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SEED AND FORAGE PERFORMANCE FOR AN ELITE GROUP OF *Paspalum notatum* INTRASPECIFIC HYBRIDS

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“Nem que tenha, cipó também é lenha!”
Pedro Luíz de Souza

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SEED AND FORAGE PERFORMANCE FOR A ELITE GROUP OF *Paspalum notatum* INTRASPECIFIC HYBRIDS[1]

Author: Cleber Henrique Lopes de Souza
Advisor: Miguel Dall'Agnol

Abstract: Previous studies for agronomy characterization in a *P. notatum* collection, selected the genotypes 'André da Rocha', 'Bagual', 'V4', '83N', '95N' and '36N' to be used in the Bahiagrass breeding program of UFRGS. Since the sexual tetraploids: C44X, Q4188 and Q4205 were available, these plants were used in crosses as mothers with the selected ecotypes as pollen donor. The progeny was evaluated for forage yield and had their reproduction mode identified. An elite group of nine intraspecific hybrids plus the controls 'Bagual', 'V4' and 'Pensacola' were arranged in a RCBD with three replications. There were evaluated over years and seasons for forage components and for two years for seed components, germination, viable seeds and dormancy. This study aimed: i) select superior agronomic *P. notatum* intraspecific hybrid for forage and seed production; ii) determine forage components of genotypes swards, cold tolerance and regrowth vigor; iii) characterize seed yield components, physiological and physical quality; iv) identify the forage components most related to herbage mass production; v) identify the variables most related to seed production; vi) provide information about reproductive phenology. Evaluations across harvest were performed during 2016/17 and 2017/18. Each time 70% of all genotypes reached 95% of luminous intercept to target the seed production, plots were deferred from the cuts when the beginning of the internode elongation was observed. Furthermore, the seed components variables were assessed in the harvest time. There were differences among genotypes, years and seasons for most of the variables assessed. The genotypes showed different performances for the traits allowing different patterns of seasonal forage yield, flowering period, seed yield and quality. The hybrids 'C15', 'C18', '336', '437' demonstrated desirable aspects in forage distribution, larger annual production being equal or better than 'Bagual' and 'V4'. 'Bagual' and hybrids 'C18', '336' stood out for seed production. 'Pensacola' showed poorly forage yield and seed performance. It was infected with high weeds not being a good check option. The hybrid 'C15' had a reasonable seed production in the first year and badly in the second year. The reason for that was negatively affected by the deferred management. Due to its highly forage yield and apomixis, more studies need to be done to establish the management to improve seed yield and quality.

Key words: forage distribution, sesonal production, seed yield, perennial grass, bahiagrass.

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Desempenho forrageiro e sementeiro de um grupo elite de híbridos intraespecíficos de *Paspalum notatum*[1]

Autor: Cleber Henrique Lopes de Souza
Orientador: Miguel Dall'Agnol

Resumo: Estudos anteriores para caracterização agronômica em uma coleção *P. notatum* selecionaram os genótipos 'André da Rocha', 'Bagual', 'V4', '83N', '95N' e '36N' para serem utilizados no programa de melhoramento genético da UFRGS. Uma vez disponíveis os os tetraplóides sexuais: C44X, Q4188 e Q4205, essas plantas foram utilizadas como mães em cruzamentos com os ecótipos selecionados os quais foram utilizados como doadores de pólen. A progênie foi avaliada quanto ao rendimento de forragem e teve seu modo de reprodução identificados. Um grupo de elite de nove híbridos intraespecíficos de grande produção forrageira, mais os controles 'Bagual', 'V4' e 'Pensacola' foram arranjados em um delineamento de blocos completos casualizados com três repetições e avaliados por anos e por estações do ano para componentes forrageiros e durante dois anos para componentes do rendimento de sementes, germinação, sementes viáveis e dormência. Este estudo objetivou: i) selecionar híbridos intraespecíficos de P. notatum agronomicamente superiores para produção de forragem e sementes; ii) determinar os componentes forrageiros, tolerância ao frio e vigor de rebrota; iii) caracterizar os componentes de produção de sementes, qualidade fisiológica e física; iv) identificar os componentes forrageiros relacionados à produção de forragem; v) identificar as variáveis relacionadas à produção de sementes; As avaliações ao longo da colheita foram realizadas durante 2016/17 (ano 1) e 2017/18 (ano 2), cada vez que 70% de todos os genótipos atingiram 95% de interceptação luminosa. Visando a produção de sementes, as parcelas foram diferidas dos cortes quando se observou o início do alongamento dos entrenós, e as variáveis dos componentes das sementes foram avaliadas por ocasião da colheita. Houve diferenças entre genótipos, anos e estações para a maioria das variáveis avaliadas. Os genótipos apresentaram diferentes desempenhos para os caracteres, permitindo identificar diferentes padrões de produção de forragem nas estações, produtividade e qualidade das sementes. Os híbridos "C15", "C18", "336", "437" mostraram aspectos desejáveis na distribuição de forragem, sendo a produção anual maior igual ou superior à de "Bagual" e "V4". "Bagual" e os híbridos "C18", "336" se destacaram na produção de sementes. "Pensacola" apresentou rendimento de forragem e desempenho de sementes ruim, abaixo do esperado, o que ocorreu devido à alta infestação de ervas daninhas, não sendo uma boa opção de testemunha. O híbrido "C15" teve uma produção razoável de sementes no primeiro ano e ruim no segundo ano uma vez que foi afetado negativamente pelo manejo de diferimento. Devido a sua alta produtividade de forragem e apomixia, mais estudos precisam ser feitos para estabelecer o manejo para melhorar o rendimento e a qualidade das sementes.

Palavras chave: distribuição forrageira, rendimento de sementes, gramínea perene, bahiagrass.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABIEC	Meat Brazilian Association
ABRASEM	Seeds and Cuttings Brazilian Association
Al	Aluminium
ANOVA	Analysis of Variance
Ca	Calcium
CEC	Capacity to Exchange Cations
CH	Canopy Height
cm	Centimeters
ColdTol	Cold Tolerance
CQFS	Soil Chemistry and Fertility Commission - RS/SC, Brazil
cv	Cultivar
CWE	Carcass Weight Equivalent
D	Dormancy of Seeds
DDM	Dead Dry Matter
DF	Deegre of Freedom
DM	Dry Matter
EEA	Agronomy Experimental Estation
EMBRAPA	Agriculture Research Brazilian Enterprise
ES	Empty Seeds
G	Germination of Seeds
g	Gram
GD	Degree Days
GDP	Gross Domestic Product
H	Hidrogenium
ha	Hectare
IBGE	Statistic and Geograph Brazilian
IBONE	Botany Northeast Institute of Argentina
IH	Inflorescence Height
INFDM	Inflorescence Dry Matter
IRFA	Index of photosynthetically active radiation intercept
ISA	Induced Sexual Autotetraploid
K	Potassium
kg	Kilogram
LBDM	Leaf Blade Dry Matter
m	Meters
M	Million
mg	Miligrams

Mg	Magnesium
mm	Millimeters
N	Nitrogen
NDVI	Normalized Difference Vegetation Index
NI	Number of Inflorescences
NR	Number of Racemes
NRI	Number of Racemes per Inflorescence
^oC	Celsius degree
OM	Organic Matter
P	Phosphorous
Perc.Leaf	Percentage of Leaf over TDM
PRNT	Relative Total Neutralizing Power
PSY	Potential Seed Yield
PW	Percentual of Weeds
RCBD	Random Column Block Design
RegVig	Regrowth Vigor
RL	Racemes Length
RS	Rio Grande do Sul
RTD	Reproductive Tillers Density
sat	Saturated
SC	Santa Catarina
SDM	Sheat Leaf and Stem Dry Matter
SULPASTO	Associação Sul Brasileira de Produtores de Sementes de Pasto
SY	Seed Yield
T	Temperature
Ton	Tonnes
TDM	Total Dry Matter
Tmax	Maximum Temperature
Tmean	Mean Temperature
Tmin	Minimum Temperature
TSW	Thousand Seed Weight
TTD	Total Tillers Density
TZ	Viable Seeds
UFRGS	Universidade Federal do Rio Grande do Sul
UNNE	Universidad Nacional del Nordeste
USDA	United States Department of Agriculture
VSY	Viable Seed Yield
VTD	Vegetative Tillers Density

WDM	Weed Dy Matter

1. CHAPTER I

1.1 GENERAL INTRODUCTION

Brazil had a cattle herd around 222 million heads, it is approximately one animal per each Brazilian citizen regarding to Instituto Brasileiro Geográfico e Estatístico (IBGE, 2017). According to Associação Brasileira da Indústrias Brasileiras de Carne (ABIEC 2018), it is estimated that 39.2 million heads are slaughtered per year, getting a beef production of 9.71 million Tons Carcass Weight Equivalent (T CWE). The exports are 20.9% of this value for main destinations such as Hong Kong (26%), China (14%), Egypt (10%), Russia (10%), Iran (9%), European Union (7%), Chile (4%), Saudi Arabia (3%), United States (1%) and othe (13%).

On the other hand, the domestic marked consumed the remaining 79.1% proving the importance of the intern consumers for this activity. Even though, the Gross Domestic Product (GDP) reached out 6.6 % of all Brazil revenues: R$ 6.56 trillion, contributing for the trade positive balance in 2018. It raised 3.6 % 2017 compared to 2016.

This big system of meat production just works on because Brazil has low-cost inputs when compared to other countries (Agribenchmark, 2018). Brazilian livestock has been using pastures as the base of the productive system since ever. The country has 158.6 M ha of pastures lands being 29.5% of natural grasslands, 63% and 7.5% of cultivated pastures at good and bad conditions, respectively (IBGE, 2017 – preliminar results). Therefore, only 10.44% of total slaughter cattle are from feedlots (ABIEC, 2018).

Year by year since 1990, Brazil has been pushing down the land use area for each unit of beef production. That is a consequence of increasing activity efficiency motivated by nature conservations law, by broad market competition, and because part of the land has been destined to grow grains such as soybean.

One of the pillars to achieve this scenario it was results from plant breeding efforts whereupon a couple of apomictic grass were selected and released, an example: the cultivar ‘Marandú’, a superior genotype of *Urochloa brizantha* collected in Africa and selected by Valle *et al*. (2009). This perennial plant adapted to acid soils and to low nutrientes requirements got success for whole Midwest and North of Brazil dominating the forage market due to the easy implementing and reasonable obtaining viable seeds.

In general, tropical forage plants have a recent domestication history exhibiting characteristics of wild plants in terms of seed production. Therefore, forage breeder should also aim at reducing the seed shattering, dormancy and uniformity in flowering, as well pursue to improve seed yield components aiming to increase the production of viable pure seeds.

The ability to produce seed in enough amounts, germination and vigor rates is important to guarantee easy establish of sward as well as for variety succeed. Therefore, the use of seeds as a vehicle for propagating forage species promotes the rapid dissemination of new materials making easy its adoption by agricultors (Carambula, 1981).

The seed market in Brazil moves approximately R$ 10 billion being behind only the USA and China. The forage market share contributes 11% of the total, being responsible for the biggest exportation of tropical forage seed in the world, wherein *Urochloa spp*. (ex-*Brachiaria spp.*) varieties are predominant

(ABRASEM, 2015). During the years 2016 and 2018 there were 4784 and 3,020 seeds fields registered at MAPA for *Urochloa spp*. and *Megathirsus maximum* (ex-*Panicum spp.*) varieties, respectively, performing a total gross production 5,313.46 and 1.057.65 tonnes of seed. Today, the most party of crop perennial pastures used in the country are from exotic genus such as *Urochloa spp*. and *Megathirsus maximum* (Jank *et al*., 2011).

It is possible to infer that beef system production is basement at two tropical perennial forage genera. The pastures formed by these swards have high genetically uniformity because these species present apomixis. The apomixis guarantee heterosis fixation and uniformity for pastures in the whole places because it is a clonal reproduction via seeds. Notwithstanding, these forages from narrow genetic bases presents great risk and vulnerability to pests, diseases, and climatic changes because they have high selection pressure against pathologies.

At the subtropical places, as Rio Grande do Sul, due the lack of summer species tolerant to cold, many experts and farmers have attempting to use tropical varieties such as *Urochloa brizantha* cv. MG-5 and *Megathirsus maximum* cv. Aruana to form perennial pastures. However, frost events could kill all the plants with a need to new sowings and more costs.

An alternative to diversify the pastures used in the country is exploring the potential of native species more adapted to different ecosystems. In the southern region of the country, the natural grassland biome called Pampa covers 7.5 M ha corresponding at 81.9% of total state pastures (IBGE, 2017 – preliminar results). Even today, it is considered the main driving force of cattle raising in the Rio Grande do Sul. The beef system economy has its spot in, the cattle herd, with 13.781.896 heads get 6.21% of Brazil total wherein 77.16% of cattle exclusively destined for slaugter. In contrast, it has been losing space for more intensive activities such as grain farming and fruit growing. This biome is a source of germplasm for obtaining promising grasses, whose has a great diversity of species with forage potential being approximately 400 grasses, mainly of the genus *Paspalum*, and more than 150 legumes (Boldrini, 2006).

However, farmer's search for increased gains and lack of knowledge of the management and quality of native species led to the adoption and implementation of exotic forages, neglecting this natural germplasm bank (Jacques; Nabinger, 2006).

An agreement among UFRGS, EMBRAPA and South-Brazilian Association for Forages Research Promotion (SULPASTO) has been working with genetic breeding of *Paspalum spp*. with the intention of genetic improvement and release cultivars suited to new market demand, in order to ensure local sovereignty in livestock production and minimize the environmental impact caused by intensive production systems.

The native grasses of this species are tetraploid and present an apomictic mode of reproduction (Quarin *et al*., 2001). For breeding purposes, it is necessary to search for and obtain sexual plants. The sexual tetraploid clones used in breeding programs are from chromosome duplication of sexual diploid genotypes (Weiler *et al*., 2015). Intraspecific hybridizations between artificially tetraploidy sexual plants and elite apomictic indigenous ecotypes result have generated hybrids populations that segregated for apomictic and sexual reproduction. The progenies presented genetic variability and have been selected to desired characteristics (Weiler *et al*., 2017; 2018).

This thesis aims to select hybrid genotypes more productive during the growth seasons in two years through agronomic evaluations proofing forage value, seed production, cold tolerance, competition and persistence ability in a Brazil southern subtropical zone.

1.2 LITERATURE REVIEW

1.2.1 *Paspalum spp.*

The genus *Paspalum L.* (family Poaceae) has its center of origin and diversification of most of its species in South America (Batista; Godoy, 1998). It includes more than 400 tropical and subtropical species, many of them with good forage value, whose importance is evidenced by their adaptability and presence in practically all herbaceous communities in the different Brazil ecosystems (Batista; Godoy, 2000). They are often dominant and responsible for producing the largest share of forage available in many of these plant formations (Valls, 1987). This large genetic diversity shows the lower risk of biological imbalance of these ecosystems (Strapasson *et al*., 2000).

The species of the genus *Paspalum L.* have a wide geographical distribution, being found in natural pastures in Uruguay, Argentina, Paraguay and Brazil, in the latter, in the states of Mato Grosso do Sul, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul and other. They occur in all physiographic regions, exhibiting approximately 70 taxonomical entities with wide and often particularized distribution (Barreto, 1974). The most species *Paspalum* with potential forage use are in the botanical groups: *Notata*, *Dilatata* and *Plicatula*, being important because they present species of excellent adaptation to drought, cold and defoliation (Valls; Pozzobon, 1987; Batista; Godoy, 2000; Penteado; Macedo, 2000).

Currently, research has been carried out to characterize germplasm and genetic improvement for forage performance, quantitative and qualitative aspects of seed production of *P. notatum*, *P. guenoarum* and *P. lepton* (Bertoncelli, 2018; Motta, 2018; Graminho, 2018; Krycki, 2019; Nexus' developing project).

Due to the importance of this genus and its contributions for the natural pastures of the Rio Grande do Sul, many studies were conducted by the Federal University of Rio Grande do Sul generating information about forage matter production, nutritive value, response to photoperiod and to nitrogen doses at several species: *P. notatum*, *P. guenoarum*, *P. lepton*, *P. urvillei*, *P. pauciciliatum* and *P. lividum* (Santos, 2005; Sawasato, 2007; Townsend, 2008; Pereira *et al*., 2011; Fachinetto *et al*., 2012; Machado, 2014; Fachinetto *et al*., 2017; Steiner *et al*., 2017).

In other studies, the mode of reproduction, the level of ploidy, the regularity of chromosomal pairing in meiosis of pollen mother cells, the pollinic viability of parents, the analysis and counting of somatic chromosome number were determined for the progenitors used in crosses and for the progeny obtained (Pereira, 2013; Krycki *et al*., 2016; Weiler *et al*., 2017; Fachinetto *et al*., 2018).

1.2.2 Paspalum notatum

P. notatum var. *notatum* has its center of origin in southern Brazil, northern Argentina and Paraguay (Parodi, 1937). It is a tetraploid, apomictic and pseudogamic species. In general, it establishes a dense lawn of 20 to 40 cm in height providing good forage from spring to autumn (Otero, 1961). The species is a perennial plant having a hot season cycle, dispersing through supraterran rhizomes and seeds (Barreto, 1974). Most of the production of this species occurs in the hottest months of the year, due to higher temperatures (25-30°C) and longer days (Newman *et al.*, 2010).

P. notatum is recognized as an excellent forage because of its nutritive value, acceptability by animals, high resistance to trampling and grazing, and tolerance to cold (Rosengurt, 1979; Pozzobon; Valls, 1997; Gates *et al.*, 2004). Due to the predominant participation in forage production among the species of the natural pastures of the Brazil southern fields (Valls, 1987), it has been highlighted in cattle feeding as one of the most important native pasture species of southern Brazil, Uruguay, northeast Argentina and southern Paraguay (Quarín *et al.*, 1984).

In the United States, *P. notatum var.* Pensacola was introduced around 1926 and was first distributed to producers in 1943 (Blount; Acuña, 2009), it is estimated that *P. notatum* turf and forage types have been used in 2.6 million hectares between livestock production and roadway cover (Newman *et al.*, 2010). It is also well adapted to the low-altitude regions of south-western Japan and used for both grazing and hay (Hirata *et al.*, 2006).

P. notatum originates in South America, concentrates its leaves production from spring to autumn and has high resistant to trampling and grazing because its rhizomes are protected by the sheath, emitting new leaves when stimulated by the temperature in the spring (Barreto, 1974; Maraschin, 2001). Growth points are located near the surface soil make the plant tolerant to low and frequent cuts, which makes it impossible to remove them, besides it requires low levels of fertility and pest control (Dall 'Agnol *et al.*, 2006). The vegetative propagation initially is slow, extending the stolons, forming a dense grassy lawn fixed to the ground in which it develops a pasture structure difficult to be invaded by other species and tending to be dominant (Boldrini, 1993).

In Brazil, only one cultivar of this species is Capim-Pensacola (*Paspalum notatum* Flügge var. *saure*). Although of South American origin, it was introduced and initially studied in Florida in 1935 (Haddad *et al.*, 1999). In the United States it was widely used for beef cattle and equines growth, being the most widespread and economically interesting species, with estimates of one million hectares cultivated in the state of Florida alone (Burton; Mulinix, 1998). In Rio Grande do Sul, it was mainly in the “Planalto” region, hence the importance of being compared from the point of view of production and forage value with the native ecotypes (Dall'Agnol *et al.*, 2006).

