-1} were applied, regardless the growing season. These results are possible because N has an effect on plants growth, development, and physiological processes (Seepaul et al., 2016), contributing for difference on DMY among then. Moreover, differences in responses observed across seasons among genotypes may be attributable to rainfall, temperature, photoperiodism, harvest frequency and ability to use N and to express productivity potential.

Hybrid 1020133 had similar DMY than Aruana, except in the fall of year 1 and summer of year 2 (Table 3 and 4). Aruana is a high-yielding species that shows DMY of 13,160 kg ha^{-1} when fertilized with 300 kg N ha^{-1} (Pontes et al., 2016). The greater DMY of Aruana in some seasons as compared with the hybrid 1020133 can be attributed to its higher stem production (data not shown), which may have increasing the herbage accumulation. Fernandes et al. (2014), who evaluated the forage production of 24 genotypes of *M. maximus*, described that Aruana produced approximately half the leaf DMY, because continuous flowering during the year favored stem DMY. On the other hand, hybrid 1020133 showed greater or similar DMY than Azulão, regardless N rates, season or year of evaluation (Table 3 and 4). Azulão is a locally-adapted ecotype that produces high DMY, as reported by Towsend (2008) that observed DMY of 11,506 kg ha^{-1} (summer of second year) when fertilized with 360 kg N ha^{-1}. The hybrid 1020133 resulted from a cross between 4PT sexual tetraploid and Azulão. The results observed in the present study suggests greater genetic potential for hybrid 1020133 compared to its male parental. Therefore, hybrids selection such as 1020133 may provide an opportunity for development novel genetic resources with larger distribution and herbage accumulation.

Nitrogen is the most limiting nutrient for forage productivity, and N supply tends to improve yields (Silveira et al., 2013). In general, N rates of 240 and 480 kg N ha^{-1} provide an increase on DMY in all seasons for 2 years. Such cumulative effects of N fertilizer on grass production have been noted previously with *Brachiaria brizantha* (Costa et al., 2010) and ecotypes of *P. guenoarum* (Towsend, 2008) and *P. notatum* (Machado, 2014) as N fertilizer rates increased. Grass-legume mixture had similar DMY compared to the higher N rates during spring (240 and 480 kg N ha^{-1}) of year 2. This result may be attributed to the harvest carried out in September (see, Table 2), which contributed for higher DMY during spring. Thus, grass-legume mixture can be an alternative practice for provide early-grazing in subtropical conditions. According to (Cox et al., 2017) ideal pastures should optimize forage production, nutritional quality, and pasture longevity, while minimizing the use of inputs. Legumes offer important opportunities for sustainable grassland-based livestock production because they can contribute to substituting inorganic N-fertilizer inputs with symbiotic N_2 fixation, increasing biomass yield and the nutritive value, and raising the efficiency of conversion of forage to animal protein (Lüscher et al., 2014). Therefore, our results reveal that grass-legume mixture can be an alternative for to replace higher N rates applied in grass monoculture, but it is dependent of the growth season.

Nutritive value

The CP contents in hybrids 103061 and 103084 were significantly higher than Azulão and its progenies (1020133 and 102069). In addition, hybrid 103061 had a greater CP content than Aruana (Figure 1a). The hybrids 103061 and 103084 resulted from a cross between 4PT sexual tetraploid and the apomictic ecotype Baio. This manifested that different male parental may influence in nutritional characteristics of its progenies through of introgression of genes, which possibly explaining the difference among genotypes. Thus, our findings suggest that CP content can be improved through artificial interspecific hybridization due to greater CP content of hybrids compared to the controls.

The NDF and ADF contents were lower in hybrids 103061 and 103084 than the other genotypes, except between Azulão and hybrid 103084 (Figures 1b and 1c). Variability for NDF and ADF contents, also was observed by Seepaul et al. (2016) in a study with different genotypes of switchgrass (*Panicum virgatum*). These results indicate that cell wall components may change in response to N supply, with genotypes responding dissimilarly. High NDF content provides a reduction in the dry matter intake whereas high ADF content decrease digestibility (Costa et al., 2010). Therefore, genotype selection that shown a lower NDF and ADF content is desirable for improved the grassland-based animal production.

The DMD concentration in hybrids 103061 and 103084 were significantly higher than Aruana, Azulão and its progenies (1020133 and 102069) (Figure 1d). Greater digestibility observed for hybrids 103061 and 103084 can be related to lower ADF and NDF contents. According to Stabile et al. (2011), different enzymes can be responsible for controlling lignification at different plant tissues and at different forage species. In addition, different male parental may have influenced on DMD concentration of the hybrids, as previously discussed and may also explain the difference between genotypes for DMD concentration. Forage digestibility is related to intake rate, and affects animal performance positively (Fernandes et al., 2014). This trait must be considered in the selection of new forage grasses for grazing systems, since it is possible to breed, or to select genotypes for higher DMD concentration without affecting biomass yield.

In general, the application of N fertilizer and mixture with legumes resulted in greater nutritive value for all genotypes. The N application rates provided an increase for CP content. However, even without N supply the average of CP on leaf blades across genotypes, was greater than the minimum content (70 g kg^{-1}) required for cattle maintenance (Costa et al., 2010). Average across N rates, CP content increased 27% with application of 480 kg N ha^{-1} compared to 0 kg N ha^{-1} (Figure 2a). Our results are consistent with several other studies that showed N application increasing CP content in warm-season forage grasses (Silveira et al., 2013; Pontes et al., 2016; Obour et al., 2017). Higher CP content was also observed in the present study in grass-legume mixture than in N fertilized grass. Results similar were reported by Adjesiwor et al. (2017) in a mixture of bromegrass (*Bromus biebersteinii*) with Alfalfa (*Medicago sativa*) and birdsfoot trefoil (*Lotus corniculatus*) when compared to the bromegrass monoculture with (56 and 112 kg N ha^{-1}) or without N.

Nitrogen supply contributed to decrease the NDF and ADF contents. The average NDF content across N rates decreased 6% (Figure 2b), and ADF content decreased 7% (Figure 2c) with application of 480 kg N ha^{-1} compared to the control (0 kg N ha^{-1}). Costa et al. (2010) evaluating *Brachiaria brizantha* cv. Marandu under increasing N rates observed that content of NDF and ADF reduced 9 and 26% with application of 300 kg N ha^{-1} compared to 0 kg N ha^{-1} respectively. Paulino et al. (2015) reported that increasing N rates (0, 150, 300 and 450 kg ha^{-1}) decreased the NDF and FDA contents in the leaf blades of Aruana. In this study grass-legume mixture produced lower NDF content than N rates of 120, 60 and 0 Kg N ha^{-1}, while ADF content was lower compared to the other treatments. Adjesiwor et al. (2017) also observed a similar result in a mixture between bromegrass with Alfalfa and birdsfoot trefoil, which exhibited lesser NDF and ADF contents than bromegrass monoculture with (56 and 112 kg N ha^{-1}) or without N. The reduction of NDF and ADF content with the increase of N rates or in mixture with legumes obtained in this study can be considered important for improving the nutritive value of the forage, i.e. improving digestibility and the dry matter intake by the animals.

The DMD concentration increased 4% when the genotypes were fertilized with 480 kg N ha^{-1} compared to treatment without N supply (Figure 2d). The effect of N fertilizer application (0, 45, 90, 135 and 180 kg ha^{-1}) in DMD concentration has been reported by Obour et al. (2017) in *Panicum virgatum*. On the other hand, Paulino et al. (2015) observed that DMD concentration of leaf blades of Aruana was not affected when fertilized with N rates of 0, 150, 300 and 450 kg ha^{-1}. The grass-legume mixture had a greater DMD concentration than that N fertilizer rates. The greater nutritive value observed in grass-legume mixture may be attributed to presence of the legumes, because these species usually have a lower fiber content than grasses. According to Pontes et al. 2016 an improvement in DMD concentration can mainly be attributed to a decrease in the ADF content, which was observed in the present study.