1.2.3 *P. notatum* ecotypes used by UFRGS

Paspalum notatum is a polymorphous species with great morphological variability with differents appearance: vigor, size and pilosity of leaves, height of floriferous stalks, number and length of racemes, size and color of spikelets (Parodi, 1948; Barreto, 1974). The apomixis presence at a poliploid level could contribute to more uniform germplasm however, great variability exists for the species on morphological, polyploid, cytogenetic and molecular levels (Barreto, 1974; Chase, 1929; Canto-Dorow *et al.*, 1993; Steiner, 2005; Cidade *et* *al*., 2008; Reyno *et al*., 2012; Fachinetto *et al*., 2017). The species is subdivided into two varieties, *P. notatum* var. *saure* (a diploid sexual) and *P. notatum* var. *notatum* (an apomictic tetraploid), other observed forms are classified as biotypes (Canto-Dorow *et al*., 1993).

Canto-Dorow *et al*. (1993) defined four biotypes for *P. notatum* based on the length and width of the leaves, presence or absence of hair, spikelets size, and a number of veins in lemma I. The biotypes set up were: i) A: long and wide leaf blades; ii) B: long and narrow leaf blades; iii) C: short and narrow leaf blades and iv) D: pubescent leaf blades. By the other hand, Steiner (2005) arranged six morphological groups for 41 accessions. The most important traits were length and width of the leaves, length of the racemes, and spikelets. Cidade *et al*. (2008) performed a morphological characterization for 95 accessions of *P. notatum* based on eight traits in which resulted in the formation of eight groups. The divergence among accessions was influenced by the height of the flowering stem, length and width of the leaf blades, and length of the racemes.

Fachinetto *et al*. (2017) studied morphological traits in a *P. notatum* germplasm composed by two groups: i) 25 Plants Introduced (PIs, denomination used by from USDA collection and ii) 28 entries from UFRGS collection (Dahmer *et al*., 2008). The genotypes were collected at locations in Argentina, Brazil, Uruguay, and Paraguay. They found 13 and 19 morphological groups based on qualitative and quantitative data, respectively, reporting greater intraspecific diversity than found by any other authors. This was attributed to the inclusion of accessions obtained from the USDA, which resulted in greater geographical coverage and, therefore, in a more comprehensive collection. The results showed variability for all morphological characteristics analyzed and in particular for the angle between the racemes (53.65% of the total variance observed), the insertion angle of the leaves (25.65%), the length of flowering stems (14%). These three characteristics alone accounted for 93.26% of the total variance of the nine traits evaluated in the study. In addition, the length of leaves contributed by 16.61% to the formation of groups. This trait is related to forage production and plant habit, could be used for selection. These deserve some attention because erect habit plants compete better for light and tend to achieve a greater height (Bernardes, 1987). In the plant community as a pasture, this could result in greater forage production. On the other hand, prostrate habit plants may be better adapted to heavy grazing and be better at colonizing new habitats (Zimmer, 1994). Since these would accumulate more dry matter in rhizomes suggesting my be tolerate to intensive grazing (Pedreira; Brown, 1996a). Prostrate habit plants would producer more roots than erect growth habit plants while these would produce more foliage. According to Pedreira; Brown (1996b), selection for increased yield in *P. notatum* resulted in increased allocation of dry matter to harvestable foliage and prossibly a greater production of non-root biomass.

Also, Fachinetto *et al*. (2017) assessed the genetic variability among the same group of accessions from this germplasm collection, based on morphological traits and genetic diversity. The traits most contributing to the genotypes discrimination were identified and the information generated guided the crosses to explore more heterosis into the breeding program.

Notwithstanding, in different ecological conditions diverse forms can be found. This may be attributed to the environment-dependent phenotypic variation of the same genetic group called biotypes (Canto-Dorow, 1993) or variations within the species adapted to survive in a particular environment defined as ecotypes (Otero, 1961). In Rio Grande do Sul, there are native *P. notatum* ecotypes adapted to various soil and climate conditions, with different intrinsic characteristics as to size and thickness of the rhizome, leaf and inflorescence size, yield and quality (Nabinger; Dall'Agnol, 2008). In a study comparing two native ecotypes, identified as 'Capivari' and 'André da Rocha' with the commercial cultivar Pensacola, Prates (1977) found that native ecotypes were superior in several aspects evaluated, with 'André da Rocha' found to be promising, showing higher canopy, high forage production, good nutritive value and good seed production.

Santos (2005) characterized the growth dynamics of *P. notatum* ecotypes ‘André da Rocha’ and ‘Comum’ plus the species: *P. pauciciliatum*, *P. lividum* and *Axonopus catharinensis*. ‘André da Rocha’ stood out because had a leaf blade linear accumulation which increased in the whole period of the experiment: spring, summer and autumn. The dead matter accumulation in 'André da Rocha' only became inconvenient after 400 degrees days indicating an ability by its to hold large forage matter of good value for a long period what it was a response of the longer leaf life duration of it. Haddad *et al*. (1999) working with Pensacola found significative effect of dry matter production until 65 days after the standard cut in the summer, even with poor quality. In the end of winter and beginning of spring ‘André da Rocha’ had the longest leaf blade length (55 cm). Among the studied ecotypes, ‘André da Rocha’ had minor phyllocron in winter and spring conditions and presented higher elongation rates in spring, followed by summer and fall. The dry matter production reached 13.9 ton.ha^{-1} with 7.8 ton.ha^{-1} of leaf blades and was higher than in *P. notatum* ‘Comum’.

During two growing seasons, acessions of *Paspalum urvillei, P. guenoarum*, *P. notatum* produced in average 17, 16.8 and 14 ton.ha^{-1} of dry matter, respectively (Sawasato, 2007). These accesses got a ratio of over 60% leaf blades corroborated by observed by Santos (2005) and aforementioned.

Steiner *et al*. (2017) compared natives *Paspalum* ecotypes and relying upon agronomic evaluations; they observed *P. guenoarum* ecotypes ('Azulão' and 'Baio') yielded 32% more dry matter than *P. notatum* ecotypes ('André da Rocha' and 'Bagual'). Nonetheless, the authors mentioned that the specie particular growth habit and the crop management imposed to the trial beneficiated *P. guenoarum* ecotypes allowing more dry matter accumulation. In a different cuts regime, more frequently, *P. notatum* could produce more. Even this way, *P. notatum* ecotype 'Bagual' produced more dry matter than 'Pensacola' variety, with yields of 14 tons of DM.ha^{-1} and 8 tons of DM.ha^{-1}, respectively.

A germplasm collection composed of 55 *P. notatum* accesses collected in South America by the United States Department of Agriculture (USDA) and Department of Forage and Agrometeorology of Agronomy College (UFRGS) was studied by Fachinetto *et al*. (2012). The higher performance ecotypes were 48N, 95N, 30N e V4. They produced more than ‘Bagual’ and ‘André da Rocha’, as well as, showed higher persistence through the winter. All genotypes aforementioned presented higher yield than cv. Pensacola. The brazilian natives ecotypes used in these experiments: V4, ‘André da Rocha’ and ‘Bagual’ are from Rio Grande do Sul, respectively, collected at 'Barra do Quaraí' and 'André da Rocha' cities and 'Missões' region, naturally found at lower latitudes than those genotypes originating from Argentine: 48N - Mercedes; 95N - Corrientes; 30N - Santa Fé. The latter places have colder and more rigorous winters with many frost events, what can explain the differences observed in dry matter production and the persistence evaluation between the brazilians and argentine ecotypes.

Fachinetto (2010) evaluated 25 accessions of *P. notatum* plus *P. guenoarum* ecotype 'Azulão' and 'Baio' and reported great variability for morphological characteristics. Most of the accessions presented high forage yields and good persistence in winter when compared to cv. Pensacola. Although the accessions of *P. guenorarum* were more productive of all the accumulated production along the evaluation period, *P. notatum* 48N and 95N were the most productive accessions in all the cuts, being in some evaluations, similar to the *P. guenoarum*. The accessions most productive (30N, 48N, 95N, and V4), also belonged to different morphological groups. This is a piece of valuable information for crosses because progenitors with more genetic distance can result in a more heterozygotic progeny. It is worth noting that the materials evaluated had higher yields than Bagual and André da Rocha of recognized forage potential (Prates, 1977; Sawsato, 2007; Towsend, 2008; Steiner *et al*., 2017).

Machado (2014) evaluated dry matter production undergoing increase doses of nitrogen fertilization (0, 60, 180 and 360 kg.N.ha^{-1}) for the selected ecotypes by Fachinetto *et al*. (2012). The evaluations were carried out by forage mass harvest, totaling seven cuts in the whole period. Nitrogen was applied as urea and fractionated in three applications during the evaluations. There were interaction ecotypes x nitrogen x cut for the variables analyzed, except for the production of total accumulated dry mass in the period, although it presented significant difference among ecotypes and nitrogen doses.

There were verified positive responses to increasing levels of fertilization. The most productive genotypes were 48N, 83N, 'André da Rocha' and 'Bagual', in dry matter production and in the production of leaf blades. These ecotypes were collected at Mercedes and Corrientes (Argentina), and Rio Grande do Sul (Brazil) (last two), respectively. Also, 83N, 'André da Rocha' and 'Bagual' presented the better forage production distribution along the cuts and produced around 20% more than the less productive genotypes including cv. Pensacola.

Although Pensacola is one of the few alternatives for summer perennial species to subtropical places in which seeds are available on the market, practically all studies comparing the native ecotypes of the species and the hybrids resulting from them with it have shown that these genotypes present higher yields than this cultivar, pointing out the need to explore the productive potential of these materials (Fachinetto *et al*., 2012; Barbosa, 2014; Machado et al., 2017; Weiler *et al*., 2018).

1.2.4 Apomixis in *Paspalum sp.*

Apomixis is an alternative breeding route of plants that produces genetically identical individuals to the parent female plant through seeds (Ortiz *et* *al.*, 2013). This mode of clonal reproduction results in genetically identical progeny derived from an unreduced and unfertilized egg cell (parthenogenesis) (Hand *et al.*, 2014). This complex feature is of great interest to the agricultural community because there is the potential to establish hybrid vigor in economically important crops. Also, it is an invaluable tool for plant breeding.

This asexual path is genetically controlled in plant reproduction. The embryo develops from mitotic divisions of egg cells, causing the formation of fertile seeds, without the union of the female gamete with the male, as occurs in the sexual reproduction (Carneiro; Dusi, 2002).

According to Savidan *et al*. (1989), in the apomictic species, breeding requires totally or highly sexual plants to cross-breed and generate genetic variability. Once they are available, it is possible to improve agronomic characteristics and to fix these characteristics through intraspecific hybridization of sexual plants with apomictic plants. Since part of F1 progeny will be presenting apomixis, besides being a heterozygous progeny, the apomictic individuals may be propagated by seeds without genetic segregation.

Most *P. notatum* biotypes are autotetraploids (2n = 4x = 40) and reproduce through apospory type of apomixis, with pseudogamy (Forbes; Burton, 1961). In pseudogamous apomixis, although the donor pollen does not contribute with genetic material, the production of viable pollen is needed for fertilization of the endosperm. In Rio Grande do Sul, tetraploid forms are predominant and all apomictic (Pozzobon; Valls, 1997; Dahmer *et al*., 2008).

Some level of variation within accessions can be expected, since apomixis is facultative in most tetraploids of the genus *Paspalum* (Sartor *et al*., 2011), and a degree of genetic diversity is present in apomictic *Paspalum* species (Sartor *et al*., 2013; Majesky *et al*., 2015).

Diploidy forms often have sexual reproduction (Adamowski *et al*., 2005) and diploidy apomictic are rare only occurring as exception arising from dihaploids obtained from an apomictic tetraploid (Martinez et al., 2001). The authors suggest certain complementarity at polyploid level caused by an alleles variation, wich could give rise to apospory expression in tetraploids.

The genic segment that controls apomixis in *P. notatum* has a strong suppression of recombination because it originated from a translocated segment of a centromeric, telomeric or heterochromatic region during the *Paspalum* genus speciation process. Therefore, apomixis of the apospory type has a tetrasomic inheritance of a single dominant gene with a pleiotropic effect and incomplete penetrance (Martinez *et al*., 2001). According to the authors, the lethal effect of this dominant allele distorts the segregation pattern, making possible the greater presence of sexual individuals in progeny than in apomictic ones. Several studies indicated the apomixis character as a monogenic inheritance, segregating for sexual and apomictic progenies in the proportion of 3:1, Sex:Apo (Grossniklaus, 2001); 2.8:1 (Martinez *et al*., 2001); 6.5:1 (Stein *et al*., 2004); 4.65:1 (Acunã *et al*., 2009); 4.35:1 (Acuña *et al*., 2011); ranging 1:1 to 7:1 among different families (Zilli *et al*., 2015); and 1:2.1 (Weiler *et al*., 2017).

The variation in the degree of apomic expression was observed as correlated with different stages throughout the flowering season in *Dichanthium aristatum* Poir. (Knox, 1967), Capim Buffel (Sherwood *et al*., 1980), *Paspalum cromyorrhizon* Trin. (Quarin, 1986) and *P. notatum* (Rebozzio *et al*., 2011).

Certain environmental interference may affect the number of sexual eggs in facultative apomicts (Koltunow, 1993). Rios *et al.* (2013; 2017) indicate that facultative apomictic plants can produce both sexual and apomictic seeds at different periods of their flowering; however, it is still not clear which environmental factors affect, and to what extent, the preference of the reproductive mode occuring in facultative apomictics of *P. notatum*. During the aposporic development, the megaspore mother cell may degenerate before or after the beginning of the differentiation of the apospory or undergoes meiosis and form a reduced embryonic sac. Therefore, the sexual process can coexist with unreduced embryonic sac (Karasawa, 2009).

Quarín *et al.* (1984) showed that some plants of *P. notatum* tetraploids are facultative apomictic with high expression of sexuality. When they suffer some kind of environmental stress, they present embryos with the sexual reproduction mode. According to the aforementioned, for breeding purposes, the management of environmental conditions of light, temperature, water deficit and photoperiod could be used to increase the sexuality level of plants purposed for crosses in which these would be used as pollen recipients. In addition, sexual reproduction in the facultative apomictics could be exploited when crosses are made at the beginning or end of the flowering season (Rebozzio *et al.*, 2011, Rios *et al.*, 2013a). When uniform progeny is desired, however, seed should be harvested at peak flowering to ensure clonal reproduction through apomixis (Rios *et al.*, 2017).

1.2.5 Obtention of *P. notatum* sexual tetraploids

The existence of sexual genotypes is obligated for any breeding program of polyploid apomictic grasses (Sartor *et al.*, 2009). *Paspalum* genus has a high correlation between ploidy level and the mode of reproduction. Diploidy individuals usually shows sexual reproduction and alogamy, while polyploidy individuals are correlated with apomixis (Adamowski *et al.*, 2005).

Among the first studies to characterize *P. notatum*, a diploid form ($2n = 2x = 20$) native of northeast and middle west of Argentina was described as sexual and meiotically stable, allogamy and self-incompatible (Burton, 1946; Forbes; Burton, 1961).

Native sexual diploid of *P. notatum* plants have been found in In Brazil and in Rio Grande do Sul (Pozzobon; Valls, 1997; Fachinetto *et al.*, 2018), but their presence is associated with modified environments, as an escape from the cultivar Pensacola (Dahmer *et al.*, 2008). In other *Paspalum* species, cytogenetic analyses have revealed diploid accessions (Pozzobon *et al.* 2008; 2013).

In *P. notatum*, new wild diploids were revealed: ‘66N’, ‘67N’ and ‘92N’ collected in Santa Fe (Argentina) and ‘87N’ in Paysandu (Uruguay) (Fachinetto *et al.*, 2018). In general, they were more productive than Pensacola and more persistence in winter (Fachinetto *et al.*, 2012). In addition, the four diploid accessions, when analyzed by morphological traits, were grouped separately from each other and Pensacola. These plants are new sources of variability can be use in crosses with other diploid entries or tetraploid genotypes if duplicated ploidy (Fachinetto *et al.*, 2017).

Among the native populations of Rio Grande do Sul state, tetraploid sexual plants were not found (Dahmer *et al.*, 2008). Due to the lack of tetraploid sexual genotypes, it was necessary to duplicate the chromosome number of the sexual diploid accesses for creation of sexual tetraploid plants, whose purpose is to use them as female parents in the crosses with apomictic plants (Quarin *et al*., 2001).

In a hybridization process published in 1960, sexual tetraploid individuals were obtained from chromosome duplication, using colchicine and subsequent crosses with tetraploid apomictic individuals (Burton; Forbes, 1960), but the cultivars were not released. In the year 2000, Quarin *et al*. (2001, 2003) obtained three novel *P. notatum* sexual tetraploids artificially polyploid using colchicine, termed ‘C4-4X’ and ‘Q4188’, ‘Q4205’.

Recently, the Pensacola variety was polyploid using colchicine, obtaining three plants, named ‘WKS3’, ‘WKS 63’ and ‘WKS 92’ (Weiler *et al*., 2015). Sexual reproduction was confirmed in ‘WKS 63’ and ‘WKS 92’ while ‘WKS 3’ became apomictic after chromosome duplication (Krycki *et al*., 2016). The expression of apomixis in the duplicated plants may be due a gene-dosage efect. Quarin *et al*. (2001) state that the apomixis gene is present at the diploid level, but it is unexpressed in the diploid plant. The ploidy-dependence may occur at a locus that controls the apomixis using a secondary locus that involves a higher allele dosage to affect the expression of the main locus.

The two sexual plants generated were new sources of genetic variability, with the possibility of crosses for breeding programs, resulting in productive exploitation of the genus *Paspalum*. Therefore, in order to perform crosses, it is essential to make evaluations of superior parents that have the capacity to transmit characteristics of superior forage to the progeny (Krycki, 2015).

Chromosome duplication of diploid sexual plants allows the level of ploidy to be equalized within intraspecific hybridizations, allowing the use of sexual individuals as female parents in apomictic genetic breeding programs (Hanna *et al*., 1995). For many species, the irregular chromosome pairing of newly induced polyploids may compromise fertility, seed production and the viability of new individuals and future crosses (Podio *et al*., 2012). However, several cytogenetic studies revealed stability and viability in meiosis in *Paspalum spp.* (Moraes-Fernandes *et al*., 1973; Pagliarini *et al*., 2001; Dahmer *et al*., 2008; Reis *et al*., 2008; Krycki *et al*., 2016; Krycki, 2019). Therefore, it is necessary in a breeding program, for the selection of individuals able to reproduce viable seeds.

These sexually duplicated plants were used in crosses generating apomictic and new sexual plants more stable of better agronomic value (Weiler *et al*., 2018; Machado, 2014). The genetic background of the sexual plant used as a pollen recipient in the crosses does not interfere with the segregation of apomixis (Forbes *et al*., 2004). Currently, these new sexual plants have been used in the breeding program of the Forage Breeding Group of the Agronomy College in the Federal University of Rio Grande do Sul (UFRGS). These genotypes have been used as female at crosses with other superior hybrids (Krycki, 2019) and natives apomictic collected at exploration excursions for new sources of germplasm in the Pampa (Nexus project, at developing by Dall'Agnol, Weiler, Brunes and Simioni - data not published).

Analyses of the reproduction mode of parents and progenies are fundamental in breeding programs aimed at enabling intraspecific hybridizations and the choice of the parents. This evaluation allows identifying the plants that will be used as female parents plants of sexual reproduction and as male parents apomictic plants (Krycki *et al*., 2016)

Determination of the reproduction mode can be carried out by phenotypic variability in F_2, molecular markers, flow cytometry and cytoembryological analyses (Matzk *et al*., 2000; Martínez *et al*., 2003; Krycki *et al*., 2016; Weiler *et al*., 2017). The last mentioned is the most trustworthy method.

Any deviation in the endosperm ratio seriously affects seed development. Besides that, apomictic of *P. notatum* forms endosperms with a ratio of 4m:1p (Quarín, 1999). Thus, a mature seed developed by sexual means has a ratio of embryo DNA content: endosperm 2n:3n, while a seed that originated by aposporia, parthenogenesis and pseudogamy shows an embryo:endosperm ratio of 2n:5n.

1.2.6 *P. notatum* hybrids developed by UFRGS

In collaboration with the Northeast Botany Institute (IBONE) of Northeast National University (UNNE) located in Corrientes, Argentina, Weiler et al. (2017) had access to genotypes C44X (Quarin et al., 2001), Q4188 and Q4205 (Quarín *et al*., 2003). These sexual tetraploid genotypes were used as female parents in artificial crosses with the native ecotypes of Rio Grande do Sul: 'André da Rocha' and 'Bagual'.

The F1 hybrid progeny generated had its agronomic potential evaluated in the field across two years by Weiler *et al*. (2018). Some genotypes had hybrid vigor, being more productive than the parents and the cultivar Pensacola. Also, they verified that the leaf arrangement of the plant could be related to the damages caused by the frost in the cold season but no association was detected.

A group of 28 individuals was selected and had their mode of reproduction determined by using cyto-embryological analyzes and molecular markers (Weiler *et al*., 2017). After that, selected group, more the progenitors: 'André da Rocha', 'Bagual', C4-4X, Q4188, Q4205 and the control, cv Pensacola, were evaluated in two places: Eldorado do Sul and São Gabriel along two years by Barbosa (2014).

The evaluations were carried out by cuts when the erect habit hybrids achieved a minimum height of 30 cm and prostrate habit plants reached 20 cm. The plots were established using seedlings vegetativelly propagated. After the fourth forage harvest in the first year at both places, the genotypes' ranking changed because the effects of the cuttings and the planting was not biased anymore. The most productive among the apomictic hybrids were: B43, B26, C9, B17, C22, D25, A16, C15, D3, B2, and C6. Among the sexual hybrids were C18, D16, B2, and C32.

A significant correlation was found among the variables analyzed. The forage yield components: leafs blade, stems and inflorescences dry matter had high correlations with total dry matter production (DMP), 0.84, 0.79 and 0.76, respectively. Plant height had a moderate correlation with DMP (0.60).

Machado *et al*. (2017) evaluated the influence of the photoperiod on the forage yield in *P. notatum* accesses. The entries were composed of intraspecific hybrids developed by Weiler *et al*. (2017; 2018), ecotypes from the United States Department of Agriculture, native tetraploid ecotypes from Rio Grande do Sul and sexual autotetraploid clones from the National University of the Northeast in Argentine totaling 19 ecotypes (Table 1). The materials evaluated were subjected to two light regimes: an extended photoperiod with 14 light hours and a natural photoperiod.

Table 1. Identification of ecotypes and intraspecific hybrids of *Paspalum notatum* adapted from Machado *et al*. (2017) and Fachinetto *et al*. (2012). * Only Pensacola exibited diploid level, all the other are tetraploid. ** Induced Sexual Autotetraploid plant.

Genotype	**Origin**	**Identification**
30N	Santa Fé - Argentina	PI 508833 (USDA)
36N	Santa Fé - Argentina	PI 508834 (USDA)
48N	Mercedes - Argentina	PI 508838 (USDA)
70N	Cordoba - Argentina	PI 508828 (USDA)
83N	Corrientes - Argentina	PI 508827 (USDA)
95N	Corrientes - Argentina	PI 424652 (USDA)
V4	Barra do Quarai, RS - Brazil	V14310 (Valls)
André da Rocha	André da Rocha, RS – Brazil	MD, CN s/n (UFRGS)
Bagual	Missões, RS - Brazil	MD, CN s/n (UFRGS)
Pensacola*	Viamão, RS - Brazil	Comercial cultivar
C44x	Corrientes - Argentina	I.S.A.** Quarin *et al*., 2001
Q4188	Corrientes - Argentina	I.S.A. Quarin *et al*., 2003
Q4205	Corrientes - Argentina	I.S.A. Quarin *et al*., 2003
C1	Q4205 x André da Rocha - Brazil	Hybrid (Weiler et *al*., 2017; 2018)
C2	Q4205 x André da Rocha – Brazil	Hybrid (Weiler *et al*., 2017; 2018)
C15	Q4205 x André da Rocha - Brazil	Hybrid (Weiler *et al*., 2017; 2018)
C17	Q4205 x André da Rocha - Brazil	Hybrid (Weiler *et al*., 2017; 2018)
D3	Q4205 x Bagual - Brazil	Hybrid (Weiler *et al*., 2017; 2018)
D16	Q4205 x Bagual - Brazil	Hybrid (Weiler *et al*., 2017; 2018)

According to the same authors, the genotypes' ranking changed when submitted to treatments and seasons of year. The highest frequency of difference between treatments in forage production of the genotypes was observed in winter followed by autumn. The hybrids were superior to progenitors across the seasons after the first year. 'André da Rocha', 'Bagual', Q4188, Q4205 and the cultivar Pensacola exhibited reductions in dry matter yield when subjected to natural photoperiod and were more responsive to an extended photoperiod than the hybrids. Although, C44X, the sexual autotetraploid induced to be more insensitive to an extended photoperiod but it was also less productive.

The major determinant in the forage production decrease in winter, observed in *P. notatum*, seems to be dormancy, induced by the change in the photoperiod (Dall'Agnol; Gomes, 1987). The cv. Pensacola and othe *P. notatum* accesses had forage production increased when induced by an extended photoperiod (Sinclair *et al*., 2001; Blount *et al*., 2001).

The sexual clones Q4188 and Q4205 had the higher dry matter production in spring in both years when submitted to the treatment, with increases of 1.3- and 1.4-fold, respectively. Q4205, though, were not in the most productive group during the other seasons. By the other hand, the ecotypes 36N, 48N, and 83N were insensitive to photoperiod reduction, maintaining a reasonable stable yield throughout the seasons. The sexual plants plus the ecotypes were used in crosses aiming more forage production and attenuation of photoperiodo sensitivity (Machado, 2014). This author also performed artificial hybridizations with female parents used by Weiler *et al*. (2017). However, the male parents used were eight apomictic genotypes selected by Fachinetto *et al*. (2012) for dry matter production: 30N, 36N, 48N, 70N, 83N, 95N e V4 (Table 1). These crosses generated 30 hybrids, whose agronomic characteristics were evaluated along with the respective parents and the controls: 'André da Rocha', 'Bagual' and Pensacola. As a result, the author selected the hybrids '437', '336', '122', '132', '332', '137', '127' and '221' due to the higher accumulated dry matter production in the period (Table 2).

Table 2. Genotypes in agronomic evaluation with their genealogies, and mode of reproduction selected by Weiler *et al*. (2018); Barbosa (2014) and Machado (2014). Hybrids names preceded by letters are from crosses by Weiler *et al*. (2017) as well as their reproduction mode, whereas, the exhibited with only numbers are from Machado (2014). The reproduction mode of Machado hybrids was determined by Kricky (data not published).

Genotype	Genealogy	Reproduction mode
A16	Q4188 x André da Rocha	Facultative apomictic
B26	Q4188 x Bagual	Highly apomictic
B43	Q4188 x Bagual	Highly apomictic
C9	Q4205 x André da Rocha	Highly apomictic
C15	Q4205 x André da Rocha	Highly apomictic
C18	Q4205 x André da Rocha	Sexual
C22	Q4205 x André da Rocha	Highly apomictic
D3	Q4205 x Bagual	Highly apomictic
122	Q4188 x 36N	Unknow
132	Q4205 x 36N	Unknow
137	Q4205 x V4	Sexual
127	Q4188 x V4	Sexual
221	Q4188 x 30N	Unknow
225	Q4188 x 83N	Facultative apomictic
336	Q4205 x 95N	Sexual
437	Q4205 x V4	Sexual
712	C44X x 36N	Facultative apomictic
10036	Q4205 x 95N	Facultative apomictic

There was interaction between cut and ecotypes for all evaluated characters that can be understood as a genotype-environment interaction effect. The results showed variations of the performance classification of hybrids along the cuts (Machado, 2014). The author suggested the cuts carried out in autumn as the cause because the climatic conditions begin to be unfavorable due to decrease temperature and luminosity. The highest yields were observed at the cuts in spring and summer seasons. Some hybrids were superior to the parents, the 'André da Rocha' and 'Bagual' controls, and to the cultivar Pensacola. The '437' hybrid produced 3.5 times higher than the controls of recognized forage potential (Townsend, 2008; Steiner *et al*., 2017). When compared to cv. Pensacola, the difference was 5.6 times. This hybrid vigor was expected because the parents had larger genetic distance (Aguilera *et al*., 2011), since, the female parent was from Argentina and the male parent was from Rio Grande do Sul, Brazil. The leaf dry mass production had high phenotypic correlation with the production of total dry mass (0.89). Also, the total dry mass and leaf dry mass were highly correlated with plant height, 0.78 and 0.81, respectively. This corroborates what was found by Barbosa (2014) and indicates that indirect selecting is a viable method for pursuung high dry matter production with those hybrids.

Graminho (2018) evaluated agronomically the apomictic intraspecific hybrids of *P. notatum* obtained by Weiler *et al*. (2017): B26 and B43 (Q4188 x Bagual) and C22 and C9 (Q4205 x André da Rocha) plus the controls: cv. Pensacola and ecotype Bagual, submitted to increasing rates of nitrogen: 0, 60, 120, 240, 480 kg of N ha^{-1}. The genotypes were evaluated over two growth years during 2014/2015 and 2015/2016. There was no interaction between genotypes and levels of nitrogen fertilization. Hence, the genotypes performance ranking remained constant across the treatments. After the establishment of pasture, the dry matter production of hybrids B26, B43, C9 and C22 and of the native Bagual ecotype responded positively and linearly to nitrogen fertilization. The level of 120 kg of N ha^{-1} $year^{-1}$ promotes better efficiency of nitrogen use of genotypes. C22 and B26 were the most productive hybrids and superior to Bagual and Pensacola in the first year. During the second year C22 retained its position but it was equal to ‘Bagual’. Together, they reached the highest productions.

Another study by the same author was the evaluation of the same genotypes submitted to nitrogen rates or intercropped with temperate climate legumes: white clover and birdsfoot trefoil. The dry matter yield in intercropped systems involving white clover plus birdsfoot trefoil and *Paspalum notatum* genotypes was similar to that of mineral nitrogen fertilized systems. The equivalence between systems varied between genotypes and years. Some genotypes resulted in better use of the grass-legume system, equating to systems with high doses of N, but this was year-dependent. In general, except for Pensacola, a mixture with white clover and birdsfoot trefoil were similar to fertilized systems with up to 240 kg N $ha^{-1}year^{-1}$. According to the study, the genotypes B26, C22, C9, and Bagual are indicated for consortiated with temperate legumes due to dry matter production and botanical characteristics and to relations among the structural components. However, the systems with C22, C9 and B26 present a higher proportion of leaf blades in the dry matter of *P. notatum* than the Bagual system. In the first year, these genotypes were superior to the Pensacola system because of total forage production and leaf blade yield.

Similar to Weiler *et al*. (2018), Barbosa (2014), and Machado *et al*. (2017), the total dry matter yield and leaves dry matter yield estimated by phenotypic correlations were high and significant (0,81; $P<0,0001$), suggesting that the separation of structural components could be unnecessary. Thus, that saved time and labor in forage breeding programs.

1.2.7 *Paspalum sp.* seed production

The production, distribution, and use of improved seeds are one of the key segments in the links of the agribusiness chain. The use of seed, as a vehicle for propagating forage species represents an important milestone in the development of national livestock raising, by promoting a rapid dissemination of new materials. It is a complex process, though, and conditioned by a series of specific and well-determined factors.

According to Jank *et al*. (2011), among the objectives of forage breeding programs should be scope seed production, because low yields lead to high implementation costs, resulting in low adoption by ranchers.

Productivity in a crop is the result of the interaction of genetic and environmental (biotic and abiotic) factors and grow techniques (Fageria *et al*., 2007). The more effective this interaction, the greater the quantitative seed production as a reflection of the proper exploitation of the genetic potential of the varieties or the hybrid in question. Attained the efficiency of the interaction, the result is high seed production per unit area, that is, high seed yield.

Excellent results of forage production and quality were obtained with ecotypes and intraspecific hybrids of *P. notatum* (Fachinetto *et al*., 2012; Barbosa, 2014; Steiner *et al*., 2017; Machado *et al*., 2017; Weiler *et al*., 2018; Graminho, 2018). On the other hand, there is a lacky of information regarding *P. notatum's* response to seed production and this is a limiting factor in the popularization of its use as a cultivated forage (Lopes, 2009). According to Souza (2015), in crops where grain is the main agronomic product, selection for increasing the efficiency of mobilization of photoassimilates to these organs has been the main objective of breeding programs, whereas in forage crops, vegetative parts are the main agronomic concern. Thus, efforts to increase the efficiency of seed production have been neglected (Martiniello; Silva, 2011).

Summer perennials grass species present a recent history of genetic and agronomic manipulation retaining certain wild characteristics that constitute obstacles to commercial seed production (Souza, 2001). The *Paspalum* seed production in southern Brazil presents difficulties due to non-synchronism in the inflorescence emission and the short interval to full bloom between the beginning and seed abscission (Lopes; Franke, 2011a; Scheffer-Basso *et al*., 2007).

According to Costa; Saibro (1984), in *P. guenoarum* ecotype ‘Azulão’, the precarious emergence of the inflorescences and the prolonged anthesis period in the individual inflorescences have repercussions that directly affect the timing of seed maturation, that is, in the wide variation of the stage of development of the seeds at any point in the plant's reproductive cycle.

These factors make it difficult to determine the ideal timing for harvesting, as delays result in low yields due to degranulation losses, whereas anticipation results in a harvesting a high number of seeds still in formation (Boonman, 1971). This lack of synchrony in seed maturation may lead to low quality lots (Souza, 2001; Humphreys, 1981).

Corroborating to aforementioned, Batista; Godoy (1998) cite aspects such as the non-flowering of plants, flowering without formation of cariopse, low amounts of cariopse per inflorescence and low rate germination due to the dormant seeds as recurrent problems in the production of seeds of *Paspalum spp*. The elevated proportion of empty seeds, in developing and dormancy, are the main obstacles to getting quality seeds (Humphreys, 1979; Maeda; Pereira, 1997).

Some techniques considering irrigation, sowing arrangement, cuts and fertilization had been developed to increase the yield and quality of seeds in *Paspalum spp.* (Pinto, 1982; Rosa, 1984; Gates; Burton, 1998; Scheffer-Basso *et al*., 2007; Beck, 2012; Lopes *et al*., 2016; Bertoncelli, 2018).

Pinto (1982) and Rosa (1984) lead a study about *Paspalum guenoarum* Azulão ecotype established by a different sowing method and fertilizated by nitrogen rates in the first and second crop year, respectively. They tested sowing on haul (throwing the seeds and covering it) and on rows with different spacings: 45, 90 and 135 cm, submitted to 0, 75, 150 and 300 kg N.ha^{-1}. In both years, a cut was made in the spring and the forage was removed from the area.

The results seen were not clear as to which row space arrangement and N level match could contribute to seed production. The seed yield achieved 254.3 kg.ha^{-1} of viable pure seeds with 75 kg N.ha^{-1} and lines spaced 90 cm in the first year. However, the seeding spacing of 45 cm with 150 kg N.ha^{-1} provided the highest germination (35.4%) and an increased to 90 cm between rows, resulted in a reduction of germination of seed produced (13.78%).

In the second year, Rosa (1984) obtained the minimum germination value with overcast sowing (11.1%) and the maximum with sowing in spaced rows 135 cm (36.3%), regardless of the N dose.

In another experiment, the same authors imposed nitrogen fertilization at different times and doses to study the components of yield and seed quality for the same species. Pinto (1982) found the maximum value of 286 kg.ha^{-1} of viable pure seeds in the control treatment without application of N in the first year of the crop. Although, there was not difference observed among the treatments for germination. The application of nitrogen (N) whole dose in the “rubber” (stem thickening phenological moment during bloom) increased germination (59%). The lowest germination (45.3%) was observed with the N whole dose applied at sowing.

Rosa (1984), attained 356 kg.ha^{-1} of viable pure seeds in the second year of cultivation. The germination values oscillated between 39.12% and 52.88%. There was no significant difference between treatments. Otegui *et al*. (2005) obtained germination percentages of 58% and 64.75% in the best treatments experimenting with light and temperature effects on germination of *P. guenoarum* seeds.

In an experiment carried out in northeast Thailand, Hare *et al*. (1999) evaluated the yield components of *Paspalum atratum* seeds submitted to cutting regimes. The higher seed yield occurred in the two cuts treatment in which the last cut was carried out in June when there was time enough for flowering and appropriated seeds to develop.

The carrying out of frequent cuts associated with the delay in deferrals leads to a reduction in the number of inflorescences per area and consequent reduction of seed yield (Carambula, 1981; Hare *et al*., 1999).

Scheffer-Basso *et al*. (2007), evaluating *Paspalum dilatatum* Poir Biotype Virasoro undergoing cutting regimes prior to deferment date for seeds production concluded that it is affected by cut management. The highest seed yields were observed with cuts at 10 cm in height, when 75% of the vegetative canopy was removed after a longer cut interval (45 days). This situation provided a greater number of inflorescences per unit area, highly associated with seeds yield.

Since the number of inflorescences per area is the yield component most associated with seed yield (Lopes *et al*., 2016), superior genotypes in forage production can be selected for higher seed production through this component (Lopes *et al*., 2018).

The high number of inflorescences per area at flowering should be pursued by breeders, but this could generate less nutritive quality of the forage due to a higher proportion of stems. An alternative would be a search for genotypes with a shorter and uniform flowering period (Acuña *et al*., 2009).

Lopes *et al*. (2016) evaluated the management of consecutive cuts in the production and quality of *P. guenoarum* seeds of the 'Azulão' ecotype. They concluded that the largest production of seeds is obtained when the plants are not cut or when they are cut only once. The production of seed was affected by the interaction between cutting management and years. In both years, the seed yield was greater when plants were not cut or when cut once: 850.3 and 794.4 kg.ha^{-1} for the first year; 784.9 and 627.3 kg.ha^{-1} for the second year, respectively. In the first year, the increase in cuts resulted in a reduction of seed production, whereas in the second one, there was no difference between the application of two and three cuts. The germination had the same tendency observed at the seed yield. The highest value observed was in the second year for zero and one cut with values of 75% and 79.3%, respectively. In the first year, 69.3% and 71% were found. The variable most highly correlated with seed production was the reproductive tillers followed by total tillers.

The ideal time for seed harvesting is the physiological maturity point, when the seed presents the highest percentage of germination, vigor and dry matter (Popinigis, 1985). One way to achieve that and to attenuate seed shattering is to determine the better point to seeds harvest.