Our findings reveal that N supply improved the nutritive value of the genotypes, since it increased the CP content and DMD concentration, and decreased NDF and ADF content compared to the control (no N supply). However, grass-legume mixture showed better values than that N fertilizer. Therefore, this approach could be an alternative to provide adequate nutrition for grazing animal. The positive influence of legume proportion on forage nutritive value has been reported by Brink et al. (2015). These authors observed that white clover had a positive additive effect on mixture digestibility due to their high digestibility relative to the other grass components in the mixture.

3.5. CONCLUSIONS

We concluded that N supply and mixture with legumes are needed to increase DMY and improve nutritive value in *Paspalum* interspecific hybrids. Grass-legume mixture has the potential to substitute for N-fertilized grass in the spring and fall from the second year. Moreover, the results also showed that grass-legume mixture could improve nutritive value contrasted to application of inorganic N fertilizers.

Hybrid 1020133 showed higher positive response to N supply for forage production and had a DMY similar to Aruana and Azulão. Hybrid 103061 showed positive response to N supply for nutritive value and had a higher CP and DMD and lower NDF and ADF than Aruana and Azulão. Therefore, we suggest that hybrids 1020133 and 103061 are indicated for new steps of evaluation within the breeding program.

ACKNOWLEDGMENTS

We acknowledge for the financial support provided by Sulpasto (Associação Sul-brasileira para o Fomento de Pesquisa em Forrageiras) and CNPq for conducting this research.

REFERENCES

Adjesiwor, A.T., M.A. Islam, V.D. Zheljazkov, J.P. Ritten, and A. Garcia y Garcia. 2017. Grass-legume seed mass ratios and nitrogen rates affect forage accumulation, nutritive value, and profitability. Crop Science 57: 1–13. doi: 10.2135/cropsci2016.09.0776

Aguilera, P.M., M.E. Sartor, F. Galdeano, F. Espinoza, and C.L. Quarin. 2011. Interspecific tetraploid hybrids between two forage grass species: sexual *Paspalum plicatulum* and apomictic *P. guenoarum*. Crop Science 51:1544–1550. doi: 10.2135/cropsci2010.10.0610

Bergamaschi, H., R.W., M.R. Guadagnin, L.S. Cardoso, M.I.G, Silva, F. Comiran, F. Dalsin, M.L. Tessari, and P.C. Brauner. 2013. Boletins agrometereológicos da estação experimental agronômica da UFRGS. Dep. de Plantas Forrageiras e Agrometeorologia, Universidade Federal do Rio Grande do Sul, Porto Alegre-RS, Brasil

Brink, G. E.; M.A. Sanderson, M. D. Casler. 2015. Grass and legume effects on nutritive value of complex forage mixtures. Crop Science 55: 1329–1337

Chase, A. 1929. The North American species of *Paspalum*. Contributions US Natl Herb 28:1–310

Comissão de Química e Fertilidade do Solo (CQFS) - RS/SC. 2004. Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina. 10 ed., Porto Alegre, RS, Brasil

Costa, K.A.P., V. Faquin, and I.P. Oliveira. 2010. Doses e fontes de nitrogênio na recuperação de pastagens do capim-marandu. (In Portuguese, with English abstract.) Arquivo Brasileiro de Medicina Veterinária e Zootecnia 62: 192–199

Cox, S., M. Peel, J.E. Creech, B.L. Waldron, J. Ean, D.R. Zobell, R.L. Miller, and D.L. Snyder. 2017. Forage production of grass-legume binary mixtures on Intermountain Western USA irrigated pastures. Crop Science 57:1742–1753. doi: 10.2135/cropsci2016.04.0235

Elgersma, A., and K. Søegaard. 2016. Effects of species diversity on seasonal variation in herbage yield and nutritive value of seven binary grass-legume mixtures and pure grass under cutting. European Journal of Agronomy 78:73–83. doi:10.1016/j.eja.2016.04.011

Fernandes, F.D., A.K.B. Ramos, L. Jank, M.A. Carvalho, G.B. Martha Jr., and G.J. Braga. 2014. Forage yield and nutritive value of *Panicum maximum* genotypes in the Brazilian savannah. Scientia Agricola 71: 23–29. doi: 10.1590/S0103-90162014000100003

Interrante, S.M., J.T. Biermacher, M.K. Kering, and T.J. Butler. Production and economics of steers grazing tall fescue with annual legumes or fertilized with nitrogen. Crop Science 52:1940–1948. doi: 10.2135/cropsci2011.11.0610

Jank, L., C.B. Valle, R.M.S. Resende. 2011. Breeding tropical forages. Crop Breeding and Applied Biotechnology 11: 27–34

Lenth, R.V. 2016. Least-squares means: the R package lsmeans. Journal of Statistical Software. 69: 1–33

Lüscher, A., I. Mueller-Harvey, J.F. Soussana, R.M. Rees, and J.L. Peyraud. 2014. Potential of legume-based grassland–livestock systems in Europe: a review. Grass and Forage Science 69: 206–228. doi: 10.1111/gfs.12124

Machado, J.M. 2014. Caracterização agronômica de ecótipos de *Paspalum notatum* Flügge em resposta ao fotoperíodo e a fertilização nitrogenada e seleção de híbridos intraespecíficos. 115 p. PhD thesis. Universidade Federal do Rio Grande do Sul, Departamento de Plantas Forrageiras e Agrometeorologia, Porto Alegre, RS, Brasil

Motta, E.A.M., M. Dall'Agnol, E.A. Pereira, J.M. Machado, and C. Simioni. 2017. Valor forrageiro de híbridos interespecíficos superiores de *Paspalum*. (In Portuguese, with English abstract.) Revista Ciência Agronômica 48: 191–198. doi: 10.5935/1806-6690.20170022

Novo, P.E., C. A. Acuña, C.L. Quarin, M.H. Urbani, F. Marcón, and F. Espinoza. 2017. Hybridization and heterosis in the Plicatula group of *Paspalum*. Euphytica 213: 198–210. doi: 10.1007/s10681-017-1983-4

Novo, P.E., F. Galdeano, J.F. Valls, A.I. Honfi, F. Espinoza, and C.L.Quarin. 2016. Interspecific hybrids between *Paspalum plicatulum* and *P. oteroi*: a key tool for forage breeding. Scientia Agricola 73:356–362. doi: 10.1590/0103-9016-2015-0218

Obour, A.K., K. Harmoney, and J.D. Holman. 2017. Nitrogen fertilizer application effects on switchgrass herbage mass, nutritive value and nutrient removal. Crop Science 57: 1754–1763. doi: 10.2135/cropsci2016.07.0582

Parsons, A.J., G.R. Edwards, P.C.D Newton, D.F Chapman, J.R. Caradus, S. Rasmussen, and J.S. Rowarth. 2011. Past lessons and future prospects: plant breeding for yield and persistence in cool-temperate pastures. Grass and Forage Science 66: 153–172. doi: 10.1111/j.1365-2494.2011.00785.x

Paulino, V.T., N.M. Rasquinho, K.M.R. Duarte, and M.A.C. Lucena. 2015. Nutritive value and digestibility of Aruana guinea grass under n fertilization of NBPT- treated urea. International Journal of Agriculture Innovations and Research 4: 415–420

Pereira, E.A., T. Barros, G.K. Volkmann, G.K. Battisti, J.A.G. da Silva, C. Simioni, and M. Dall'Agnol. 2012. Variabilidade genética de caracteres forrageiros em *Paspalum.* (In Portuguese, with English abstract.) Pesquisa Agropecuária Brasileira 47: 1533–1540. doi: 10.1590/S0100-204X2012001000017

Pontes, L.S., T.C. Baldissera, A. F. Giostri, G. Stafin, B.R.C. Santos, and P.C.F. Carvalho. 2016. Effects of nitrogen fertilization and cutting intensity on the agronomic performance of warm-season grasses. Grass and Forage Science 1: 1–13. doi: 10.1111/gfs.12267