In a study to determine the harvesting time of *P. guenoarum* ecotype Azulão, Pinto *et al*. (1984) observed that the inflorescence dry matter (seeds plus racemes) increased steadily until 17.5 days after anthesis reaching the maximum value of 61.8%. This could indicate that the viable moment closest to the point of physiological maturation.

These authors indicated that the interval between 14 and 21 days after full anthesis is the most advisable for the harvest of seeds of this grass, in order to avoid losses by threshing and the harvest of pips seeds.

Batista; Godoy (1998), in a study to determine the maturation curve of seeds of the same species, concluded the seed harvest should be performed between 18 and 28 days after full anthesis. In addition, they emphasize that the seeds must be harvested before the maturation cycle ends, as the natural threshing occurs reaching values above 90% at 60 days after anthesis.

It is possible to use the beginning of threshing and the brown coloring of the seeds as easy criteria to indicate the moment of harvest (Carambula, s.d.). Being on the field, this information is a viable alternative in making the decision to harvest the seeds.

Some authors tell of the absence of established cultural practices for the production of *Paspalum sp*. seeds to corroborates with their limited commercial multiplication (Pizarro, 2000; Soster, 2009; Lopes; Franke, 2011).

Studies in technology and the ecology of forage seed are important to know more about the species and may contribute to dissemination of *Paspalum* grasses.

Although the optimum time to seed harvest from *Paspalum* species could be established by studies determining days, visual aspects or morphological traits, varieties presenting elevated seed shattering will make it difficult multiplied by seeds. The pursuiy by breeders of the increase in the proportion of seeds formed and an increase in the retention period of mature seeds in the inflorescence should be achieved (Valle, 2009). Moreover, it could result in significant increases in seed production without interfering with flowering characteristics per se (Pereira *et al*., 2003).

There is great variability in yields of *P. notatum* seeds. According to Loch; Ferguson (1998), for Pensacola and *P. notatum* cv. Argentine, yields ranged from 60 to 100 kg.ha^{-1}, reaching up to 400 kg.ha^{-1}. Gates; Burton (1998), in a Pensacola seed production experiment, by fertilization rates, obtained the production of 514 kg.ha^{-1} of seeds applicating 224 kg.N.ha^{-1}, 25 kg. P. ha^{-1} and 47 kg.K. ha^{-1}. No benefit was achieved by fertilizing above 224 kg.N.ha^{-1}.

Bertoncelli (2018) studied seed production and the quality of *P. notatum* INIA Sepé undergoing increase rates of nitrogen fertilization: 0, 75, 150 and 225 kg N.ha^{-1}, and different times for cut application arranged by factorial experimental design. The cuts were carried out based on the index of photosynthetically active radiation intercept (IRFA): i) control, without cutting; ii) early cutting when the canopy reaches IRFA 50% and iii) late cutting, IRFA 80%. The experiment was conducted during the years 2016 and 2017. The late cut application allowed higher seed yield in a single harvest. Early cutting treatment, however, allowed the realization of one more harvest, getting a higher production of seeds in the crop. According the author, the delay effect caused by late cutting on the flowering, pushes the ideal window of seed maturation to autumn when the plant growth rate is decreasing and a new productive cycle is not possible anymore.

The inflorescences number, one thousand seed weight and seeds per inflorescence were positively affected by nitrogen fertilization compared to not using it. The cleaned seeds ratio increased with N application dose.

The highest pure seed yield (1,000 kg.ha^{-1}) was achieved by applying 75 and 150 kg.ha^{-1} (2016 and 2017, respectively) with an early cut treatment. Even when no fertilization was supplied, the seed yields were around 380 kg.ha^{-1} and 547 kg.ha^{-1} at the first and the second year, respectively. The author suggested that difference betweem years was caused by hydric defficit in 2016.

Bertoncelli (2018) describes a model suggesting that the forage matter increased by nitrogen fertilization results at more tillers. The later cuts treatment would have a synchronizing effect providing a larger number of inflorescences resulting in higher pure seed yield and one thousand seed weight. Corroborating with other authors that the number of inflorescences is the most determinant variable to seed production.

Regarding the germination and their related traits, the authors concluded the effect caused by climate did not determine variation among the treatments in the second year of evaluations. According to them, the absence of adverse weather events could be contributing to perennial plants invest more in vegetative structures, since seed production would as a secondary need. This year resulted in an average germinated seeds percentage of 49%. Considering the first year, the values variationed between 41% (late cut and no nitrogen) and 74% (late cut and 225 kg.N.ha^{-1}) of germination affected by cut moment and fertilization combinations.

They concluded that application of nitrogen fertilizers in *P. notatum* INIA Sepé improves the seed germination rate when associated with late cutting in the presence of water deficit. In the case of absence of cutting or early cutting, the application of N reduces the germination potential of seeds.

The factors associated with seed dormancy in *Paspalum spp.* are the impermeability of structures involving caries (lemma and palea), which physically restrict the embryo development, and the physiological dormancy of the embryo (Popinigis, 1985; Maeda; Pereira, 1993). Seeds of *P. notatum* present this type of dormancy called "hard seed", which block to the gas exchange of the interior of the seed with the external environment (Andrade; Vaughan, 1980). However, when they were scarified, they present satisfactory germination due to the penetration of water in the lemma (Soares, 1972).

The expression of dormancy is linked to physiological causes in the newly harvested seeds, which are progressively suppressed with the storage time (Munhoz *et al*., 2009). Franke; Nabinger (1996), characterizing seeds germination of *P. notatum* at several accesses got the highest value of 60.5% of germination when treated with KNO_3 (0.2%) in an access of an unknown source. When their seeds were submitted to the tetrazolium test, they showed that 76.5% were viable. This difference was attributed to embryo mortality during the germination test. On the other hand, Carvalho; Carvalho (2009) obtained 12.2% germination for this specie indicating tha great variability is reported for germination rate.

Seed dormancy is recurrent among species of the genus *Paspalum*. For *P. guenoarum*, one of the causes of seed dormancy is embryo immature (Coradin; Ferreira, 1984; Mecelis *et al*., 1991; Batista; Godoy, 1998). Coradin; Ferreira (1984) observed a period of 210 days of storage to overcome the dormancy whereas, Batista; Godoy (1998) studied the seed dormancy period and the minimum storage period required before planting. The seeds presented dormancy for 90 days after harvest, according to them, after this period the seeds remained in accordance with the commercial standards of the time until 405 days after their harvest. Mecelis *et al*. (1991), in a study for the same species to overcome dormancy, tested time and doses of H_2SO_4 and KNO_3 in seeds from different doses of nitrogen: 0, 75 and 150 kg.ha^{-1}, shortly after harvest and stored for sixteen months. It was observed that until two and a half months after the harvest, there was the presence of dormancy, attributed to the probable stiffness of the external structure of the seed. They found that increasing nitrogen doses contributed to the physiological quality of seeds stored at low temperature and humidity. Also, the dormancy was overcome after the six months corroborating with aforementioned. The authors suggest this period should be expected, but at the laboratory level in, in freshly harvested seeds, dormancy may be overcome by chiseling with sulfuric acid for ten minutes in the presence of 0.2% KNO_3. In stored seeds, however, whose external structures could already present a degeneration, it is not necessary to use the technique.

Among the various issues related to seed quality, germination occupies a prominent place (Ticelli, 2001). The most used test to determine seed quality is the germination test. This test informs the potential of a seed lot to form normal seedlings under favorable conditions (Peretti, 1994). The aim is to get seed quality information for field sowing purposes and to provide data that can be used, along with other information, to compare different seed lots (Brazil, 2009).

Other parameters used to analyze the quality of seed lots are: germination rate and purity, the tetrazolium test, the sanity test, among othe (Brazil, 2009). Seed quality is defined as the sum of all genetic, physical, physiological and sanitary attributes that affect the ability to generate vigorous, productive and plants free from undesirable characteristics (Popinigis, 1985). On one hand, it has techniques to solve this obstacle, on the other hand, the breeding efforts pursuing genotypes no dormancy may solve the question once and for all.

1.3 HYPOTHESIS AND OBJECTIVES

The evaluation and selection of superior genotypes in plots will allow to test agronomic superiority from previously selected materials in spaced plants. The environment provided by the sward plot is closer to the formation of a pasture and represents better competition for water, light and nutrients.

P. notatum intraspecific hybrids submitted to successive harvests along the year to evaluate the production throughout the year seasons, demonstrate different agronomic performances and variation in forage yield components. The growth seasons could be interpreted as different environments contributing to select genotypes more adapted and stable.

The study of seed yield components, physiological and physical quality of seeds provides useful information to characterize a group of elite hybrids and to confirm whether the best-ranking forage genotypes have the potential to produce viable seeds to guarantee the successful adoption by farmers.

The objectives were to select apomictic and sexual hybrids of *P. notatum* of superior forage value based on the production of dry matter and seeds, productive stability, persistence and tolerance to cold and frost for future use in crosses or variety release.

2. CHAPTER II[1]

Forage mass components from *P. notatum* intraspecific hybrids across growth seasons in a subtropical zone of Brazil southern

Forage mass components from *P. notatum* intraspecific hybrids across growth seasons in a subtropical zone of Southern Brazil

ABSTRACT

The UFRGS forage breeding program selected genotypes to forage production, cold tolerance and persistence. An elite of hybrids, the ‘Bagual’, ‘V4’ and ‘Pensacola’ controls were arranged in a random complete block design with three replications and evaluated over years and seasons. This study aimed to: i) select the superior agronomic *P. notatum* intraspecific hybrid across seasons of the year; ii) determine botanical composition of forage mass, total tillers density, canopy height, cold tolerance and vigor regrowth pos-winter. Also, iii) characterize the relationship between botanical composition components and forage production. Evaluations were performed during 2016/17 and 2017/18 each time 70% of all genotypes reached 95% of luminous intercept. Genotype, season factors and their interaction had an effect for all variables evaluated ($p \leq 0.001$). ‘Pensacola’ was inferior than the best hybrids in most part of the variable during all seasons. Summer had larger forage dry mass and leaf blade dry mass, and the hybrids did not differ from the ecotypes in this season. The larger values of stem dry mass and inflorescence dry mass was observed for Bagual’ and ‘V4’. They presented the lowest percentage of leaves too. The biggest differences among hybrids and ecotypes were noticed during the spring. Forage dry mass and leaf blade dry mass presented strong correlations across the seasons furthermore, there were differences for percentage of leaves among genotypes. The hybrids ‘C15’, ‘C18’, ‘336’, ‘437’ showed desirable aspects in forage distribution, larger annual production and cold tolerance.

Key words: forage distribution, cold tolerance, warm grass

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[1] Prepared in accordance with the standards of the International Journal of Plant Production.

2.1 INTRODUCTION

Paspalum notatum is recognized as an excellent forage because of the nutritive value, acceptability by animals, high resistance to trampling and grazing, and tolerance to cold (Pozzobon & Valls, 1997; Gates et al., 2004). Due the predominant participation in forage production among the species of the natural pastures of southern Brazil (Valls, 1987), it has been highlighted in cattle feeding as one of the most important native pasture species of southern Brazil, Uruguay, northeast Argentina and southern Paraguay (Quarín et al., 1984).

Previous studies evaluated *Paspalum spp.* ecotypes to genetic variability, morphological traits, genetic diversity, forage production, persistence and cold tolerance (Fachinetto et al., 2012, 2017; Steiner et al., 2017). As results, the genotypes 'André da Rocha', 'Bagual', '83N', '95N' and '36N' were selected as pollen donors to be used with the sexual tetraploids: C44X (Quarín et al., 2001), Q4188 and Q4205 (Quarín et al., 2003) in artificial crosses (Weiler et al., 2017, 2018). The generated F_1 hybrid progeny had agronomic potential evaluated in the field (Weiler et al., 2018) and a group of 28 individuals was selected and had their mode of reproduction determined (Weiler et al., 2017).

This study aimed: i) select superior agronomic *P. notatum* intraspecific hybrids across seasons of the year of an elite group composed of hybrids and ii) determine botanical composition of forage mass, cold tolerance and regrowth vigor.

MATERIAL AND METHODS

Experimental Design

The study was conducted at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul (EEA-UFRGS), Rio Grande do Sul State, Brazil (30°05´S, 51°40´W, 46 m alt.).

This region's climate is classified as subtropical humid (Cfa according to Köppen classification). The minimum and maximum monthly temperature for the last 40 years varied between 8.5^{o}C (July) to 30.2^{o}C (January) respectively. The average annual precipitation was 1450 mm (Bergamaschi et al., 2013).

The experiment consisted of a group of twelve *P. notatum* genotypes arranged in a randomized complete block design with three replications. The germplasm evaluated included nine *P. notatum* intraspecific hybrids (‘A16’, ‘C15’, ‘C18’, ‘D3’, ‘225’, ‘336’, ‘437’, ‘712’ and ‘10036’), *P. notatum* ecotype ‘V4’ and ‘Bagual’, and the commercial variety Pensacola (*P. notatum* var. saure), were used as controls.The hybrids are results of two cycles of crosses and selection: ‘A16’, ‘C15’, ‘C18’, and ‘D3’, originated from crosses by Weiler et al. (2017, 2018), which were selected from agronomic evaluations in individual plants and later had their agronomic value proven through evaluations of plants conducted on lines in two locations in Rio Grande do Sul (southern Brazil). Another source of hybrids, nominated 225, 336, 437, 712 and 10036 come from crosses and selection for forage production made by Machado (2014). The tetraploid ecotypes: 36N, 83N, V4 and 95N, selected by the production of dry matter yield and ploidy level determined by Fachinetto et al. (2012) were used as male genitors, and the female ones of these crosses were also those used by Weiler et al. (2017).

Crop management

The experiment was planted in November 2015. The plots were 1.5 m by 2 m with 40 cuttings obtained by vegetative tillers division. They were arranged in rows spaced 30 cm and 25 cm between plants. On 14 January 2016, the trial was mowed to eliminate any variations related to stablishment. Then was fertilized with nitrogen and potassium as needed. The experimental area was fertilized prior to the experiment implementation according to needs of the perennial grasses of Soil Chemistry and Fertility Commission–RS/SC, Brazil (CQFS, 2004). Also, in the course of the experiment, was applied, 40 kg.ha^{-1} of N in each cut.

Data collection

Evaluations were performed during 2016/17 (year 1) and 2017/18 (year 2) (Table 1). Everytime 70% of all genotypes reached 95% of luminous intercept, they were all evaluated and mowed. This luminous inrercept is the level that indicates the best point between herbage accumulation and the nutritive value reached in warm-season forages (da Silva and Nascimento, 2007).

The data were recorded at field (Table 1), using a square of 0.25 m^2 (0.5 x 0.5 m) representative per plot and later extrapolated to square meters.

Table 1. Forage harvests date.

Year	Season	Harvest Dates
2016/17	Spring	31 October, 24 November and 15 December 2016
	Summer	04 and 26 January, 22 February and 15 March 2017
	Fall	19 April, 21 June 2017
2017/18	Spring	26 September, 31 October, 30 November and 21 December 2017
	Summer	09 January, 07 and 27 February, and 27 March 2018
	Fall	05 May 2018

Two random samples with 0.25 m^2 were picked from the plots every harvest to determine dry matter yield and their forage components. The samples were separated into leaf blades (LBDM), stems and sheat leaves (SDM), weeds (WDM), inflorescences (INFDM) and, when applicable dead material (DDM). After that, the samples were dried in a forced-air oven at 65°C for 72 hours and weighed to obtain the botanical composition of forage dry mass, the total forage dry mass of genotypes (TDM) was expressed in kg.ha^{-1}.

Frost events occurred on 10 June 2016 and 18, 19 and 20 July 2017. The Cold Tolerance (ColdTol) was assessed by the damage level, which was caused to the plants by frost and low temperatures on 14 June 2016, 22 July 2017. Visual scores from one to five were estimated with the greatest value for the highest tolerance (Acuña et al., 2009). Regrowth Vigor (RegVig) was obtained right after the winter, in the first harvest of spring. It was assigned by visual scores (1-5), being 1 for the smaller and 5 for the greater.

Data analysis

The forage data from each harvest were summarized by the sum of seasonal and annual production. ColdTol and RegVig were assessed one time per year, these variables were analyzed similarly to variables for annual production. The forage components: TDM, LBDM, SDM, INFDM and WDM were summed up by year to the accumulated annual production and by season to accumulated seasonal production. Other variables, whereas Percentual of Leaves over TDM (Perc.Leaf) and Weeds over TDM plus WDM (Perc.Weed) were obtained by this ratio using annual data. Data were subjected to tests of normality and variance homogeneity, which did not show the transformation needed. Linear models were implemented in R statistical software (R Core Team, 2015).

Analysis of variance (ANOVA) were performed for all variables to test the statistical significance for all the factors and their interactions. The year factor was imposed in the statistic model as the main-plot and the genotypes as subplots. When the season was included to the model, it was considered the subplots and genotypes the sub-subplots.

Data from each season were used to do Pearson's correlations among the variables using the Corrgram package (Wright, 2016). The graphs were created using the package ggplot2 in R (Vu, 2011). A Tukey-HSD test was used to separate genotypes means at $p \leq 0.05$ using the lsmeans package (Lenth, 2016) in R.

2.2 RESULTS

Genotype, season and their interaction had effect for all variables evaluated ($p \leq 0.005$). There was no year effect for Inflorescence (INFDM). The year-genotype interaction was significant for all forage traits. The triple interaction, year-season-genotype was significant for TDM, LBDM, INFDM, WDM, Perc.Leaf and Perc.Weed.

Total Dry Matter (TDM), Leaf Blade Dry Matter (LBDM) and Percentual of LBDM over TDM (Perc.Leaf)

The genotypes' ranking for TDM varied during the seasons and years (Table 2). The summer and fall seasons had the greater and lower TDM in both years, respectively. Also, the average year 2 allowed more TDM than year 1.

Table 2. Genotypes' ranking averaged by blocks for Total Dry Matter (TDM).

Genotypes	Year 1			Year 2			Year 1	Year 2
	Spring	Summer	Fall	Spring	Summer	Fall		
C15	3854 a	6711 a	1529 abc	5609 a	5546 ab	767NS	12094 a	11922 a
C18	2637 bc	6331 abc	1668 ab	5021 ab	6345 ab	878	10636 ab	12245 a
V4	2035 cde	6245 abc	1575 abc	3872 abcd	7161 a	916	9855 abc	11949 a
336	2491 bcd	6389 ab	1834 a	3824 abcd	5920 ab	1100	10713 ab	10845 a
A16	3100 ab	6700 a	1657 ab	3299 bcd	4792 bc	843	11457 a	8934 abc
Bagual	1738 de	6250 abc	1824 a	3201 bcd	5996 ab	932	9812 abc	10129 a
437	2609 bc	4512 bcd	1298 abc	4839 abc	5616 ab	771	8418 bcd	11226 a
10036	1605 e	4825 abcd	1318 abc	3302 bcd	5095 abc	907	7748 cde	9304 ab
712	2052 cde	4261 cd	1069 bcd	3287 bcd	4754 bc	950	7383 cde	8991 abc
225	1416 e	4545 bcd	1026 cd	3042 bcd	4976 abc	1180	6986 cde	9198 abc
D3	1581 e	3842 de	1361 abc	2663 cd	3110 c	822	6784 de	6595 bc
Pensacola	2612 bc	2027 e	582 d	2018 d	3203 c	652	5221 e	5873 c
Mean	2311	5220	1395	3665	5210	893	8926	9767

*Mean values with same letter do not differ statistically ($P \leq 0.05$) by Tukey Test; NS, Not Significant.

The cultivar ‘Pensacola’ was at the worst performing group in both years, but on spring of the first year, it produced more TDM than ‘Bagual’ (2,612 kg.ha^{-1} vs 1,738 kg.ha^{-1}), ‘10036’ (1,605 kg.ha^{-1}), ‘D3’ (1,581 kg.ha^{-1}) and ‘225’ (1,416 kg.ha^{-1}), being statistically equal to other hybrids, except the ‘C15’. In spring of the first year, the hybrid ‘C15’ reached a higher TDM (3,854 kg.ha^{-1}) yielding 1.9 and 2.2-fold, than the natives ecotypes ‘V4’ and ‘Bagual’, respectively, but did not differ from ‘A16. In spring of the second year, ‘C15’ was at first group, joined by ‘C18’, ‘V4’, ‘336’ and ‘437’ for TDM. Other genotypes, as ‘437’ and ‘V4’ rose up in the classification, being not statistically different from ‘C15’, although this latter outyielded ‘437’, 1.2-fold (5,378 kg.ha^{-1} vs 4,401 kg.ha^{-1}), and the ecotype ‘V4’, 1.4-fold (5,378 kg.ha^{-1} vs 3,709 kg.ha^{-1}).

In summer, there was variation among the genotypes' positions between the years (data not presented). For most of the genotypes, there was an increase or maintenance of forage production. In summer of the first year, the hybrids ‘C15’ and ‘A16’ obtained the highest values for TDM, respectively, 6,711 kg.ha^{-1} and 6,700 kg.ha^{-1}, although did not differ from genotypes’ values upper than 4,512 kg.ha^{-1}.

In summer of the second year, however, the hybrids ranked better in previous seasons were overcome by ‘V4’ (7,161 kg.ha^{-1}). In the same cluster, ranked from the greatest (6,345 kg.ha^{-1}) to the lowest TDM (5,546 kg.ha^{-1}), were ‘C18’, ‘Bagual’, ‘336’, ‘437’ and ‘C15’. In this season, ‘Pensacola’ and ‘D3’ presented the lowest TDM, but did not differ statistically from ‘10036’, ‘225’, ‘A16’ and ‘712’.

In fall of the first year, there was a change in the TDM ranking, according to the latter spring and summer yields from the same year. The biggest part of genotypes were ‘336’ (1,834 kg.ha^{-1}) and ‘Bagual’ (1,824 kg.ha^{-1}), being statiscal similar to other genotypes and superior to ‘712’, ‘225’ and ‘Pensacola’. In contrast, ‘Pensacola performed inferior (582 kg.ha^{-1} of TDM) all the others, but was statiscally equal to ‘712’ and ‘225’. Overall, there were not significant differences among genotypes for TDM in the second fall.

Across the seasons in both years, it was possible to see a tendency of growing herbage production of the ‘336’ hybrid. As noticeable, during the spring, it was in the sixth and fifth position, in year 1 and 2, respectively. In summer, it came up to third and fourth place and finally, in fall, it achieved the first (year 1) and second place (year 2). Observing the TDM by year, it was possible to see a group formed in year 1 by hybrids ‘C15’ and ‘A16’ with herbage accumulation of 12,094 kg.ha^{-1} and 11,457 kg.ha^{-1}, respectively. Those hybrids outyielded by 1.2 and 1.2-fold the ecotype ‘Bagual’, and by 2.32 and 2.19-fold the cultivar ‘Pensacola’. In year 2, ‘C15’ got the third position and kept in the same group as ‘V4’, ‘437’, ‘336’ and ‘Bagual’. Although ‘C18’ got the highest numerical value (12,245 kg.ha^{-1}), there was no statiscally difference from the aforementioned genotypes. The hybrid ‘A16’ performed poorly in the second year, getting a herbage production of 8,934 kg.ha^{-1}, that was not possible to differentiate from the less productive genotypes, as hybrid ‘D3 ’(6,595 kg.ha^{-1}) and the cultivar ‘Pensacola’ (5,873 kg.ha^{-1}).

The major detection of differences for LBDM (Table 3) occurred in the first summer and in the second spring. ‘Pensacola’ performed poorly in both years but showed a reasonable performance in the spring of the first year, wherein it was statistically equal to the genotypes with the highest and the lowest values.

Table 3. Genotypes’ ranking for Leaf Blade Dry Matter (LBDM).

Genotypes	Year 1			Year 2			Year 1	Year 2
	Spring	Summer	Fall	Spring	Summer	Fall		
C15	2666 ab	4914 ab	1399 ab	5378 a	4392 abc	737NS	8979 abc	10507 a
C18	2539 ab	5142 a	1606 a	4961 ab	5440 a	877	9288 ab	11278 a
V4	1751 bc	4156 bc	1421 ab	3709 bcd	4661 abc	744	7329 bcd	9115 abc
336	2075 abc	5151 a	1649 a	3688 bcd	4852 ab	1087	8875 abc	9627 ab
A16	2834 a	5363 a	1587 a	3251 cde	3694 bc	827	9785 a	7771 bc
Bagual	1518 c	4006 bcd	1659 a	3142 cde	3737 bc	884	7183 bcde	7763 bc
437	1904 abc	3524 cd	1254 ab	4401 abc	4012 abc	736	6682 cde	9149 abc
10036	1245 c	3607 cd	1236 ab	2778 de	3353 cd	720	6088 def	6852 cde
712	1782 bc	3190 de	1005 ab	3004 cde	3487 bcd	934	5976 def	7425 bcd
225	1239 c	3353 cde	1020 ab	2817 de	3322 cd	998	5612 def	7136 cd
D3	1171 c	2477 e	1297 ab	2534 de	2188 d	697	4944 ef	5419 de
Pensacola	1981 abc	1263 f	510 b	1834 e	2094 d	625	3754 f	4554 e
Mean	1892	3846	1304	3458	3769	822	7041	8050

*Mean values with same letter do not differ statistically (P≤0.05) by Tukey Test; NS, Not Significant.

In spring of the second year, ‘C15’ was at the first place, but did not differ from ‘C18’, ‘V4’, ‘336’ and ‘437’ for LBDM. Other genotypes as ‘437’ and ‘V4’ rose up in the classification, being not statistically different from ‘C15’, although this latter outyielded ‘437’, 1.2-fold (5,378 kg.ha^{-1} vs 4,401 kg.ha^{-1}), and the ecotype ‘V4’, 1.4-fold (5,378 kg.ha^{-1} vs 3,709 kg.ha^{-1}). In addition, the highest values for LBDM were obtained by the ‘A16’, ‘336’, ‘C18’ group and ‘C15’ (5,363 kg.ha^{-1} to 4,914 kg.ha^{-1}).

The Perc.Leaf was lower in summer and higher in fall, but did not differ from spring on the second year (Table 4). Also, variation among genotypes were higher in the first spring and in the second summer. The average value in the fall was high in both years (0.93). The hybrid ‘C15’ had the lowest Perc.Leaf (0.69) in the first spring although it was not significant different from ‘10036’, ‘Pensacola’, ‘D3’ and ‘437’ with value until 0.78. The highest value for the trait was observed for ‘C18’ (0.96), statiscally equal to ‘A16’, ‘225’, ‘Pensacola’, ‘712’, ‘V4’ and ‘336’ (0.83). In general Perc.Leaf was higher in the second spring than in the first one, except for ‘C18’ and ‘A16’, which already had a high Perc.Leaf in the first season (Table 4). In summer of year 1, the highest values were presented by the 'C18’, ‘336’, ‘A16’ and ‘437’ group (0.81 to 0.78). The hybrid ‘C15’ presented value equal to the overall average, and did not differ from the other hybrids, except ‘Pensacola’ (0.63). In the following year, ‘C18’ also presented the highest value, but was statiscally equal to ‘336’, ‘C15’, ‘A16’.

Table 4. Genotypes' ranking averaged by blocks for Percentual of Leaf Blade over Total Dry Matter (Perc.Leaf).

Genotypes	Year 1			Year 2			Year 1	Year 2
	Spring	Summer	Fall	Spring	Summer	Fall		
C18	0.96 a	0.81 a	0.96 ab	0.99 a	0.86 a	1.00 a	0.87 a	0.92 a
336	0.83 abcd	0.81 a	0.90 ab	0.96 a	0.82 ab	0.99 a	0.83 ab	0.89 ab
C15	0.69 e	0.73 abcd	0.91 ab	0.95 ab	0.79 abc	0.96 ab	0.74 bcd	0.88 ab
A16	0.91 ab	0.80 a	0.96 ab	0.99 a	0.77 abcd	0.98 a	0.85 a	0.87 abc
712	0.87 abcd	0.75 ab	0.94 ab	0.91 ab	0.74 bcde	0.98 a	0.81 abc	0.83 bcd
D3	0.74 de	0.66 bcd	0.96 ab	0.96 ab	0.70 cdef	0.85 bc	0.74 bcd	0.82 bcde
437	0.73 de	0.78 a	0.97 ab	0.91 ab	0.71 bcdef	0.95 ab	0.79 abcd	0.82 bcde
Pensacola	0.76 cde	0.63 e	0.87 b	0.91 ab	0.66 def	0.96 ab	0.72 d	0.78 cde
225	0.87 abc	0.74 abcd	1.00 a	0.94 ab	0.67 def	0.86 bc	0.80 abcd	0.78 de
BAGUAL	0.87 abc	0.64 cd	0.91 ab	0.98 a	0.62 f	0.95 ab	0.73 cd	0.77 de
V4	0.86 abcd	0.66 bcd	0.90 ab	0.96 a	0.65 ef	0.83 c	0.74 bcd	0.76 de
10036	0.78 bcde	0.75 abc	0.94 ab	0.84 b	0.66 def	0.80 c	0.78 abcd	0.74 e
Mean	0.82	0.73	0.93	0.94	0.72	0.93	0.78	0.82

*Mean values with same letter do not differ statistically ($P \leq 0.05$) by Tukey Test. NS, not significant.

In the first fall, '225' presented the highest value, 1.0, and 'Pensacola the lowest one, 0.87, while the middle group was formed by the range from 0.97 to 0.90. In the second year, the differences among genotypes were clearer compared to the first year. The first group was composed of 'C18', '336', '712', 'A16' and 'C15' with values between 1,00 to 0.98. The 'Bagual' ecotype and 'Pensacola' cultivar had similar performances and stayed in the middle. The lowest value was performed by 'V4' and '10036' (0.83 and 0.80).

Stem Dry Matter (SDM) and Inflorescence Dry Matter (INFDM)

The genotypes' rank for SDM ranged across the seasons and years, but there was no triple interaction (Table 5). The first year showed more SDM production than the second year, although this did not occur for the hybrids '10036', '225', '437'. Even with no differences evident, the highest values over the years in the average, were obtained by the ecotypes 'V4' and 'Bagual'; with the same classification observed in the summer. At this time, the ecotypes reached 1311 and 1300 kg.ha^{-1}, the highest values observed among all seasons. The lowest SDM in summer was performed by 'C18', 'D3', '225', '712', '336' and 'Pesacola', and ranged between 656 and 573 kg.ha^{-1}, which did not differ among themselves and from '437', '10036' and 'A16'. In spring, different from summer and from the total of two years yield, the hybrids 'C15', '437' ranked at the top and accumulated more SDM than 'Bagual' and 'C18'. In fall, there were no significantly differences, although materials ranged between 0 kg.ha^{-1} (‘A16’ and ‘C18’) and 76 kg.ha^{-1} (‘V4’).

Table 5. Genotypes’ ranking averaged by blocks of each season and sum of each year for Stem and Leaf Sheat Dry Matter, expressed in kg.ha^{-1} (SDM).

Genotypes	Spring	Summer	Fall	Year 1	Year 2
V4	98 bc	1311 a	76 NS	1414 ab	1555 a
Bagual	59 c	1300 a	42	1461 ab	1341 ab
437	330 ab	807 bc	20	1137 bc	1178 abc
10036	155 abc	788 bc	67	853 bc	1167 abc
225	89 bc	632 c	61	604 c	962 abc
C15	388 a	963 b	23	1854 a	895 abc
712	122 bc	623 c	5	788 bc	712 bc
Pensacola	191 abc	573 c	7	853 bc	690 bc
A16	98 bc	734 bc	0	1009 bc	656 bc
D3	106 bc	635 c	57	992 bc	604 c
336	105 bc	609 c	19	894 bc	573 c
C18	33 c	656 c	0	864 bc	515 c
Mean	148	803	32	1060	904

*Mean values with same letter do not differ statistically ($P \leq 0.05$) by Tukey Test. NS, not significant.

The top positions for INFDM were occupied by ‘C15’, ‘V4’ and ‘Bagual’ in the first year and occupied by ‘10036’, ‘V4 and ‘Bagual’ in the second year (Table 6). The highest values occurred in summer and there were no differences between spring and fall. In spring ‘C15’ reached the highest value, but it was not different from ‘437’, ‘Pensacola’, ‘10036’ and ‘712’. In summer, similar trend was observed for SDM, ‘V4’ and ‘Bagual’, which accumulated more INFDM than all others, while ‘C15’ was ranked at third position, but statiscally equal to ‘437’, ‘10036’ and ‘A16’. The rest of the genotypes oscilated between 656 and 573 kg.ha^{-1} and did not differ from ‘437’, ‘10036’ and ‘A16’. In fall, there were no differences among the genotypes.

Table 6. Genotypes’ ranking averaged by blocks for Inflorescence Dry Matter expressed in kg.ha^{-1} (INFDM).

Genotypes	Year 1			Year 2			Year 1	Year 2
	Spring	Summer	Fall	Spring	Summer	Fall		
V4	70 b	826 a	43 NS	31 b	947 a	41 NS	938 ab	1020 a
Bagual	31 b	761 ab	28	8 b	865 ab	3	820 abc	876 ab
10036	71 b	485 bcd	11	256 a	741 abc	65	567 bcd	1062 a
C15	502 a	548 abc	17	11 b	326 ef	3	1068 a	341 de
437	211 b	256 de	0	155 ab	604 cd	3	467 cd	762 abcd
225	51 b	354 cde	0	80 ab	683 bcd	34	406 cd	797 abc
712	39 b	410 cde	2	63 ab	498 de	0	451 cd	560 bcde
336	134 b	415 cde	25	25 b	362 ef	0	574 bcd	387 cde
D3	7 b	502 bcd	19	5 b	313 ef	28	528 bcd	346 de
Pensacola	198 b	132 e	0	44 b	319 ef	3	330 d	365 cde
A16	30 b	313 cde	0	9 b	312 ef	0	343 d	321 de
C18	9 b	333 cde	0	0 b	259 f	0	342 d	259 e
Mean	113	445	12	15	519	15	569	591

*Mean values with same letter do not differ statistically ($P \leq 0.05$) by Tukey Test.

The ‘C15’ hybrid had a strong decrease in the INDFM in the second year, while ‘10036’ increased in this trait. Some genotypes, such as ‘C15’, ‘437’ and ‘Pensacola’ produced a good portion of INFDM in spring, having values more distributed between spring and summer in the first year than the other genotypes, while, this behavior was not evident in the second year. Similar to this variable, the summer had the biggest INFDM values for biggest part of genotypes, followed by spring and fall, wich did not differ from each other in the second year.

Cold Tolerance and Regrowth Vigor

The ColdTol were lower in the first year, although this year proportionated bigger detection of differences between materials, unlike in year 2 (Table 7). The only exception was ‘437’ which do not differ over years. All the other genotypes presented values considerably greater than the values from the first year. In the first year, ‘437’ performed the highest value but did not differentiate from ‘C15’, ‘C18’ and ‘712’, in contrast, ‘V4’ exhibited the lowest ColdTol, however, it was statiscally equal to ‘A16’, ‘D3’, ‘Bagual’, ‘225’, ‘10036’ and ‘Pensacola’. In the second year, ‘C15’ was more tolerant than ‘Pensacola’ and the difference among the other hybrids and them was not detected. The hybrids 'C15' and '437', had a good performance in both years. The hybrid ‘10036’ and cultivar ‘Pensacola’ performed weakly.

Table 7. Genotypes' visual scores (1 to 5) averaged by blocks for cold tolerance after froze events in the winter and for regrowth in the spring beggining.

Genotypes	Cold Tolerance		Genotypes	Regrowth
	Year 1	Year 2		
C15	4.2 ab	5.1 a	C15	3.67 a
437	4.5 a	4.8 ab	C18	3.47 ab
C18	3.8 abc	4.9 ab	437	3.38 ab
A16	2.9 bcde	4.5 ab	V4	3.38 ab
D3	2.9 bcde	3.9 ab	A16	3.33 ab
336	2.4 de	5.0 ab	336	3.18 ab
Bagual	2.5 cde	4.9 ab	Bagual	3.02 ab
V4	1.7 e	4.7 ab	712	3.00 ab
712	3.1 abcd	4.6 ab	Pensacola	2.93 ab
225	2.7 cde	4.7 ab	10036	2.63 ab
10036	2.4 de	4.5 ab	225	2.58 ab
Pensacola	2.3 de	3.7 b	D3	2.20 b
Mean	2.95	4.61		3.07

The RegVig, obtained in early spring, remained constant over the years for the genotypes and interaction or year effect were not observed. Similar to cold tolerances evaluations, 'C15' had the highest value and stayed at the top of ranking. 'D3' had the worst performance. The rest of genotypes did not differ from each other.

Correlations

Most of the variables were positively correlated among themselves (data not shown). Common to the three seasons (spring, summer and fall), TDM was strongly correlated with LBDM, ranging from 0.99 in the fall to 0.93 in the summer. In this season, other forage compounds, that did not have significance in spring or fall had moderate associations to TDM such as SDM (0.59) and INFDM (0.52). These latter traits were highly correlated to each other in all of three seasons (spring: 0.73; summer: 0.81 and fall: 0.89). Also, RTD were associated with them, INFDM (0.75) and SDM (0.66). Though DDM makes up part of forage production components, its participation had two different responses. In spring, it was slightly negatively associated with LBDM and TDM, -0.38 and -0.32, while in the fall was moderately correlated to these variables, 0.58 and 0.64, respectively.

In summer, WDM was low to moderate correlated to other forage components: SDM (-0.33), TTD (-0.41), INFDM (-0.49), LBDM (-0.66) and TDM (-0.71). The negative value was also observed in the fall, though not significant, except for TTD (-0.38).

Analysing the correlations over seasons, ColdTol and RegVig at the beginning of spring had significant correlations only with data from spring. Although not correlated between themselves, the nature of the correlations and other variables were similar. They were negatively associated to DDM (-0.44 and -0.22) and positively moderate correlated to LBDM (0.66 and 0.47), TDM (0.64 and 0.47) and to CH (0.43 and 0.38), respectively.

The ColdTol had low correlation during the years although it was significant indicating that some association exists. The ColdTol had a low correlation between years although it was significant indicating that some association exists. The ColdTol obtained in the first year had a correlation with TDM in the harvests: 0.46 for 31 October 16, relative to the first harvest in the first year; 0.60 for 26 September 17, relative to the first harvest in the second year. Also, presented a correlation of 0.48 and 0.55 with TDM spring for both years. The ColdTol assessed in the second year present lower value for the correlations with the harvests and the season: 0.34 for the harvest on 26 September 17 and 0.41 for the TDM spring in the second year. However, exhibited moderate correlation with the annual TDM in the first and second year: 0.41 and 0.54, respectively.