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Saraiva, K.M. 2015. Caracterização agronômica e qualidade de forragem de híbridos interespecíficos do gênero *Paspalum*. 95 p. PhD thesis. Universidade Federal do Rio Grande do Sul, Departamento de Plantas Forrageiras e Agrometeorologia, Porto Alegre, RS, Brazil

Sartor, M.E., C.L. Quarin, and F. Espinoza. 2009. Mode of reproduction of colchicine-induced *Paspalum plicatulum* tetraploids. Crop Science 49:1270–1276. doi: 10.2135/cropsci2008.05.0270

Schroder, J.J. 2014. The position of mineral nitrogen fertilizer in efficient use of nitrogen and land: a review. Natural Resources 5: 936–948. doi: 10.4236/nr.2014.515080

Seepaul, R., B. Macoon, K.R. Reddy, W.B. Evans. 2016. Nitrogen application rate and genotype effects on switchgrass production and chemical characteristics. American Journal of Plant Sciences 7: 533–546. doi: 10.4236/ajps.2016.73047

Silveira, M.L., J.M.B. Vendramini, B. Sellers, F.A. Monteiro, A.G. Artur and E. Dupas. 2013. Bahiagrass response and N loss from selected N fertilizer sources. Grass and Forage Science 70: 154–160. doi: 10.1111/gfs.12078

Stabile, S.S., A.P. Bodini, L. Jank, F.P. Renno, M.V. Santos and L.F.P. Silva. 2011. Expression of genes from the lignin synthesis pathway in guineagrass genotypes differing in cell-wall digestibility. Grass and Forage Science 67: 43–54. doi: 10.1111/j.1365-2494.2011.00817.x

Steiner, M.G., M. Dall'agnol, C. Nabinger, S.M. Scheffer-Basso, R.L. Weiler, C. Simioni, M.T. Schifino-Wittmann, and E.A.M. da Motta. 2017. Forage potential of native ecotypes of *Paspalum notatum* and *P. guenoarum*. Anais da Academia Brasileira de Ciências 89: 1753–1760. doi: 10.1590/0001-3765201720160662

Towsend, C. 2008. Características produtivas de gramíneas nativas do gênero *Paspalum*, em resposta a disponibilidade de nitrogênio. 255 p. PhD thesis. Universidade Federal do Rio Grande do Sul, Departamento de Plantas Forrageiras e Agrometeorologia, Porto Alegre, RS, Brazil

Vu, V.Q. 2011. ggbiplot: A ggplot2 based biplot. R package version 0.55. Retrieved from: http://github.com/vqv/ggbiplot

Table 1. Chemical characteristics of soil before starting and during the accomplishment of the experiment.

Year	Clay	pH H_2O	OM	P	K	H+Al	Al	Ca	Mg	CEC	Base sat.	Al sat.
	g kg^{-1}		g kg^{-1}	mg dm^{-3}		cmol$_c$ dm^{-3}					%	
*	220	5,5	15,0	8,9	105	2,5	0,0	2,4	1,0	6,2	60	0,0
1	260	5,3	14,0	15,0	72	2,8	0,1	2,8	1,1	6,8	59	2,4
2	190	5,2	17,0	15,0	108	3,9	0,2	2,5	0,8	7,5	48	5,3

*Soil analysis before starting experiment (2014); [1]Soil analysis during the first year of the experiment (2015/16); [2]Soil analysis during the second year of the experiment (2016/17).

Table 2. Harvests carried out in four interspecific *Paspalum* hybrids, Azulão and Aruana during years 1 and 2.

N rate	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Kg/ha	Year 1								
0	x	x	-	x	x	x	x	x	-
60	x	x	-	x	x	x	x	x	-
120	x	x	-	x	x	x	x	x	-
240	x	x	-	x	x	x	x	x	-
480	x	x	-	x	x	x	x	x	-
Leg*	x	x	-	x	x	x	x	x	-
	Year 2								
0	-	x	-	x	x	-	x	-	-
60	-	x	-	x	x	-	x	x	-
120	-	x	-	x	x	x	x	-	x
240	-	x	x	x	x	xx	x	x	-
480	-	x	x	x	x	xx	x	x	x
Leg*	x	x	x	x	x	x	-	x	-

Year 1 = September 2015 to April 2016; Year 2 = September 2016 to May 2017.
*Leg = grass–legume mixture; x= number of harvest.

Table 3. Genotype, N rate and Genotype x N rate effects on dry matter yield by season of four interspecific *Paspalum* hybrids, Azulão and Aruana in year 1.

Season	N rate (kg ha^{-1})	Genotypes						Mean
		1020133	102069	103061	103084	Aruana	Azulão	
		Dry matter yield (kg ha^{-1})						
Spring	0	5425 d-A	4955 c-AB	4012 d-B	5418 d-A	5785 c-A	4793 c-AB	5065
	60	6117 cd-A	5877 bc-A	4719 cd-B	6182 cd-A	6551 bc-A	6289 b-A	5956
	120	7129 bc-A	6269 b-AB	5263 bc-B	6800 bc-A	7307 b-A	6635 b-A	6567
	240	8248 b-AB	7992 a-AB	6439 ab-C	7598 ab-B	8881 a-A	8187 a-AB	7891
	480	9560 a-A	8745 a-AB	6661 a-C	8058 a-B	8751 a-AB	7865 a-B	8273
	Leg*	6579 cd-A	6688 b-A	5561 abc-AB	5989 cd-A	6096 c-A	4668 c-B	5930
	Mean	7176	6754	5443	6674	7229	6406	
Summer	0	3742	3853	3369	3747	3603	3751	3678 d
	60	4842	5419	4763	4815	4177	4693	4785 c
	120	5503	5407	5453	5435	5069	5881	5458 b
	240	6741	7248	6635	6369	6073	7200	6711 a
	480	7071	7273	6467	7284	7533	7108	7123 a
	Leg*	4488	4327	4474	4697	4189	4199	4396 c
	Mean	5398 AB	5588 A	5194 AB	5391 AB	5107 B	5472 AB	
Fall	0	1047 c-A	1115 bc-A	888 c-A	970 c-A	991 d-A	917 d-A	988
	60	1413 bc-A	1310 bc-A	1213 bc-A	1335 bc-A	1265 cd-A	1300 bc-A	1306
	120	1537 b-A	1478 b-A	1414 ab-A	1327 bc-A	1690 b-A	1491 b-A	1490
	240	2061 a-B	2209 a-B	1549 ab-C	1614 ab-C	2833 a-A	2321 a-B	2098
	480	2213 a-B	2335 a-B	1739 a-C	1733 a-C	3075 a-A	2366 a-B	2244
	Leg*	1367 bc-AB	1076 c-AB	1297 b-AB	1046 c-B	1418 bc-A	1024 cd-B	1205
	Mean	1606	1587	1350	1338	1879	1570	

Means followed by the same lowercase letter in columns and uppercase letters in the rows are not different ($P > 0.05$), *Leg = grass–legume mixture.

Table 4. Genotype, N rate and Genotype x N rate effects on dry matter yield by season of four interspecific *Paspalum* hybrids, Azulão and Aruana in year 2.

Season	N rate (kg ha^{-1})	Genotypes 1020133	102069	103061	103084	Aruana	Azulão	Mean
		Dry matter yield (kg ha^{-1})						
Spring	0	2445	2451	2133	2251	2614	2453	2391 d
	60	3269	2667	2393	2672	2929	2621	2759 c
	120	3827	3621	2677	2944	3126	3694	3315 b
	240	5510	4956	4187	4775	4944	5131	4917 a
	480	6617	5563	4652	5119	5539	6013	5584 a
	Leg*	5350	5137	5019	5822	5539	4898	5294 a
	Mean	4503 A	4066 B	3510 C	3931 B	4115 AB	4135 AB	
Summer	0	3358 b-AB	3185 b-AB	3055 b-B	3392 b-AB	3217 c-AB	3932 c-A	3357
	60	3921 b-AB	3413 b-AB	3247 b-B	3366 b-AB	3479 c-AB	4051 c-A	3580
	120	5847 a-AB	5104 a-AB	5387 a-AB	5020 a-B	5157 b-AB	6386 b-A	5484
	240	7575 a-AB	6101 a-BC	6165 a-BC	5962 a-C	7554 a-AB	7767 ab-A	6854
	480	6988 a-BC	6477 a-C	5967 a-C	6529 a-C	9240 a-A	8517 a-AB	7286
	Leg*	2446 c-A	2005 c-AB	1793 c-B	1795 c-B	2027 d-AB	2285 d-A	2059
	Mean	5023	4381	4269	4344	5112	5490	
Fall	0	-	-	-	-	-	-	-
	60	1045 c-A	1046 b-A	915 b-A	925 b-A	1110 c-A	1052 c-A	1016
	120	1177 c-AB	1047 b-ABC	871 b-BC	791 b-C	1146 c-AB	1397 bc-A	1072
	240	1505 bc-A	1487 b-A	1031 b-B	1168 b-AB	1461 bc-A	981 c-B	1272
	480	2899 a-ABC	2549 a-ABC	2277 a-BC	2099 a-C	3315 a-A	3099 a-AB	2706
	Leg*	1796 b-A	1193 b-B	1041 b-B	1140 b-B	1835 b-A	1709 b-A	1452
	Mean	1684	1464	1227	1225	1773	1648	

Means followed by the same lowercase letter in columns and uppercase letters in the rows are not different ($P > 0.05$), *Leg = grass–legume mixture.

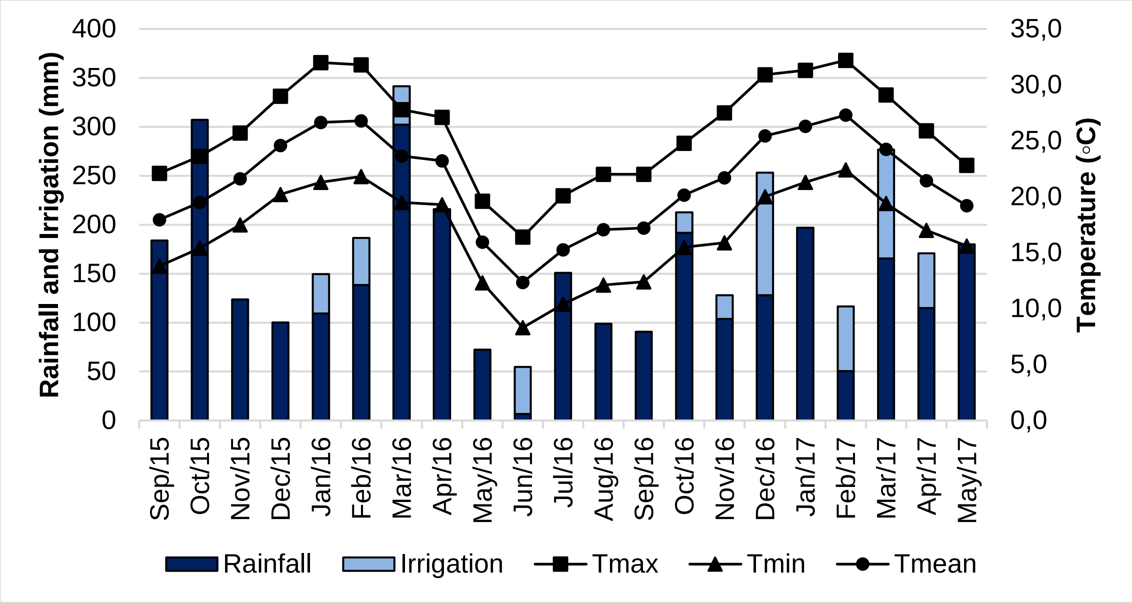

Figure 1. Maximum (Tmax), minimum (Tmin) and mean (Tmean) monthly temperature (ºC), rainfall and irrigation (mm) during the experimental period (September 2015–May 2017).

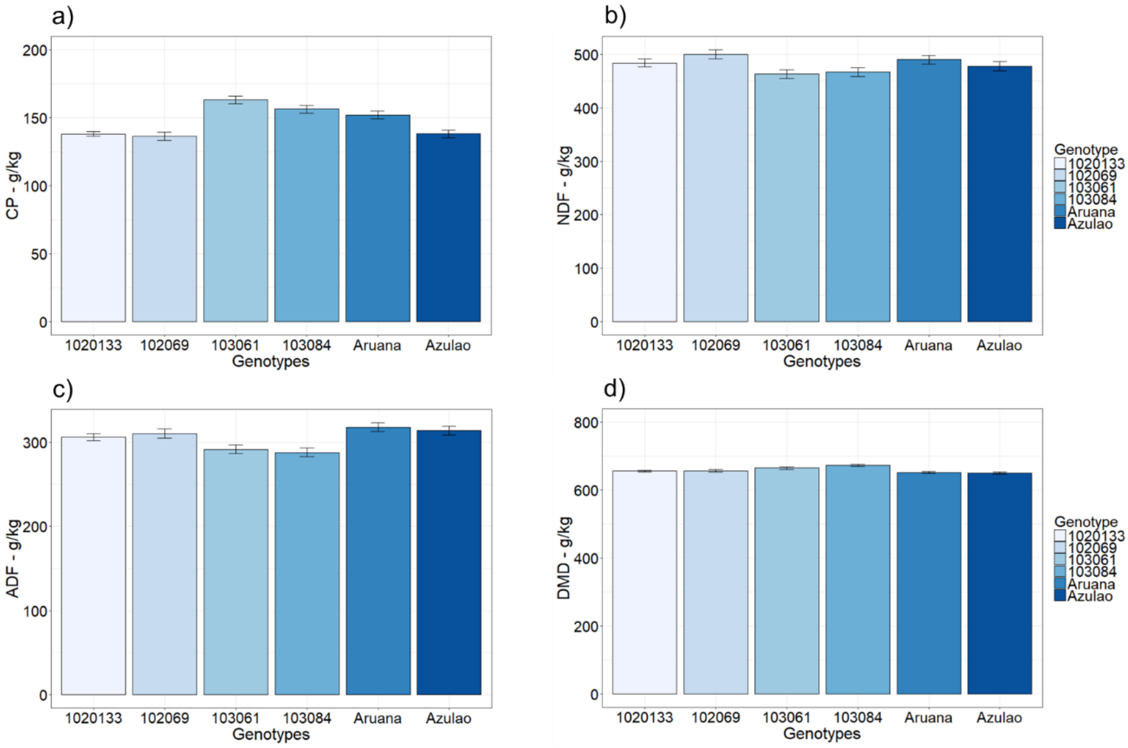

Figure 2. The effect of genotypes on (a) CP = crude protein, (b) NDF = neutral detergent fiber, (c) ADF = acid detergent fiber and (d) DMD = dry matter digestibility of four interspecific hybrids *Paspalum*, Azulão and Aruana.