RegVig presented correlation of 0.42 between years. Also, the first year presented fewer significant correlations for this trait with other TDM variables: 0.44 for 26 September 17, 0.40 for TDM in spring of the first year and 0.41 for TDM in the first year. In the second year the RegVig showed correlations for TDM of 0.66 at 26 September 17; 0.46 and 0.56 for TDM in spring in first and second years, respectively; 0.52 for TDM of fall in the first year, 0.65 and 0.49 for annual TDM in first and second year, respectively.

High and moderate correlations among TDM in harvests, seasons and year were observed. For instance, between spring and year value, the ranged among 0.56 to 0.83 while between fall and year value were from 0.35 to 0.84. Also, between years value, a correlation of 0.64 was observed.

2.3 DISCUSSION

Annual production

The annual yield values obtained in this study were in accord to those found by other authors, although the annual TDM for ‘Pensacola’ and ‘Bagual’ was a little bit lower than related by the literature (Vendramini et al., 2013 and Steiner et al., 2017). The values for ‘Pensacola’ were 5221 and 5873 kg.ha^{-1} , while ‘Bagual’ exhibited 9812 and 10129 kg.ha^{-1} in the first and second year, respectively. In an experiment conducted in the same region of the current study, ‘Bagual’ reached in the first and second year, respectively, 14337 kg.ha^{-1} between 23 January and 21 July; and 7712 kg.ha^{-1} amid 11 October and 19 January (Steiner et al., 2017).Other authors also reported annual TDM similar to the ones found in this study. Interrante et al. (2009) reported total dry matter yield for diploid and tetraploid cultivars, and the highest values reported for the cultivars were: ‘Argentine’, 12300 kg.ha^{-1}; ‘Tifton 7’, 12000 kg.ha^{-1}; ‘Pensacola’, 11500 kg.ha^{-1}; ‘Tifton 9’, 11800 kg.ha^{-1} and ‘PCA Cycle 4’ 10300 kg.ha^{-1} future ‘Uf Riata’. Moreover, Vendramini et al. (2013) testing grazing frequency inputting low fertilization in Ona, Florida, reported annual forage yields of *P. notatum* cv. ‘Argentine’ between 4300 and 6400 kg.DM.ha^{-1}, cv. ‘Pensacola’ between 3400 to 5900 kg.ha^{-1}; cv. ‘Tifton 9’ 3700 to 6900 kg.ha^{-1}; UF Riata 4000 to 7000 kg.ha^{-1}.

In both years, the ecotypes ‘Bagual’ and ‘V4’ did not differ from most productive hybrids, however differences in the values around 2000 kg.ha^{-1} were observed among the hybrids and ecotypes. ‘Pensacola’ was overcomed in both years by the hybrids: ‘C15’, ‘C18’, ‘336’ and ‘437’. The hybrids ‘C15’ and ‘C18’ are progenie from Q4205 x ‘André da Rocha’ (Weiler et al., 2017; 2018), the first one present apomixis while the other is a sexual plant. In previous studies, ‘André da Rocha’ was tested for forage production against ‘Pensacola’ and ‘Bagual’ (Fachinetto et al., 2012; Steiner et al., 2017). ‘André da Rocha’ was more productive than ‘Pensacola’ but, in general, lower than ‘Bagual’. Also, Q4205 has a poorly forage performance but was one of the few sexual tetraploids available at that time (Weiler et al., 2018). According to (Weiler et al., 2018), the hybrids ‘C15’ and ‘C18’ present heterosis degree which although the parents were not tested in the current study is possible to deduce that they would yield more than the parent yieldest, ‘André da Rocha’.

Other most productive hybrids are, ‘336’ and ‘437’, also progeny from ‘Q4205’ but from crosses with ‘95N’ and ‘V4’, respectively. In both years, there were no differences for annual TDM between ‘437’ and ‘V4’. The ecotypes ‘95N’ and 'V4' stood out for agronomy performance and was selected by Fachinetto et al. (2012) and were used in crosses that resulted in these hybrids. These hybrids also shown sexual reproduction mode and joined with 'C18' are valuable tools to be used in crosses because distinguish at genetic background due to the father used, besides proving to be agronomic superior.

Although TDM production is important, part of forage includes inflorescences, stems and dead materials, which are not able to provide a basis for assessing animal performance. LBDM is preferred because represents the fraction with the higher digestibility, crude protein and intake, as well, is strongly associated with animal performance (Martucello et al., 2015) and a high correlation between LBDM and TDM was observed over seasons (spring, 0.97, summer, 0.93, fall, 0.99). The ‘C18’ hybrid had a great Perc.Leaf, indicating its high potential to good nutritive value. The ‘A16’ and ‘336’ hybrids did not differ from ‘C18’ in the two years but were not so productive for LBDM. The leaf blades are the most important component in the sward because high animal performance is direct related to increase consumption of leaves. Indeed, leaf component is also the most important in the evaluation of forage plants, given that it is a better indicator of quality than the other morphological components of the plant. (Martuscello et al., 2015).

Forage production by season

The annual herbage accumulation is the most common data used to verify yield differences in genotypes tests (Hubber et al., 2016; Motta et al. 2016, 2017). However, our data shows that differences between seasons were also found and had a considerably impacted in the final value of total production.

The Brazil mid-west cultivars were developed considering just two seasons in the year, classified as "waters" and "droughts" (summer and winter) while southern Brazil has the four seasons well defined (Valle et al., 2009). The fluctuation in precipitation regimes, temperatures, and photoperiod over seasons of the year have several impacts in plant growth and in forage distribution.

The magnitude effects caused by seasons periods over forage variables were larger than observed by year factor for all traits (Table 4 and 5). Season of year is considerably a shorter period of time, well defined by climate environment as observed in spring, summer, fall and winter in subtropical and temperate regions. On the other hand, the differences related to the same season among years were closer to each other than observed for the years per se.

The interactions perceived between year versus genotype and season versus genotype shown genotype’s performance can change regarding environment imposed, as observed in several studies with *Paspalum spp.* (Venuto et al., 2003; Pereira et al., 2012; Hubber et al, 2016; Motta et al., 2016 and 2017). The triple interaction among these factors indicates that there were enough differences among seasons to observe different patterns of behavior among the variable for the genotypes tested. Therefore, there is variance enough to treat those periods as different environments.

The ‘C15’, ‘C18’ and ‘336’ hybrids stood out among other genotypes across the year and in the seasons for main variables related to forage performance. They showed high TDM with a great part of it derived from the LBDM, as well a good production of biomass in the spring and better forage distribution and good tolerance.

In the current study, ‘C15’ and ‘437’ had 40% and 37% of TDM produced in spring, respectively, while ‘V4’ and ‘Bagual’ presented 27% and 25%, respectively. In fall for genotypes with great herbage production, ‘336’ and ‘Bagual’ had 14% while ‘C15’ showed 10% and ‘437’, 11%. Although, ‘D3’ was not the yieldest, performed 16% of all its production in the fall, value superior to all other genotypes. According to Machado et al. (2017), the lower TDM observed for this hybrid in spring subjected to the extended photoperiod would be due to consuming its energy reserves to vegetative production during fall and winter, which may have affected the forage yield in the subsequent season. Although winter production was not measured because the genotypes' plot canopy did not reach the cut height, it is comprehended the part of forage produced in the winter was allocated to spring contributing to highlight some hybrids such as the 'C15'.

A major range among best hybrids and ecotypes for TDM was observed in the spring. In both years, ‘C15’ and ‘C18’ displayed earlier in the spring, yielding numeric more than the ecotypes ‘V4’ and Bagual’. ‘C15’ achieved on average 1800 kg.ha^{-1} and 2300 kg.ha^{-1}, respectively. More TDM than performed by ‘V4’ and ‘Bagual’ in spring of the two years, although in the second spring has not detected the differences between ‘C15’ and ‘V4’, and among ‘C18’ and the ecotypes. Hybrids showing increase forage production just after winter are important because, in general, summer perennial forages display an initial slow growth in the begginning of the spring. Similar results were obtained for *P. notatum* ‘Boyero UNNE’ compared to ‘Argentine’ (Urbani et al., 2017) As observed by the authors, the ecotypes showed lower production in the spring than the hybrids. Naturally, *Paspalum notatum* reduce its forage yield in the winter period because the specie is characterized as a long-day species (Newman et al., 2007). While, a greater temperature and daylength in summer result in rapid growth, greater forage production (Vendramini et al., 2010) as registered for the ecotypes.

In the fall of year 1, ‘336’ had a better performance among the hybrids but did not differ from the other genotypes. In the second year, it performed great TDM, but was not possible to confirm because there were no differences between the genotypes.

The evaluation across spring, summer and fall for two years, showed different agronomic performances among the genotypes. The most part of the genotypes performed weak, due to low temperatures and decreased photoperiod. The selection of the superior genotypes with more production in this period is a valuable alternative to reduce costs, labor for the producer and improving the profits. This period between growth cycles is commonly known as “forage emptiness”, when there is a lack of forage production between different forage growing cycles (Nabinger et al., 2006; Nabinger and Carvalho, 2009).

The high Perc.Leaf of some genotypes for ‘C18’ and ‘336’ are importat to maintain a minimum nutritive value. Also, high production of biomass as observed for ‘C15’ (in both years) are important. Pastures can present a drop in nutritive value and a decrease in plant growth reducing animal performance and leading to overgrazing in the fall resulting in forage shortage as old summer pastures are ending and new winter growing pastures are being sown.

Forage components

In general, *Paspalum* hybrids have presented high ratios of leaves over stems (Pereira et al.,2015; Hubber et al., 2016; Motta et al., 2017; Weiler et al., 2018). Some hybrids in this study presented Perc.leaf upper 80%, in special, 'C18' that showed high percentual of leaves (ranging between 81% to 96%) and larger LBDM, even in summer, when this trait was lower in the mean among other seasons. Although the ecotype 'V4' obtained the greatest TDM, its LBDM was lower than that of 'C18', '336' and close to 'C15'; as a consequence, the Perc.Leaf of this ecotype was low, 0.65, which was not statistically different from the lowest value, obtained by 'Bagual' (0.62) whereas the hybrids got 0.86, 0.82 and 0.79, respectively

The SDM and INFDM had environment effects caused by season and year, more proeminent than the genotype effect alone. 'C15' had the high SDM and INFDM production in spring and summer reaching the highest value among genotypes in the spring while 'V4' and 'Bagual' played the first and second place in the summer for the same traits. Steiner et al., (2017) reported that the 'Bagual' ecotype was superior to all other ecotypes for INFDM, indicating a high potential for seed production which evidences the great potential to multiplication by seeds of the native plants.

The 'C15' INFDM in the spring of year 2 was dramatically lower than observed in year 1, indicating a strong influence from the environment, pointing out that this hybrid can present alteration in leaf blade-stem ratio dependent on management. The values obtained by year for INFDN can related to seed yield production because there are a high association between number of inflorescences per area and seed yield (Lopes et al., 2011; Lopes et al., 2016; Lopes et al., 2018). Therefore, it is expected, in an average of years, a large seed production from 'V4', 'Bagual' and '10036'.

Cold Tolerance (ColdTol) and Regrowth vigor (RegVig)

In South America region, winter with frosts are frequent and tropical grasses are not well adapted. Several native *Paspalum* species with great variability for desirable traits were proposed as warm-season forage crops (Pizarro, 2000). The response to photoperiod found in *P. notatum* seems to be a physiological response to avoid freeze damage by winter time (Sinclair et al. 2001). Efforts in plant breeding to reduce photoperiod sensitivity, increase freeze tolerance, forage production during the cool-season and improve the seasonal distribution of forage production have been made at the diploid and tetraploid level (Blount and Acuña 2009).

The hybrids showed variability for tolerance to cold, with the amplitude for the trait in year 1 been higher than in year 2, when the genotypes exhibited higher CT in an average. This was expected, because the mean temperatures in the winter of the second year were higher (Figure 1). Also, *P. notatum* plants in the first year were not fully established, which could increase the sensitivity to the freezing stress. Among the hybrids, ‘C15’ had the highest tolerance in both years while ‘Bagual’, ‘437’ and ‘C18’ performed well only in the second year. These hybrids are progenies from crosses of ‘Q4205’ with the ‘V4’ or ‘André da Rocha. The ecotype ‘V4’ presented poorly ColdTol in the first year, but improved at the second one, perhaps consequences of warmer temperatures in the winter. By the other hand, ‘André da Rocha’s forage production seemed to be less cold tolerant than ‘Bagual’ and ‘Pensacola’ (Steiner et al., 2017). Although not observed in this study, ‘V4’ and ‘Bagual’ demonstrated rapid regrowth capacity during the transition from winter to spring in the first year of evaluation under extended or normal photoperiod (Machado et al., 2017). In contrast, the sexual induced tetraploid ‘Q4205’ showed certain level of tolerance to cold, which could be inherited by its progenie, since a large variability for cool-season regrowth and freeze resistance was observed among the hybrids (Acunã et al., 2009; Acuña et al., 2011; Machado et al., 2017).

Finally, the hybrids tested in this study are the first generation of crosses between sexual autotetraploid induced genotypes and highly apomictic ecotypes (Weiler et al., 2017). As argued by Acuña et al (2009), this technique of hybridization allows exploiting and fix heterosis previously blocked by apomixis. The results obtained by Acunã et al. (2009 and 2011), Weiler et al. (2018) and in the present study, have shown that some hybrids performed better than parentals and controls, and therefore, would be possible to fix desirable traits selecting superior genotypes highly apomictic.

2.4 CONCLUSIONS

The forage components presented alterations among seasons over years. Summer had more SDM and INFDM, regargless of the genotypes. LBDM in *P. notatum* intraspecific hybrids had a high correlation with TDM showing that indirect selection could be attainable. The apomictic hybrid ‘C15’ has the highest potential to be used as cultivar because produced more forage and also had a good forage yield in the spring. In addition, the sexual hybrids ‘C18’ and ‘336’ should be used at crosses, because showed a high percentage of leaves and also had an important forage production in the fall, respectively. Those materials, also, showed reasonable cold tolerance and regrowth vigor compared to the ecotypes and to the cultivar ‘Pensacola’. Additional research is needed to evaluate these hybrids further in terms of persistence under grazing and animal performance.

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3. CHAPTER III

Seed yield components, germination and dormancy of *P. notatum* intraspecific hybrids

Seed yield components, germination and dormancy of *P. notatum* intraspecific hybrids

ABSTRACT

An elite germplasm collection of *Paspalum notatum* was selected for forage production, cold tolerance and regrowth vigor post-winter. Nine hybrids, 'Bagual', 'V4' and 'Pensacola' were arranged in a RCBD with three replications. The period analysed covered spring, summer and fall in two years. These genotypes were evaluated for its seed yield, seed components, germination, viable seeds and dormancy. This study aimed to i) characterize seed yield components, physiological and physical quality of a *P. notatum* intraspecific hybrids elite group; ii) identify the most related variables to seed production and iii) select the most productive genotypes for future experiments. When the beginning of the internode elongation was observed, the plots – previously used for forage trial – were deferred from the cuts,. The seed yield components were assessed at the seed harvest. The genotypes differed among themselves in relation to seed yield, germination, viable seeds, dormancy and blooming period. Some traits showed a significant year x genotype interaction, whereas others exhibited a high dependence of the genotype, such as rate of viable seeds and percentage of full seeds. The seed yield components were highly influenced by the year. In the second year there was decrease in seed yield, weight of thousand seeds and germination rate, but also exhibited an increase on dormancy rate. This was attributed to adverse climatic events, the incidence of *Claviceps paspali* and the deferment management carried out. Finally, the ecotype 'Bagual', the hybrids 'C18' and '336' stood out for seed production, while the cultivar 'Pensacola' showed a poor performance for all traits.

Key words: bahiagrass, warm forage grasses and seed production

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[1] Prepared in accordance with the standards of the International Journal of Plant Production.

3.1 INTRODUCTION

One of the key segments in the links of the agribusiness productive chain is the production, distribution and use of improved seeds. The use of enhanced seeds, as a vehicle for the propagation of forage species, represents an important milestone in the development of a national livestock system. It promotes a rapid dissemination of new materials. Though, it is a complex process and conditioned by a series of specific and not well-determined factors. Excellent results of forage production and quality were obtained in southern Brazil with ecotypes and intraspecific hybrids of *P. notatum* (Fachinetto et al., 2012; Steiner et al., 2017; Machado et al., 2017; Weiler et al., 2018). Yet, the efforts to increase the efficiency of seed production have been mostly neglected for forage species (Martiniello & Silva, 2011). The *Paspalum spp*. seed production in southern Brazil presents difficulties hard to overcome. There is a lack of synchronism in the inflorescence emission and the short interval to full bloom between the beginning and seed abscission (Scheffer-Basso et al., 2007; Lopes & Franke, 2011a). Moreover, problems such as flowering without the formation of caryopsis, low amounts of caryopsis per inflorescence, low rate germination and dormant seeds are recurrent in the production of seeds of *Paspalum spp.* (Batista and Godoy, 1998).

These factors make it difficult to determine the ideal timing for harvesting. Delays may result in low yields due to shattering losses, whereas anticipation results in harvesting a high number of seeds still under development (Boonman, 1971). This lack of synchrony in seed maturation may lead to low quality seeds lots (Humphreys, 1981; Souza, 2001). the yield component most associated with seed yield in *Paspalum spp.* Is the number of inflorescences per area (Lopes et al., 2016). Similarly, superior genotypes in forage production can be selected for higher seed production through this component (Lopes et al 2018). Among other traits, a high number of inflorescences per area is pursued by breeders. The problem is that this could generate less nutritive quality of the forage due to a higher proportion of stems. An alternative would be a search for genotypes with a shorter and uniform flowering period (Acuña et al, 2009).

There is great variability in seed yields of *P. notatum* seeds. According to Loch & Ferguson (1998), for Pensacola and *P. notatum* cv. Argentina, yields ranged from 60 to 100 kg.ha^{-1}, reaching up to 400 kg.ha^{-1}. Gates & Burton (1998), in a Pensacola seed production experiment, with fertilization rates, obtained the production of 514 kg.ha^{-1} of seeds when applying 224 kg.N.ha^{-1}, 25 kg.P.ha^{-1} and 47 kg.K.ha^{-1}. No benefit was achieved fertilizing it with more than 224 kg.N.ha^{-1}.

Although seed yield is a complex trait, affected by agricultural practices as well as environmental factors, it reveal considerable genetic variation, which is a prerequisite for improvement by direct or indirect selection (Boller et al, 2010).

This study goals are to i) characterize seed yield components, physiological and physical quality of a *P. notatum* intraspecific hybrids elite group; ii) identify the most related variables to seed production and iii) select the most productive genotypes.

3.2 MATERIAL AND METHODS

Experimental Design

The study was carried out 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 November 2015 and data collection was conducted from October 2016 to May 2018. The tillering – obtained from adult plants on the field – were transplanted in pure stands in ten rows (10 cuttings row^{-1}) with 25 cm apart in the row and 30 cm among rows (6 m^2 plot size). Weeds were manually removed from the plots until the full establishment of the plants. In January 2016, one cut at was used to standardize the plots. Prior to each crop year, the experimental area was fertilized per Soil Chemistry and Fertility Commission–RS/SC, Brazil (CQFS, 2004). The soil at the experimental site was classified as Dystrophic Red Argisol.