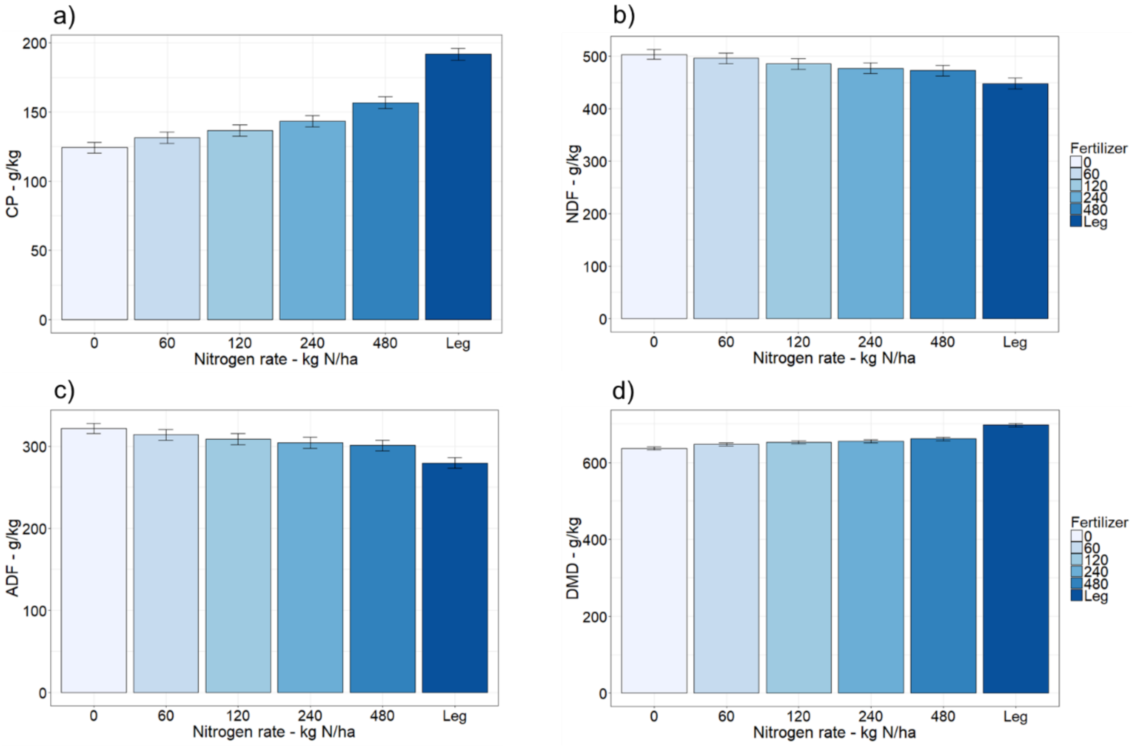

Figure 3. The effect of N rates and mixture with legumes on (a) CP = crude protein, (b) NDF = neutral detergent fiber, (c) ADF = acid detergent fiber and (d) DMD = dry matter digestibility of four interspecific hybrids *Paspalum*, Azulão and Aruana. Leg = grass-legume mixture.

4. CHAPTER IV

4.1. FINAL CONSIDERATIONS

Results presented in this thesis showed a greater variability among genotypes for all traits was detected when different N rates were applied. These results are important for the selection of genotypes tested at various N rates, especially when plants are under N deficiency stress, because phenotypic variability among genotypes decreases, as their yield potential cannot be expressed. Moreover, the results revealed that N fertilizer application is needed to optimize and maintain biomass yield, cold tolerance and persistence of the genotypes.

It is essential an improving NUE of plants for reducing input of fertilizers, while maintaining an acceptable yield and sufficient profit margin for the producers. Results of this study revealed that hybrid 1020133 had NUE greater or similar than Aruana and Azulão, i.e. showed efficient in utilizing N and converting to forage production. Therefore, genotype selection is an important tool to improve this trait.

Our findings also showed that grass-legume mixture had biomass yield similar to the rate of 60 and 120 kg N ha^{-1}. In addition, grass-legume mixture had greater nutritive value than grass monoculture fertilized with N. Thus, grass-legume mixture can be an alternative to replace N fertilizer application, because legumes can contribute to raising the efficiency of conversion of forage to animal protein and especially substituting inorganic N-supply with symbiotic N_2 fixation.

The artificial hybridization technique used in native species provides novel genetic resources for livestock production systems. Besides that, our findings revealed that hybrids had a positive response for biomass yield and nutritive value when subjected to additional N rates, especially, hybrid 1020133 that showed Total-DMY similar to Aruana and Azulão, as well as greater Leaf-DMY greater than Aruana. In addition, hybrid 103061 had greater CP and DMD and lower NDF and ADF than Aruana and Azulão. Therefore, hybrids 1020133 and 103084 are indicated to new steps within the breeding program, such as evaluation of seed production and animal performance.

It is suggested the replication of the study at additional sites and years to provide information about results that could be expected under different soil types, environmental conditions, and grazing management. More research is also needed to evaluate profitability of the grass–legume system to a grass–N fertilizer system. In order, for the grass-legume system to be profitable for livestock system, the price of annual legume establishment must be lower than the price of N fertilizer. Moreover, more research is needed to identify legume species that may be more promising for a *Paspalum*–legume mixture, under other climate and soil conditions.

5. REFERENCES

ACUÑA, C. A. et al. Bahiagrass tetraploid germplasm: reproductive and agronomic characterization of segregating progeny. **Crop Science**, Madison, v. 49, n. 2, p. 581-588, 2009.

ADJESIWOR, A. T. et al. Grass-legume seed mass ratios and nitrogen rates affect forage accumulation, nutritive value, and profitability. **Crop Science**, Madison, v. 57, n. 1, p. 1-13, 2017.

AGUILERA, P. M. et al. Interspecific tetraploid hybrids between two forage grass species: sexual *Paspalum plicatulum* and apomictic *P. guenoarum*. **Crop Science**, Madison, v. 51, p. 1544-1550, 2011.

ALISON Jr., M. W.; HOVELAND, C. S. Birdsfoot trefoil management. II. Yield, quality and stand evaluation. **Agronomy Journal**, Madison, v. 81, n. 5, p. 745-749, 1989.

AROEIRA, L. J. M. et al. Disponibilidade, composição bromatológica e consumo de matéria seca em pastagem consorciada de *Brachiaria decumbens* com *Stylosanthes guianensis*. **Pesquisa Agropecuária Brasileira,** Brasília, v. 40, n. 4, p. 413-418, 2005.

ASHWORTH, A. J. et al. Biologically fixed nitrogen in legume intercropped systems: comparison of nitrogen-difference and nitrogen-15 enrichment techniques. **Agronomy Journal**, Madison, v. 107, n. 6, p. 2419-2430, 2015.

ASSMANN, A. L. et al. Produção de gado de corte e acúmulo de matéria seca em sistema de integração lavoura-pecuária em presença e ausência de trevo branco e nitrogênio. **Revista Brasileira de Zootecnia**, Viçosa, v. 33, n. 1, p. 37-44, 2004.

ASSMANN, T. S. et al. Fixação biológica de nitrogênio por plantas de trevo (*Trifolium* spp) em sistema de integração lavoura-pecuária no Sul do Brasil. **Revista Brasileira de Zootecnia**, Viçosa, v. 36, n. 5, p. 1435-1442, 2007.

BARBERO, L. M. et al. Produção animal e valor nutritivo da forragem de pastagem de *coastcross* consorciada com amendoim forrageiro. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 62, n. 3, p. 645-653, 2010.

BARCELLOS, A. O. et al. Sustentabilidade da produção animal baseada em pastagens consorciadas e no emprego de leguminosas exclusivas, na forma de banco de proteína, nos trópicos brasileiros. **Revista Brasileira de Zootecnia**, Viçosa, v. 37, (suplemento especial), p. 51-67, 2008.

BARRETO, I. L. **O gênero *Paspalum* (Gramineae) no Rio Grande do Sul**. 1974. 258 f. Dissertação (Livre-Docência - Fitotecnia) - Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 1974.

BARTL, K. et al. Agronomic performance and nutritive value of common and alternative grass and legume species in the Peruvian highlands. **Grass and forage Science**, Oxford, v. 64, n. 2, p. 109-121, 2009.

BATISTA, L. A. R.; GODOY, R. Capacidade de produção de sementes em acessos do gênero *Paspalum*. **Revista Brasileira Zootecnia**, Viçosa, v. 27, n. 5, p. 841-847, 1998.

BIERMACHER, J. T. et al. Expected economic potential of substituting legumes for nitrogen in bermudagrass pastures. **Crop Science**, Madison, v. 52, n. 1, p. 1923-1930, 2012.

BORTOLINI, F. et al. Caracterizações morfológica e agronômica e divergência genética em germoplasma de trevo branco. **Revista Brasileira de Zootecnia**, Viçosa, v. 35, n. 4, p. 1601-1610, 2006.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Proteção de Cultivares no Brasil/MAPA**. Secretaria de Desenvolvimento Agropecuário e Cooperativismo. Brasília: MAPA/ACS, 2011. 202 p.

BRASIL. Ministério da Saúde. Portaria n° 2.914, de 12 de dezembro de 2011. **Diário Oficial da União**, Brasília, DF, 12 dez. 2011. Disponível em: <http://bvsms.saude.gov.br/bvs/saudelegis/gm/2011/prt2914_12_12_2011.html>. Acesso em: 12 out. 2017.

BRENNAN, J. P.; EVANS, J. Measuring the relative economic costs of legume and fertilizer nitrogen. **Australian Journal of Experimental Agriculture**, Melbourne, v. 41, n. 1, p. 383-390, 2001.

BRINK, G. E. et al. Growth of white clover ecotypes, cultivars, and germplasms in the Southeastern USA. **Crop Science**, Madison, v. 39, n. 1, p. 1809-1814, 1999.

BRINK, G. E.; SANDERSON, M. A.; CASLER, M. D. Grass and legume effects on nutritive value of complex forage mixtures. **Crop Science**, Madison, v. 55, n. 3, p. 1329-1337, 2015.

BRUGNOLI, E. A. et al. Diversity in diploid, tetraploid, and mixed diploid-tetraploid populations of *Paspalum simplex*. **Crop Science**, Madison, v. 53, n. 4, p.1509-1516, 2013.

BURSON, B. L. Apomixis and sexuality in some *Paspalum* species. **Crop Science**, Madison, v. 37, n. 1, p. 1347-1351, 1997.

BUTLER, T. J.; MUIR, J. P. Perspective on forage legume systems for the tallgrass and mixed-grass prairies of the southern Great Plains of Texas and Oklahoma. **Crop Science**, Madison, v. 52, n. 1, p. 1971-1979, 2012.

CARVALHO, N. L.; ZABOT, V. Nitrogênio: nutriente ou poluente? **Revista Eletrônica em Gestão, Educação e Tecnologia Ambiental**, Santa Maria, v. 6, n. 6, p. 960-974, 2012.

CARVALHO, P. C. F. et al. Forrageiras de Clima Temperado. In: FONSECA, D. M.; MARTUSCELLO, J. A. (Org.). **Plantas Forrageiras**. Viçosa, MG: Ed UFV, 2010. p. 494-537.

CASSMAN, K. G.; DOBERMANN, A.; WALTERS, D. T. Agroecosystems, nitrogen-use efficiency, and nitrogen management. **Ambio**, Stockholm, v. 31, n. 2, p. 132-140, 2002.

CASTAGNARA, D. D. et al. Produção de forragem, características estruturais e eficiência de utilização do nitrogênio em forrageiras tropicais sob adubação nitrogenada. **Semina: Ciências Agrárias**, Londrina, v. 32, n. 4, p. 1637-1647, 2011.

CHASE, A. **The North American species of *Paspalum***. [S.l.]: GPO, 1929. 310 p. v. 28. Contributions from the United States National Herbarium.

COSTA, A. R. et al. **Uso do nitrogênio na agricultura e suas implicações na emissão do gás de efeito estufa óxido nitroso (N_2O)**. Santo Antônio de Goiás: Embrapa Arroz e Feijão, 2009. 47p. (Documentos, 249).

COSTA, K. A. P.; FAQUIN, V.; OLIVEIRA, I. P. Doses e fontes de nitrogênio na recuperação de pastagens do capim-marandu. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 62, n. 1, p. 192-199, 2010.

COSTA, N. L. et al. Forage productivity and morphogenesis of *Axonopus aureus* under different nitrogen fertilization rates. **Revista Brasileira de Zootecnia**, Viçosa, v. 42, n. 8, p. 541-548, 2013.

COSTA, N. L.; SAIBRO. J. C. Adubação nitrogenada, épocas e alturas de corte em *Paspalum guenoarum* Arech. **Agronomia Sulriograndense**, Porto Alegre, v. 20, n. 1, p. 35-49. 1984.

COSTA, N. L.; SAIBRO, J. C. Estabelecimento e regimes de corte de alfafa e *Paspalum guenoarum* sob cultivo estreme e consorciado. **Pesquisa Agropecuária Brasileira,** Brasília, v. 20, n. 12, p. 1433-1442, 1985.

CUOMO, G. J. et al. Nitrogen fertilization impacts on stand and forage mass of cool-season grass-legume pastures. **Forage and Grazinglands**, Madison, v. 3, n. 1, p. 1-10, 2005.

DALL'AGNOL, M.; SCHEFFER-BASSO, S. M. Utilização de recursos genéticos de leguminosas para ruminantes. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 41., 2004, Campo Grande. **Anais**... Campo Grande: Sociedade Brasileira de Zootecnia, 2004. p. 115-128.

DALRYMPLE, E. J.; GOPLEN, B. P.; HOWARTH, R. E. Inheritance of tannins in Birdsfoot trefoil. **Crop Science**, Madison, v. 24, n. 1, p. 921-922, 1984.

DOE, J. Evaluation of coatings to control ammonia volatilization from surface-applied urea. **Crops & Soils**, Madison, v. 46, n. 1, p. 32-36, 2013.

ENRIQUEZ-HIDALGO, D. et al. Herbage and nitrogen yields, fixation and transfer by white clover to companion grasses in grazed swards under different rates of nitrogen fertilization. **Grass and Forage science**, Oxford, v. 71, n. 1, p. 559-574, 2015.

ERGON, A. et al. Species interactions in a grassland mixture under low nitrogen fertilization and two cutting frequencies. I. dry matter yield and stability and dynamics of species composition. **Grass and Forage Science**, Oxford, v. 71, n. 1, p. 667-682, 2016.

ERGON, A. et al. Species interactions in a grassland mixture under low nitrogen fertilization and two cutting frequencies. II. Nutritional quality. **Grass and Forage Science**, Oxford, v. 72, n. 1, p. 333-342, 2017.

ESPINOZA, F. et al. The breeding system of three *Paspalum* species with forage potential. **Tropical Grasslands**, Brisbane, v. 35, n. 4, p. 211-217, 2001.

FAGERIA N.; BALIGAR, V. Enhancing nitrogen use efficiency in crop plants. **Advances in Agronomy**, Madison, v. 88, n. 1, p. 97-185, 2005.

FERNANDES, G. et al. Impacto da fertilização nitrogenada em pastagens perenes na contaminação dos recursos naturais. **Revista Brasileira de Tecnologia Agropecuária**, Frederico Westphalen, v. 1, n. 1, p. 3-14, 2017.

FAO. Food and Agricultural Organization of the United Nations. **Climate change and food security: risks and responses**. [S.l.]: FAO, 2016. <http://www.fao.org/3/a-i5188e.pdf>. Acesso em: 29 nov. 2017.

FONTANELI, R. S.; SANTOS, H. P.; FONTANELI, R. S. **Principais forrageiras para integração lavoura-pecuária-floresta na região sul-brasileira**. Passo Fundo: Embrapa Trigo, 2009. 340 p.

GALLOWAY, J. M.; COWLING, E. B. Reactive nitrogen and the world: 200 years of change. **Ambio**, Stockholm, v. 31, n. 2, p. 64-71, 2002.

GARNETT, T.; CONN, V.; KAISER, B.N. Root based approaches to improving nitrogen use efficiency in plants. **Plant, Cell and Environment**, Oxford, v. 32, n. 9, p. 1272-1283, 2009.

GASTAL, F.; LAMAIRE, G. N uptake and distribution in crops: an agronomical and ecophysiological perspective. **Journal of Experimental Botany**, Oxford, v. 53, n. 370, p. 789-799, 2002.

HIREL, B. et al. Improving nitrogen use efficiency in crops for sustainable agriculture. **Sustainability**, Basel, v. 3, n. 9, p. 1452-1485, 2011.

HUBER, K. G. C. et al. Variabilidade agronômica e seleção de progênies F_1 de *Paspalum*. **Revista Brasileira de Ciências Agrárias,** Recife, v. 11, n. 4, p. 374-380, 2016.