The experiment was directed for forage performance. The forage was cut every time the canopy reached 95% luminous intercept at ten centimeters from the ground. In the flowering season, it was applied 40 kg.ha^{-1} of N and an area with 3 m^2 of each plot was separated to study the seed production characteristics.

The region climate 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). The average annual precipitation was about 1450 mm (Bergamaschi et al., 2013). Irrigation, was applied after planting, when needed, until the soil was saturated. Equally as needed to keep plant growth. The experiment consisted of a group of twelve *P. notatum* genotypes (Table 1) arranged in a randomized complete block design (RCBD). There were three replications evaluated for two years. The RCBD model was completed using a split-plot design, imposing the year factor as main-plot and the genotypes as sub-plots. The germplasm evaluated included nine *P. notatum* intraspecific hybrids, ('A16', 'C15', 'C18', 'D3', '225', '336', '437', '712', '10036'), *P. notatum* ecotype 'V4' and 'Bagual', and the cultivar Pensacola (*P. notatum*) used as controls. The ecotypes used as controls have recognized agronomic value (Fachinetto et al., 2012; Steiner et al., 2017). Pensacola is the only registered variety of this species available in the Brazilian market. The hybrids were the results of crosses and selection for forage production and cold tolerance made by the UFRGS breeding program (Table 1):

Table 1. Genotypes in agronomic evaluation with their origins, genealogies and mode of reproduction.

Genotype	Source	Origin	Reproduction mode
A16	Weiler et al., 2017	Q4188 x André da Rocha	Facultative apomictic
C15	Weiler et al., 2017	Q4205 x André da Rocha	Apomictic
C18	Weiler et al., 2017	Q4205 x André da Rocha	Sexual
D3	Weiler et al., 2017	Q4205 x Bagual	Apomictic
225	Machado, 2014	Q4188 x 83N	Facultative apomictic
336	Machado, 2014	Q4205 x 95N	Sexual
437	Machado, 2014	Q4205 x V4	Sexual
712	Machado, 2014	C4-4X x 36N	Facultative apomictic
10036	Machado, 2014	Q4205 x 95N	Facultative apomictic
Bagual	Planalto region, RS (Brazil)	Native	Apomictic
V4	Barra do Quaraí, RS (Brazil)	Native	Apomictic
Pensacola*	Cultivar of *P. notatum*		Sexual

* 'Pensacola' is the unique diploid evaluated in this experiment.

Crop management and data collection

In both years the plots were deferred from the cuts when the beginning of the internode elongation was observed, which in general, corresponded to 5% of reproductive tillers. The deferment dates were December 15th, 2016 for the first evaluation year and December, 21th, 2017 for the second. The seed yield components were evaluated from randomly collected samples in the center of the plots, using two squares with 0.25 m^2.. The harvest was performed when more than 50% of the inflorescences had a straw coloring and signs of caryopses dehiscence. The collected inflorescences were dried in an oven with forced ventilation at 30 °C for 72 h. Other components were manually threshed. The variables measured were i) seed yield (SY): the samples were processed using sieves to separate the impurities (glume fragments, paleas, lemma, rachis, pedicels, etc.) and a type "South Dakota"- blower for the fractionation of full and empty seeds. ii) number of inflorescences/m^2 (NI): direct count of all the inflorescence totally emerged; iii) number of racemes by inflorescence (NRI): the ratio between the total racemes amount and inflorescence of samples; iv) weight of thousand seeds (WTS): average weight of eight subsamples of 100 seeds multiplied by 10, according to rules for seed testing - "RAS" (Brasil, 2009); v) number of seeds/inflorescence (NSR) ; vi) racemes length (RL): distance from the inflorescence intersection to raceme apex from 14 random inflorescences, expressed in centimeters; vii) Percentage of full seeds (FS): the ratio of full seeds over the sum of empty plus full seeds expressed in percentage; viii) Viable pure seeds (VSY): product from SY and germination of the lot, expressed in kg.ha^{-1}; Potential seed yield (PSY): product from SY and viable seed by tetrazolium test, expressed in kg.ha^{-1}.

The inflorescence height (IH), was calculated as the average of the distance from the ground to the apex of inflorescence height, it was measured on three representative points per plot expressed in centimeters. The weed infestation was expressed as percentage, and obtained by the ratio of weed dry mass and the sum of itself and total dry mass assessed in a previous study for forage production.

The germination test was performed with four replications of 100 seeds per experimental unit (from the "pure seed" portion). The "germitest" used type sheets of paper moistened with KNO_3 (0.2%) solution in an amount equivalent to 2.5 times the mass of the non-hydrated paper, in clear plastic boxes with a lid (11 × 11 × 3.5 cm). The boxes were stored in a germinator under a regime of alternating temperature and light (20 °C/16 h in the dark and 30 °C/8 h with light). The geminator had four fluorescent lamps (20 W; 1060 lm) located inside the germinator. The results were expressed as percentage of normal seedlings obtained in the twenty-eight day (Brasil, 2009).

Tetrazolium test was carried out with four replications of 50 seeds per plot. The seeds were first presoaked in water for 16h at 25ºC. The samples were conditioned at 30ºC exposed to 2-3-5 triphenyl chloride of tetrazolium (1%) by 24 hours. After the pre-imbibition period, with the aid of a scalpel. Then, they were sectioned longitudinally through the distal half of the endosperm and washed successive times in distilled water to remove the tetrazolium solution. The seeds whom the embryos were discolored in the vital areas to germination were classified as unviable. Seeds whose tissues contained mucilaginous, flaccid or dark red stains were considered as deteriorated. Both groups were not computed as the final percentage of viable seeds. In the second year of assessment, ocurred the *Claviceps paspali* (Ergot) infestation (Erg). The scores were based on symptoms levels of this disease which were visually rated. It was used a scale 1 to 5, being: 1 = no visible honeydew or sclerotia; 2 = <10%; 3 = 11–50%; 4 = 51–80%; and 5 = >80% of the seed head completely covered with honeydew and/or sclerotia, according to Rios et al. (2015).

Data analysis

The data were subjected to tests of normality and homogeneity of variance, which did not indicate the need for transformation, except for the trait Erg (logarithm transformation). Variance analysis were performed using aov function of R Stats package (version 3.0.2, Chambers et al. 1992) of R Program. The averages were calculated using the LSMEANS package (version 2.16, Lenth, 2016) and their comparison was made using the Tukey test (P<0.05).

3.3 RESULTS

According to the variance analysis, all the variables had a significant effect on the genotype factor. Year factor was not significant for the number of racemes per inflorescence (NRI), viable seeds rate (TZ) and percentage of full seeds (FS).

The interaction year-genotype was effective for seed yield (SY), viable seed yield (VSY), potential seed yield (PSY), germination rate (G), dormancy rate (D), thousand seed weight (WTS), number of inflorescences (NI), number of seeds per raceme (NSR) and weed infestation (PW). In contrast, the other traits had differences only for genotypes.

Seed Yield (SY), Viable Seed Yield (VSY), Potential Seed Yield (PSY)

The first year provided greater values than the second for the variables listed in Table 3. Still, some genotypes did not differ between years. SY values remained statistically equal for ‘225’, ‘10036’, ‘437’, ‘D3’ and ‘Pensacola’ while the other genotypes followed the year factor behavior. Major differences among the materials were perceived in the first year. VSY is a product between SY and germination rate (G), due to lows G in the second year, there were no differences among genotypes. The first-year classification suffered alterations when compared to SY rank. On the other hand, PSY resulted in bigger values than VSY, because this trait results from product between SY and viable seeds rate (TZ) obtained by tetrazolium test.

Table 2. Genotypes’ values averaged by blocks of each year for Seed Yield (SY), Viable Seed Yield (VSY) and Potential Seed Yield (PSY) expressed at kg.ha^{-1}.

Genotype	SY		VSY		PSY	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
			kg.ha^{-1}			
Bagual	832 a	356 ab	129 cde	12 NS	630 a	238 ab
C18	609 ab	205 ab	275 ab	10	481 ab	176 ab
336	546 b	401 a	319 a	44	408 bc	315 a
V4	549 b	328 ab	233 abc	94	364 bcd	170 ab
C15	490 b	143 ab	133 bcde	25	353 bcde	98 b
225	425 bc	348 ab	72 de	59	320 bcdef	234 ab
10036	431 bc	215 ab	137 bcde	60	239 cdefg	123 ab
712	379 bc	183 ab	69 de	23	208 defg	111 b
437	453 bc	361 ab	179 abcd	56	180 defg	176 ab
A16	444 bc	271 ab	106 cde	12	166 efg	50 b
D3	205 cd	250 ab	61 de	90	154 fg	180 ab
Pensacola	64 d	103 b	31 e	72	35 g	75 b
Mean	452	264	145	46	295	162

*Letters followed by the same letter in the column did not different by Tukey test ($p \leq 0.05$). NS – Not Significative.

In the year 1, ‘Bagual’ and ‘C18’ led the SY, producing almost the double of the mean (832 and 609 kg.ha^{-1} vs 452 kg.ha^{-1}, respectively). In the second year, ‘Bagual’ dropped to third position and ‘C18’ to the ninth place. They did not differ from each other and from the other genotypes, with exception of ‘336’ and

‘Pensacola’. There were significant differences between them. Also in the second year, ‘336’ presented the highest for SY, 401 kg.ha^{-1}, and ‘Pensacola’the lowest, 103 kg.ha^{-1}, respectively. The highest value in VSY was also showed by ‘336’, which was 319 kg.ha^{-1} joined by ‘C18’, ‘V4’ and ‘437’. The ‘C18’ remained at the second place mainly due to the high G rate. ‘Bagual’ attained seventh position producing 129 kg.ha^{-1} of VSY, approximately the mean of the first year. In the second year, the differences were not significant and they ranged from 10 to 94 kg.ha^{-1}.

The PSY exhibited a greater breadth of values between years, and in the average, in the first year, presented 295 kg.ha^{-1} against 162 kg.ha^{-1} in the second year. ‘Bagual’ presented the biggest PSY, 630 kg.ha^{-1}, except than ‘C18’ (481 kg.ha^{-1}) which in turn was statistically equal to ‘336’, ‘V4’, ‘C15’ and ‘225’. In contrast, ‘Pensacola’ presented the lowest value, 35 kg.ha^{-1}, but did not differ from ‘A16’, ‘437’, ‘712’ and ‘10036’ (239 kg.ha^{-1}). In the second year, the differences were significant for PSY, in oposition from what was observed to VSY. ‘336’ presented higher PSY (315 kg.ha^{-1}) than ‘712’, ‘C15’, ‘Pensacola’ and ‘A16’ (50 kg.ha^{-1}). The genotypes: ‘Bagual’, ‘225’, ‘D3’, ‘437’, ‘C18’, ‘V4’ and ‘10036’ did not differ significantly when compared to ‘336’. ‘A16’ only was different from ‘336’.

Germination rate (G), Viable seeds (TZ), Dormancy seed (D), Full Seed (FS) and Weight of Thousand Seeds (WTS)

There was no interaction between genotypes and years factors for full seeds rate (FS) and viable seeds (TZ). Germination rate (G), however, varied among genotypes during the periods. In the first year presented in average, a higher rate than in the second year (Table 3). ‘Bagual’ had the lower G showing the half of the mean in the first year, 15% and was not different from the others, except ‘336’, which reached the higher G, 59%, which was higher than ‘712’, ‘225’ and ‘Bagual’. On the other hand, in the second year, ‘336’ presented just 11% of G being statistically equal to ‘Bagual’ (3%) and to ‘225’ (17%) and ‘Bagual had the lowest value in this year for this rate. The highest G in the second period was observed in ‘Pensacola’ – 56%, which was statistically equal to ‘D3’, ‘V4 ’and ‘10036’. The cultivar already had a reasonable rate in year 1 – 34%.

Table 3. Genotypes’ values averaged by blocks for germination rate (G), viable seeds (TZ), dormancy rate (D), percentage of full seeds (FS) and thousand seed weight (WTS).

Genotype	G (%)		TZ (%)	D (%)		FS (%)	WTS (g)	
	Year 1	Year 2		Year 1	Year 2		Year 1	Year 2
Pensacola	34 ab	56 a	51 bcd	34 abc	7 a	49 cd	1.67 e	1.77 e
V4	43 ab	35 ab	63 abc	36 abc	45 b	54 bcd	3.79 ab	3.34 ab
336	59 a	11 b	77 ab	21 a	86 cd	79.5 a	3.37 bcd	3.17 abc
D3	30 ab	35 ab	74 ab	60 bcd	51 bc	56.3 bcd	3.41 bcd	3.01 abc
10036	32 ab	28 ab	57 abc	51 abcd	52 bc	49.8 cd	3.43 bcd	3.33 ab
437	37 ab	15 b	45 cd	23 ab	68 bcd	57.8 bcd	4.02 a	3.32 ab
C18	44 ab	5 b	83 a	44 abcd	94 d	63.7 abc	4.03 a	3.5 a
C15	27 ab	16 b	72 abc	62 cd	77 bcd	59.8 bcd	3.08 d	2.78 d
225	17 b	17 b	70 abc	77 d	73 bcd	60.7 bcd	3.04 d	2.93 cd
712	18 b	14 b	58 abc	68 cd	76 bcd	56.2 bcd	3.19 cd	3.22 abc
A16	26 ab	4 b	29 d	37 abc	77 bcd	46.5 d	3.05 d	2.81 d
Bagual	15 b	3 b	71 abc	79 d	95 d	67.8 ab	3.64 abc	3.21 abc
Mean	32	20	62	49	67	58.43	3.31	3.03

*Letters followed by the same letter in the column did not different by Tukey test ($p \le 0.05$). NS – Not Significative.

The highest value for TZ was performed by ‘C18’ (82.7%), although it was only statistically higher than ‘Pensacola’, ‘437’ and ‘A16’. ‘A16’ presented the lowest value (29.4%) and stay at the same group than the lowest ones before mentioned. The average was 62.4%. Six genotypes were upper than this value: ‘C18’, ‘336’, ‘D3’, ‘C15’, ‘Bagual’ and ‘225’. 'C18' exhibited a difference from '336' of 6.1%, while to other qualified better genotypes the differences were around 2%. In contrast, 'A16' presented 15.83% less than the penultimate position, '437' (45.3%), but there were no differences among them.

In average, Dormancy rate (D) presented higher values in the second year than the first one. Genotypes’ classification was altered between years (Table 3). Some genotypes maintained high D rate over the period such as ‘10036’, ‘225’, ‘712’, ‘Bagual’, ‘C15’, ‘D3’ and ‘V4’. In the contrary, ‘Pensacola’ had a decrease from 34% to 7%. ‘336’, ‘437’, ‘A16’ and ‘C18’ increased with differences as high as 65% at this character expression. ‘336’ increased from 21% to 86%. ‘Bagual’ exhibited the larger D rate in both years, although it was equal to ‘225’, ‘712’, ‘C15’, ‘D3’, ‘10036’ and ‘C18’ in the first year and equal to ‘10036’, ‘D3’, ‘V4’ and ‘Pensacola’ in the second year. On the other hand, in the first year, the lowest D was performed by ‘336’, ‘437’, ‘Pensacola’, ‘V4’, ‘A16’, ‘C18’ and ‘10036’, while in the second year , ‘Pensacola exhibited the lowest D rate.

The mean percentage of full seeds (FS) was 58.4%, the highest value was achieved by ‘336’ being 79.5%, followed by ‘Bagual’ with 67.8% and ‘C18’ with 63.7%. In contrast, ‘A16’ presented the lowest value, 46.5% but differed only from the highest values mentioned. The other controls, ‘V4’ and ‘Pensacola’ had values lower than the mean: 54% and 49%, respectively.

Weight of Thousand Seeds (WTS) in the first year was higher than in the second one (Table 3). The genotypes ‘437’, ‘Bagual’, ‘C15’, ‘C18’, ‘D3’ and ‘V4’ had a decreased in the values in the second period while the others had no differences. In both years, ‘Pensacola’ showed the lighter weights for WTS while ‘C18’ presented the heavier values. In the first year, ‘C18’ did not differ from ‘437’, ‘V4’ and ‘Bagual’. In the second year , it did not differ from ‘V4’, ‘10036’, ‘437’, ‘712’, ‘Bagual’, ‘336’ and ‘D3’. Still in the first year, ‘C15’ and ‘A16’ presented light values just overcame by ‘Pensacola’, in both years, and ‘225’ in year 2. ‘336’ showed heavier seeds in the second year when compared to other genotypes getting superior classification and was statistical equal to the heaviest genotype, ‘C18’.

Number of Inflorescences (NI), Number of Racemes per Inflorescence (NRI), Number of seed per Raceme (NSR), Raceme Length (RL) and Inflorescence Height (IH).

The genotypes’ rank for Racemes Length (RL) and Inflorescence Height (IH) were constant across the years (Table 4). The genotypes’ values for Number of Inflorescences (NI) and Number of seeds per Racemes (NSR) varied between the years, while Number of Racemes per Inflorescence (NRI) were constant. In the first year had bigger values for NI whereas the NSR had lower values. The greatest NI in the first year was observed for ‘C15’, ‘Bagual’ and ‘V4’ being different just from ‘Pensacola’ and ‘D3’ which had the lowest NI amounts. In the second year, ‘336’ presented the greatest NI and ‘C18’ presented the lowest values. The other genotypes did not differ from themselves and from these hybrids.

Table 4. Genotypes' values averaged by blocks for number of inflorescences (NI), number of racemes per inflorescence (NRI), number of seeds per raceme (NSR) expressed at units and racemes length (RL) and inflorescence height (IH) expressed at cm.

Genotype	NI		NRI	NSR		RL	IH
	Year 1	Year 2		Year 1	Year 2		
C15	356 a	169 ab	2,00 c	15 bc	12 cd	10.84 f	85.67 bc
Bagual	349 a	214 ab	2.01 bc	12 bc	26 ab	13.22 cd	100.5 a
V4	345 a	272 ab	2.04 abc	18 b	17 abcd	14.42 bc	93.5 ab
225	299 ab	272 ab	2.01 bc	15 bc	20 abc	12.8 cde	86.17 bc
712	293 ab	171 ab	2.02 abc	11 bc	16 bcd	12.2 def	82.17 bc
336	284 ab	328 a	2.01 bc	7 c	27 a	11.44 ef	78.67 c
C18	281 ab	108 b	2.01 bc	16 bc	27 a	13.1 cde	85.5 bc
10036	265 abc	207 ab	2.05 abc	20 b	15 cd	14.23 bc	90.17 abc
437	235 abc	269 ab	2.05 ab	15 bc	20 abcd	14.94 ab	92.17 abc
A16	225 abc	223 ab	2.02 abc	30 a	18 abcd	16.6 a	87.33 abc
Pensacola	160 bc	196 ab	2.07 a	16 bc	9 d	12.84 cde	85.17 bc
D3	106 c	210 ab	2.02 bc	19 b	21 abc	12.8 cde	86.83 abc
Mean	266	220	2.02	16	19	13.29	87.82

*Letters followed by the same letter in the column did not different by Tukey test ($p \le 0.05$). NS – Not Significative.