INTERRANTE, S. M. et al. Production and economics of steers grazing tall fescue with annual legumes or fertilized with nitrogen. **Crop Science**, Madison, v. 52, n. 1, p. 1940-1948, 2012.

JANK, L. et al. The value of improved pastures to Brazilian beef production. **Crop and Pasture Science**, Melbourne, v. 65, n. 11, p. 1132-1137, 2014.

JANK, L.; VALLE, C. B. do; RESENDE, R. M. S. Breeding tropical forages. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 11, Special edition 1, p. 27-34, 2011.

LADHA, J. K. et al. Efficiency of fertilizer nitrogen in cereal production: retrospects and prospects. **Advances in agronomy**, San Diego, v. 87, p. 85-156, 2005.

LEMAIRE G.; JEUFFROY M. H.; GASTAL, F. Diagnosis tool for plant and crop N status in vegetative stage. **European Journal Agronomy**, Conthey, v. 28, n. 4, p. 614-624, 2008.

LOPES, R. R. et al. Management of consecutive cuts in the production and quality of wintergreen paspalum seeds. **Revista Brasileira de Zootecnia**, Viçosa, v. 45, n. 10, p. 587-595, 2016.

LUGÃO, S. M. B. et al. Acúmulo de forragem e eficiência de utilização do Nitrogênio em pastagens de *Panicum maximum* Jacq. (Acesso BRA-006998) adubadas com Nitrogênio. **Acta Scientiarum. Animal Sciences**, Maringá, v. 25, n. 2, p. 371-379, 2003.

LÜSCHER, A. et al. Potential of legume-based grassland–livestock systems in Europe: a review. **Grass and Forage Science**, Oxford, v. 69, n. 2, p. 206-228, 2014.

MACHADO, J. M. **Caracterização agronômica de ecótipos de *Paspalum notatum* Flügge em resposta ao fotoperíodo e a fertilização nitrogenada e seleção de híbridos intraespecíficos**. 2014. 115 f. Tese (Doutorado) – Programa de Pós-Graduação em Zootecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2014.

MARTINELLI, L. A. Os caminhos do nitrogênio**:** do fertilizante ao poluente. **Informações Agronômicas**, Piracicaba, n. 118, p. 6-10, 2007.

MARTUSCELLO, J. A. et al. Produção de biomassa e morfogênese do capim-Braquiária cultivado sob doses de nitrogênio ou consorciado com leguminosas. **Revista Brasileira Saúde e Produção Animal**, Salvador, v. 12, n. 4, p. 923-934, 2011.

MCSWINEY, C. P. et al. Greenhouse gas emissions calculator for grain and biofuel farming systems. **Journal of Natural Resources and Life Sciences Education**, Madison, v. 39, n. 1, p. 125-131, 2010.

MELLA, S. C. **Resposta de uma mistura de gramíneas e leguminosas subtropicais a diferentes sistemas de pastejo**. 1980. 160 f. Dissertação (Mestrado) - Programa de Pós-Graduação em Fitotecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 1980.

MOTA, J. F. A. S. **Caracterização morfológica e fisiológica de *Paspalum guenoarum* Arech**. Porto Alegre, UFRS, 1980. 94 p. Dissertação (Mestrado) - Programa de Pós-Graduação em Fitotecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 1980.

MOTTA, E. A. M. et al. Forage performance of *Paspalum* hybrids from an interspecific cross. **Ciência Rural**, Santa Maria, v. 46, n. 6, p. 1025-1031, 2016.

MOTTA, E. A. M. et al. Valor forrageiro de híbridos interespecíficos superiores de *Paspalum*. **Revista Ciência Agronômica**, Fortaleza, v. 48, n. 1, p. 191-198, 2017.

MUIR, J. P.; PITMAN, W. D.; FOSTER, J. L. Sustainable, low-input, warm-season, grass–legume grassland mixtures: mission (nearly) impossible? **Grass and Forage Science**, Oxford, v. 66, n. 3, p. 301-315, 2011.

NABINGER, C.; DALL’AGNOL, M. Principais gramíneas nativas do RS: Características gerais, distribuição e potencial forrageiro In: SIMPÓSIO DE FORRAGEIRAS E PRODUÇÃO ANIMAL, 3., 2008, Porto Alegre. **Anais**... Porto Alegre: [s.n.], 2008. p. 7-54.

NOVO, P. E. et al. Hybridization and heterosis in the Plicatula group of *Paspalum*. **Euphytica**, Wageningen, v. 1, n. 1, p. 198-213, 2017.

NOVO, P. E. et al. Interspecifc hybrids between *Paspalum plicatulum* and *P. oteroi*: a key tool for forage breeding. **Scientia Agricola**, Piracicaba, v. 73, n. 4, p. 356-362, 2016.

OBOUR, A. K.; HARMONEY, K.; HOLMAN, J. D. Nitrogen fertilizer application effects on switchgrass herbage mass, nutritive value and nutrient removal. **Crop Science**, Madison, v. 57, n. 1, p. 1754-1763, 2017.

ORTIZ, J. P. A. et al. Harnessing apomictic reproduction in grasses: what we have learned from *Paspalum*. **Annals of Botany**, Oxford, v. 112, n. 5, p. 1-21, 2013.

PAIM, N. R.; RIBOLDI, J. Duas novas cultivares de trevo-branco comparadas com outras disponíveis no Rio Grande do Sul, em associação com gramíneas. **Pesquisa Agropecuária Brasileira**, Brasília, v. 29, n. 1, p. 43-53, 1994.

PAIM, R. N.; NABINGER, C. Comparação entre duas formas de *Paspalum guenoarum* Arech. **Agronomia Sulriograndense**, Porto Alegre, v. 18, n. 2, p. 103-114, 1982.

PAULINO, V. T. et al. Nutritive value and digestibility of Aruana guinea grass under n fertilization of NBPT- treated urea. **International Journal of Agriculture Innovations and Research**, New Delhi, v. 4, n. 3, p. 415-420, 2015.

PEREIRA, E. A. et al. Variabilidade genética de caracteres forrageiros em *Paspalum.* **Pesquisa Agropecuária Brasileira,** Brasília, v. 47, p. 1533-1540, 2012.

PEREIRA, E. A. et al. Agronomic performance and interspecific hybrids selection of the genus *Paspalum*. **Científica**, Jaboticabal, v. 43, n. 4, p. 388-395, 2015.

PEREIRA, E. A. **Melhoramento genético por meio de hibridizações interespecíficas no grupo Plicatula – gênero *Paspalum*.** 2013. 166 f. Tese (Doutorado) – Programa de Pós-Graduação em Zootecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2013.

PITMAN, W. D. Bahiagrass (*Paspalum notatum* Flugge) management combining nitrogen fertilizer rate and defoliation frequency to enhance forage production efficiency. **Grass and Forage Science**, Oxford, v. 68, n. 3, p. 479-484, 2012.

PONTES, L. S. et al. Effects of nitrogen fertilization and cutting intensity on the agronomic performance of warm-season grasses. **Grass and Forage Science**, Oxford, v. 1, n. 1, p. 1-13, 2016.

QUARIN, C. L. The nature of apomixis and its origin in panicoid grasses. **Apomixis Newsletter**, Cidade do México, v. 5, p. 8-15, 1992.

RAMÍREZ-RESTREPO, C. A. et al. Production of *Lotus corniculatus* L. under grazing in a dryland farming environment. **New Zealand Journal of Agricultural Research**, Wellington, v. 49, n. 1, p. 89-100, 2006.

REIS, V. M. et al. Fixação biológica de nitrogênio simbiótica e associativa. In: FERNANDES, M. S. (Ed.). **Nutrição mineral de plantas**. Viçosa: Sociedade Brasileira de Ciência do Solo, 2006. p. 153-174.

ROCHA, M. G. et al. Avaliação de espécies forrageiras de inverno na Depressão Central do Rio Grande do Sul. **Revista Brasileira de Zootecnia**, Viçosa, v. 36, n. 6, p. 1990-1999, 2007.

ROTZ, C. A. et al. Ammonia emission model for whole farm evaluation of dairy production systems. **Journal of Environmental Quality**, Madison, v. 43, n. 1, p. 1143-1158, 2014.

SANDERSON, M. A. et al. Economic analysis of forage mixture productivity in pastures grazed by dairy cattle. **Forage and Grazinglands**, Madison, v. 3, n. 1, p. 1-9, 2006.

SANDERSON, M. Nutritive value and herbage accumulation rates of pastures sown to grass, legume, and chicory mixtures. **Agronomy Journal**, Madison, v. 102, n. 2, p. 728-733, 2010.

SANTOS, D. T. et al. Eficiência bioeconômica da adubação de pastagem natural no sul do Brasil. **Ciência Rural**, Santa Maria, v. 38, n. 2, p. 437-444, 2008.

SARTOR, L. R. et al. Nitrogen fertilizer use efficiency, recovery and leaching of an alexander grass pasture. **Brazilian Journal Soil Science**, Viçosa, v. 35, n. 3, p. 899-906, 2011.

SARTOR, M. E.; QUARIN, C. L.; ESPINOZA, F. Mode of Reproduction of Colchicine-Induced Tetraploids. **Crop Science**, Madison, v. 49, n. 4, p. 1270-1276, 2009.

SARTOR, M. E. et al. Ploidy levels and reproductive behaviour in natural populations of five *Paspalum* species. **Plant Systematics and Evolution**, New York, v. 293, n. 1, p. 31-41, 2011.

SCHEFFER-BASSO, S. M. et al. Comportamento de leguminosas (*Adesmia, Lotus, Trifolium*) em mistura com festuca. **Revista Brasileira de Zootecnia**, Viçosa, v. 31, n. 6, p. 2197-2203, 2002.

SCHEFFER-BASSO, S. M.; BRUSTOLIN, R.; DALL'AGNOL, M. Performance of *Lotus corniculatus* L. genotypes submitted to cutting interval: subsidies to a breeding program. **Revista Brasileira de Zootecnia**, Viçosa, v. 40, n. 8, p. 1645-1650, 2011.

SCHEFFER-BASSO, S. M.; VENDRUSCOLO, M. C.; CECCHETTI, D. Desempenho de leguminosas nativas (*Adesmia*) e exóticas (*Lotus, Trifolium*), em função do estádio fenológico no primeiro corte. **Revista Brasileira de Zootecnia**, Viçosa, v. 34, n. 6, p. 1871-1880, 2005.

SCHEFFER-BASSO, S. M.; GALLO, M. M. Aspectos morfofisiológicos e bromatológicos de *Paspalum plicatulum*. **Revista Brasileira de Zootecnia**, Viçosa, v. 37, n. 10, p. 1758-1762, 2008.

SCHNEIDER, R. et al. Avaliação agronômica de progênies de policruzamento de trevo-branco em dois locais do Rio Grande do Sul. **Revista Brasileira de Zootecnia**, Viçosa, v. 40, n. 9, p. 1879-1885, 2011.

SCHRÖDER J. J. The position of mineral nitrogen fertilizer in efficient use of nitrogen and land: a review. **Natural Resources**, Hubei Province, v. 5, n.15, p. 936-948, 2014.

SCHWINNING, S.; PARSONS, A. J. Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. **Journal of Ecology**, London, v. 84, n. 6, p. 799-813, 1996.

SEEPAUL, R. et al. Nitrogen application rate and genotype effects on switchgrass production and chemical characteristics. **American Journal of Plant Sciences**, Irvine, v. 7, n. 1, p. 533-546, 2016.

SILVEIRA, M. L et al. Bahiagrass response and N loss from selected N fertilizer sources. **Grass and Forage Science**, Oxford, v. 70, n. 1, p. 154-160, 2013.

SNYDER, C. S. **Eficiencia de uso del nitrogeno:** desafios mundiales, tendencias futuras. Informaciones agronomicas. [S.l.]: International Plant Nutrition Institute, 2009. 5 p. Disponível em: <http://www.ipni.net>. Acesso em: 18 ago. 2017.

SOSTER, M. T. B.; SCHEFFER-BASSO, S. M.; DALL'AGNOL, M. Caracterização agronômica de genótipos de cornichão (*Lotus corniculatus* L.). **Revista Brasileira de Zootecnia**, Viçosa, v. 33, n. 6, p. 1662-1671, 2004.

STEINER, M. G. et al. Forage potential of native ecotypes of *Paspalum notatum* and *P. guenoarum*. **Anais da Academia Brasileira de Ciências**, Rio de Janeiro, v. 89, n. 1, p. 1753-1760, 2017.

STRAPASSON, E.; VENCOVSKY, R.; BATISTA, L. A. R. Seleção de descritores na caracterização de germoplasma de *Paspalum sp*. por meio de componentes principais**. Revista Brasileira de Zootecnia**, Viçosa, v. 29, n. 2, p. 373-381, 2000.

TEKELI, A. S.; ATES, E. Yield potential and mineral composition of white clover (*Trifolium repens* L.) tall fescue (*Festuca arundinacea* Schreb.) mixtures. **Journal of Central European Agriculture**, Zagreb, v. 6, n.1, p. 27-34, 2005.

TEUTSCH, C. D.; FIKE, J. H.; TILSON, W. M. Yield, digestibility, and nutritive value of crabgrass as impacted by nitrogen fertilization rate and source. **Agronomy Journal**, Madison, v. 97, n. 6, p. 1640-1646, 2005.

TOWNSEND, C. **Características produtivas de gramíneas nativas do gênero *Paspalum*, em resposta a disponibilidade de nitrogênio.** 2008. 255 f. Tese (Doutorado) – Programa de Pós-graduação em Zootecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2008.

URBANI, M. H. et al. Registration of 'Boyero UNNE' Bahiagrass. **Journal of Plant Registrations**, Madison, v. 11, n. 1, p. 26-32, 2017.

VAN BUEREN, E. T. L.; STRUIK, P. C. Diverse concepts of breeding for nitrogen use efficiency. A review. **Agronomy for Sustainable Development**, Paris, v. 37, n. 1, p. 1-24, 2017.

VAN SOEST, P. J. **Nutritional ecology of the ruminnant**. 2. ed. Ithaca: Cornell University Press, 1994. 476 p.

VERHULST, N. et al. **Eficiencia del uso de nitrógeno y optimización de la fertilización nitrogenada en la agricultura de conservación**. [S.l.]: CIMMYT, 2015.12 p.

WEILER, R. L. et al. Intraspecific tetraploid hybrids of *Paspalum notatum*: agronomic evaluation of segregating progeny. **Scientia Agricola**, Piracicaba, v. 75, n. 1, p. 36-42, 2018.

6. AUTHOR BIOGRAPHY

Eder Alexandre Minski da Motta, was born in November 15, 1987, in Palmeira das Missões, Rio Grande do Sul, Brazil, son of Antonio Carlos Abron da Motta and Mariza Lucia Minski da Motta.

In October 2006, he started his Undergraduate Degree in Animal Science at the Federal University of Santa Maria (Palmeira das Missões Campus) and finished in December 2010.

From May 2011 to January 2012, he worked with dairy cattle in the company Käfer Agrícola (Representative - Gea Farm Technologies of Brazil). In this period, he worked with sales of hygiene products and equipment for milking, as well as technical assistance in management of milking and milk quality.

In April 2012, he started his Master of Science Degree in Animal Science at the Federal University of Rio Grande do Sul (UFRGS), under supervision of Dr. Miguel Dall'Agnol. In March 2014, he completed his Master of Science Degree.

In April 2014, he started his Ph.D. in Animal Science at the UFRGS, under supervision of Dr. Miguel Dall'Agnol. In 2017, had a "sandwich" period at the University of Florida, Gainesville, FL, USA, under the supervision of Dr. Esteban Fernando Rios. In March 2018, he submitted his Ph.D. thesis to the defence committee at the UFRGS, Porto Alegre, RS, Brazil.