The highest value for NRI was presented by ‘Pensacola’, which was not different from ‘437’, ‘10036’, ‘V4’, ‘712’ and ‘A16’. A high value for this trait means that there was more inflorescence with three or four racemes. In contrast, ‘C15’ presented the lowest value, but only differentiated from ‘437’ and ‘Pensacola’. In the second year had more NSR on average, though some genotypes produced less than in the first year. The values in this period ranged between 7 to 30, corresponding to ‘336’ and ‘A16’. The controls ‘V4’, ‘Pensacola’ and ‘Bagual’ produced 18, 16 and 12, not differentiating from the mean. In the second period, ‘Pensacola’ presented the lowest value, 9, being statistically equal to the value of 20 performed by ‘437’. ‘C18’ and ‘336’ reached the highest NSR (28 and 27) and differed from ‘712’, ‘10036’, ‘C15’ and ‘Pensacola’. From the first to the second year, ‘Bagual’ rose up to third place while ‘V4’ got down to eighth position, ‘A16’ stayed above ‘V4’s rank but did not differ statistically.

The average high of inflorescence (IH) was 87.8 and varied from 78.67 cm to 100.5 cm. ‘Bagual’ presented the highest that did not differ from ‘V4’, ‘437’, ‘10036’, ‘A16’ and ‘D3’. ‘336’ exhibited the lowest value and just differed from ‘V4’ and ‘Bagual’. The RL varied from 10.84 cm and 16.6 cm, which were performed by hybrids ‘C15’ and ‘A16’. The second longest raceme was observed for ‘437’, which was similar to ‘A16’, ‘V4’ and ‘10036’. ‘C15’ did not differ from ‘336’ and ‘712’. The average for this trait was 13.29 cm, close to ‘Bagual’s value and above ‘Pensacola’s value, although they did not differ from each other. The control ‘V4’ showed a value bigger than ‘Bagual’ but not statistically different.

Percentage of Weeds (PW) and Ergot Infestation (Erg)

The weed infestation (PW) varied between 5% and 65% being bigger in the first year. Even tough there were no differences for some hybrids over the years (Figure 1), the highest PW values were observed for ‘Pensacola’ in both years and ‘D3’ just for the second year. In general, the lowest PW occurred to ‘V4’, ‘336’, ‘C18’ and ‘Bagual’.

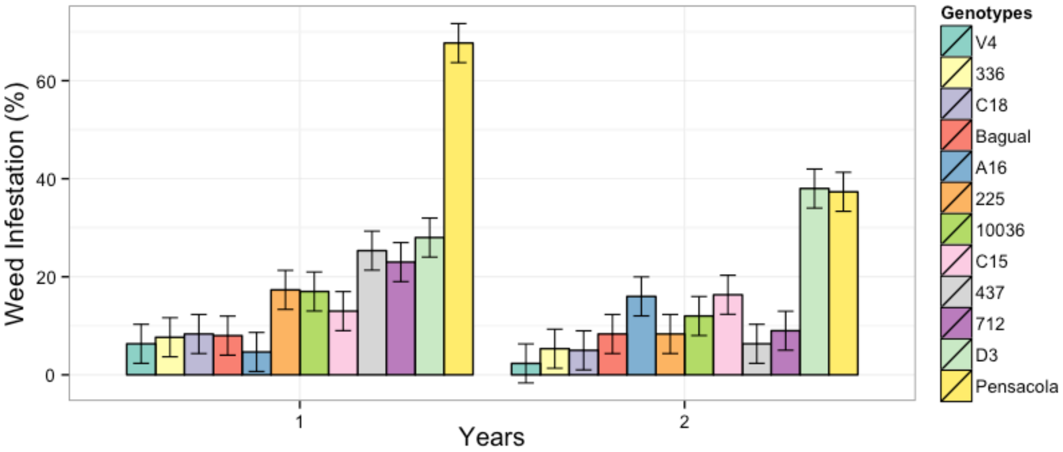

Figure 1. Weed Infestation level (0 to 100%) among genotypes over years 1 and 2.

In general, Ergot infestation (Erg) (Figure 2) level was observed only in the second year. The differences were detected among the genotypes. The hybrid ‘437’ presented the highest infestation, followed by ‘A16’. The lowest levels of symptoms occurred for ‘C18’ followed by ‘D3’ and ‘C15’ which did not differ from ‘C18’. ‘Pensacola’ and ‘V4’ showed to be more sensitive than ‘Bagual’, but they did not differ among themselves.

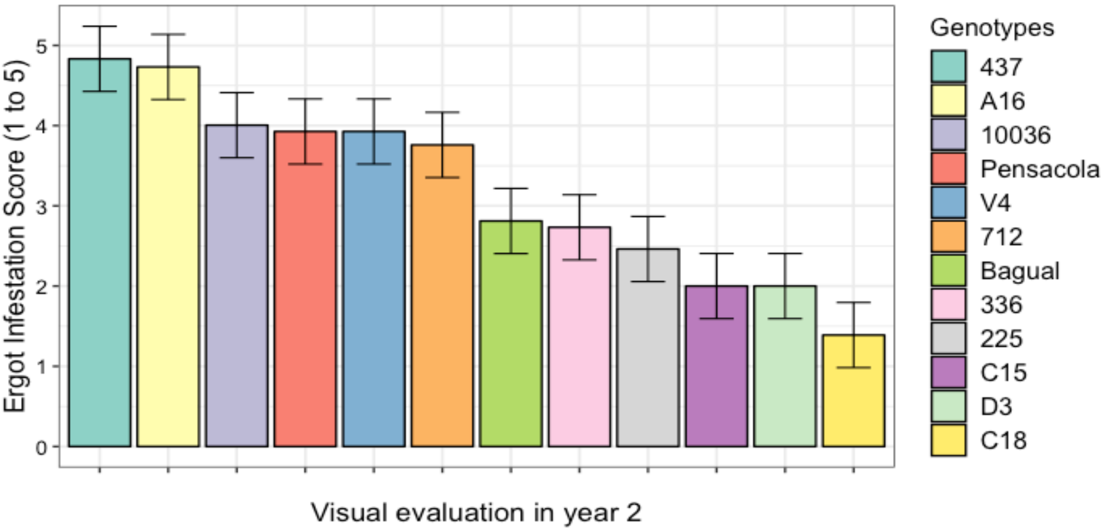

Figure 2. Ergot Infestation score assessed by visual evaluation during the second year.

Correlations

The correlations values and significance varied between the years and among variables (data not shown). Some variables were associated in both years with values up to 0.7, such as NI and NR, SY and G with VSY. The correlations with SY in the first year, orderly, were NI (0.74), WTS (0.63) and FS (0.53), while NRI had a negative and low correlation to SY (-0.44). In the second year: NI (0.75) remained most closed to SY, NSR (0.51) became significant. Conversely, WTS (0.38) and FS (0.47) decreased. The highest value observed for FS was a negative correlation with NSR (-0.65) that become positive in the second year (0.52). In the first year, it was also observed a positive correlation between FS and NSI (0.61). G was negative correlated in the first year to NSR (-0.33), which became more evident in the second year when the correlation was -0.56.

3.4 DISCUSSION

The seed yield components are traits regulated by polygenes. Certain variations in the magnitude of the genetic effect were observed. Interactions between year and genotype were expected for the variables, mainly, those ones more correlated to seed yield (SY) in *Paspalum* hybrids (Lopes et al., 2017 and 2018). It is indicated more than one year of assessment to determine seed production traits in germplasm of *Paspalum* hybrids. Some variables were not influenced by the year genotype interaction which suggests that the genotypes' rank for that trait was not altered. The Number of Racemes per Inflorescence (NRI) and % of Viable Seeds (TZ), were mainly defined by genotype factor, demonstrating that these are characteristics inherent to genetic background. In addition, Weigh of Thousand Seeds (WTS) and percentage of Weeds (PW) were affected by year x genotype interaction. Genotypes had more influence. Indeed, some traits are more influenced by genetic than main environment effects. The existence of interactions between these factors can be different for the same variables among species or germplasm.

Lopes et al. (2017) reported the genotypes' number of seeds per inflorescence showed different patterns in different years, whereas tiller height and inflorescence rachis length, were inherent to the genotype in hybrids of *P. plicatulum x P. guenoarum*. At the same time, other variables showed higher influence from year than the genotype factor, despite the hybrid genetic background. Thus, the environment strongly determined traits performance. The year exerted a greater influence in phenotypic expression of SY, VSY, PSY, G, D, NI, NSR and PW over the hybrids of *P. notatum*. Similar behavior was reported for total tillers density, number of inflorescences, number of seeds per inflorescence, thousand seed weight and seed yield in hybrids of *P. plicatulum x guenoarum* (Lopes et al., 2017). The traits most contributed to genetic divergence among a progeny of *P. plicatulum x P. guenoarum* hybrids were inflorescence height (23.59%), inflorescence rachis length (21.63%), thousand seed weight (16.67%) and seed yield (14.23%). Corroborating to that, Lopes et al. (2018) stated the high genetic variance estimates were also found for thousand seed weight, number of seeds per inflorescence and number of inflorescences. This indicates that the improvement of these traits is a goal attainable in *Paspalum* hybrids.

Therefore, there are a group of characteristics exhibited strong link with genetic effects, which are not much influenced by environment, other that are moderate influenced by environment. Finally, another one highly influenced by environment such as SY, VSY, PSY, NI, NSR and G. For this reason, several techniques could be used to improve the yields in seed production (Gates &

Burton, 1998; Phaikaew et al., 2002; Lopes et al., 2016.). For instance, the height of canopy and inflorescences may affect the seed yield. It is important to consider the height of inflorescences as a target in plant breeding. Taller plants are more prone to lodging while short plants make mechanical harvest difficult due to the accumulation of plant matter and can be more prone to diseases. Interestingly, ‘A16’ presented the highest RL, 16.6 cm, while ‘C15’ showed the shortest, 10.84 cm. NSR had the highest value for ‘A16’, which may be because there were other components of seed yield were involved. It may attempted to compensate the relations among themselves to target the maximum quantity of viable seeds. In counterpart, ‘A16’ showed the lightest WTS among the tetraploid genotypes, evidencing different strategies in the partition of photoassimilates among the parts of the plant. The most important seed yield component to predict seed yield is the number of reproductive units per area (Humphreys, 1981). Relying upon our correlations, NI and NR stood out in association with SY in both years and was clear to see this pattern on ecotype ‘Bagual’, that presented elevated NI and SY. Though, when examining the genotypes' seed yield, there was some variation due to the genotype, such as observed for 'C18' and '336'. These hybrids produced, on the average, 407 and 473.5 kg.ha^{-1} and presented medium values for NI whose reduction was compensated by NSI and WTS. In contrast, ‘C15’ showed the biggest NI over years but performed on average for SY, VSY and PSY, and poorly in the second year.

Lopes & Frank (2011b) reported a negative correlation between seed yield and vegetative tillers (-0.83). According to them, the tillering dynamic along a plant cycle is variable, depending on the frequency of new tillers appearance and the elongation of reproductive tillers. In this study, the correlation between SY and weed infestation was negative and achieved -0.70 in the first year, decreasing in the second one, probably due to sprayed of herbicides. Others negative correlations performed with SY were perceived with NRI and G indicating that the increase of NRI can frail the investment in seed development in favor to creation of racemes, as well as the production of more amount of seed can reduce seed quality due to the dilution effect.

The average values reported in the current study are like most common warm-season perennial grass cultivated in the tropics and subtropics. Marcon et al. (2018) found a mean seed yield of 280 $kg.ha^{-1}$. The values reported to *P. lenticulare* varied from 90 $kg.ha^{-1}$ to 540 $kg.ha^{-1}$ wherein the mean seed yield of the group was 280 $kg.ha^{-1}$. *P. atratum* displayed 310 $kg.ha^{-1}$. The germination under field conditions ranged between 28% and 11%. *P. atratum* U39, displayed the highest germination, while *P. lenticulare* L347 and *P. atratum* U44 exhibited the lowest. Overall mean germination percentage was 16% for *P. atratum* and 19% for *P. lenticulare.* The seed yield for *P. atratum* cv. Suerte yielded between 110 and 220 $kg.ha^{-1}$ (Kalmbacher et al., 1997) while *P. atratum* BRA 9610 produced in average and nitrogen treatments 704 $kg.ha^{-1}$ but the harvests occurred 3 times at 3-5-day intervals (Phaikaew et al., 2002). According to Loch & Ferguson (1998), for Pensacola and *P. notatum* cv. Argentine, yields ranged from 60 to 100 $kg.ha^{-1}$, reaching up to 400 $kg.ha^{-1}$. Gates & Burton (1998), a Pensacola seed production experiment obtained the production of 514 $kg.ha^{-1}$ of seeds applicating 224 $kg.N.ha^{-1}$, 25 $kg.\ P.\ ha^{-1}$ and 47 $kg.K.\ ha^{-1}$ by fertilization rates. Adjei et al. (2000) reported the best yield for *P. notatum* 'Argentine' reached 145 $kg.ha^{-1}$, when sward residue from previous growing season was removed between late April and late May, almost in the middle of spring for conditions in Florida - USA, and then immediately fertilized with 100 kg N ha^{-1} using a base 0-50-190 NPK kg ha^{-1} in study whom attempt different combinations of defoliation management and nitrogen fertilization.

In the current study, there was not a permanent management to control weeds. The plot areas destined to seed production were previously cultivated for forage production. In an overview, all genotypes produced more seeds than 'Pensacola', yet, the yield observed to this cultivar were lower than expected (Gates & Burton, 1998). The low values reported in this study were adversely affected by weed infestation (figure 2). Weed control is mandatory to grow seeds. On the other hand, weed infestation is valuable information to evaluate genotypes' competition and persistence skills. The pastures with these genotypes will need less use of herbicides and labor to control weeds, resulting in savings to farmers. In the first year, 60% of the 'Pensacola' plots were infested by weeds, when was observed the lowest SY, 62 kg.ha^{-1}. In the second year due to 2,4-D spray, there was a reduction of weeds, but the impact over SY was not evident. A great portion of weeds in 'Pensacola's plot was composed of *Setaria sphacelata* which is not affected by 2,4-D. In the second year, these plots were also prejudiced, though less. The lowest infested genotypes were 'V4', '336', 'C18' and 'Bagual'They also had a higher SY. Interestingly, '336' and 'C18' had high growth up habits and were not expected to present a low weed infestation. Because, competitive biotypes have prostrate growth habit, as 'V4' and 'Bagual'. In fact, '336'and 'C18' produced more tillers than 'V4' and 'Bagual', covering better the ground and preventing the emergence of weeds.

In general, in the second year of assessments it was observed more production of reproductive tillers, as well as among hybrids of *P. plicatulum x P. guenoarum* (Lopes et al., 2017). Indeed, it was expected that the tillers density and SY to be higher because the plants were perennial and well stablished in the second year of the crop. Conversely, the genotypes had in general lower seed production in the second year.

On the other hand, stress at flowering later stages can affect the cell division and cell enlargement after fertilization limiting the size and storage capacity of the seed. The photosynthetic activity of the vegetative plant may be reduced affecting the production of assimilates for the developing of seeds, causing a decrease in seed production and seed viability and vigor (Bewley and Black, 1994). Also, the amount of photoassimilate supply into grain filling can suffer decreases since the starch amount in the endosperm is related to the variation in WTS. Nevertheless, a minimum is needed to produce a viable individual which implies in the reduction in the final total number of grains that can be formed. For instance, the NI, RL, NSI, WTS and SY showed decreased in the second year but TZ was not affected. There was a moderate correlation between SY and empty seeds showing the most part of productive genotypes produced a larger amount of empty seeds. Comparing the effect of hydric stress, it was not observed a larger empty seeds in the second year, with the year effect for FS and TZ indicated that the problems due to the environment in seed production resulted merely in a reduction of the seeds amount.

There was variability to NI and number of reproductive tillers among hybrids and years. These characteristics suffered a strong influence by the environment and are the focus in crop management to improve seed yield, testing different fertilizer levels, sowing rates, row spacing, cutting heights and irrigation patterns (Phaikaew et al., 2001a, 2001b and 2002; Canto et al., 2012; Gbenou et al., 2013; Lopes et al., 2016).

On the other hand, the partitioning among the sinks in the perennial grasses, as *Paspalum spp,* are not elucidated at all. Indeed, plant changes imposes for management to increase seed production with high quality. It still needs to be understood. Some authors stated that in normal conditions, perennial plants give priority to rhizomes propagation because there are higher chances to survive and occupy adjacent patches when compared to seeds dispersion (Gardner & Mangel, 1999; Zandt et al., 2003). Although it is known that when the plant's own survival condition is at risk, all stored energies are directed to the production of seed descendants. In addition, in *P. notatum*, the number of sexual eggs increases in proportion to the apomictic ones, because of environmental stresses as a way of trying new genetic combinations that could be better adapted to the imposed new environmental scenario (Rios et al., 2013).

The hybrid ‘A16’ exhibited a decrease in forage dry mass in the second year, when occurred a strong reduction of forage production in summer. In the current study, ‘A16' showed a larger decrease in the SY and PSY, as well as a different flowering behavior when compared to the previous year. The emergence of inflorescences heads started later and occurred for a shorter time than in the previous year for the same genotypes. It was observed for most of the genotypes, that greatest SY were followed by greatest quantities of empty seeds. Weight of thousand seeds (WTS) was highly variable among genotypes, but did not have strong correlations with quality traits of seeds.

The results found to WTS, FS, G, D and their relationship with TZ shows that a quantity of minimum nutrients is needed to normal seed development, although environment effect can alter the characteristic expression, such as observed for D rate. In the second year, this trait increased probably due to the problems in watering supply which caused drought to the plants. Environmental signals guide the dormancy expression by signaling that environmental conditions are becoming unfavorable for seedling establishment and growth. Hence, seed dormancy prevents seeds from germinating at inappropriate times. It is an adaptive trait that optimizes the distribution of germination time and population persistence (Copete et al., 2011).

Different expression of dormancy (D) was observed among the genotypes between the years, suggesting that exist variability enough to select the trait, even in a small group of elite genotypes. In the second year, it was detected the existence of more correlations with D. The trait showed a moderate positive correlation with NSI, NSR and WTS, respectively, 0.45, 0.63 and 0.58, which may indicate that plants facing adversity, such as drought, could guide their efforts to generate and fill seeds with higher D expression.

P. notatum present impermeability of the seed coat, which restricts the water entry. (Mathews, 1947; Anderson, 1953). Seed coat structure and thickness are extremely variable between different species and in the seeds of the same species grow under different environmental conditions. (Bewley and Black, 1994). The expression of D is regulated by genes being modified by external environmental factors, and genotype and environment interactions. Dormancy not only varies with genotype but also with maturation environment (Fenner and Thompson, 2005). Heritability of dormancy is complex because parts of the seeds differ genetically since a part receive nutrients from endosperm with maternal and paternal DNA and the embryo is surrounded by maternal tissues as the testa (Debeaujon et al., 2007).

In the second year, ergot infestation was observed in the field and visually rated. The hybrids ‘437’ and ‘A16’ were the most affected, with reductions in SY, G and WTS observed for the second year, although other genotypes also presented a decrease for these variables and were less prejudiced by Ergot.

The incidence of ergot increases as stigmas of unfertilized ovaries remain receptive and became more prone to infection by the ergot pathogen (Burton and Lefebvre, 1948). Facultative apomictics and sexual tetraploids spend more time to start the embryo development compared to the highly apomictic plants, which can become it more vulnerable to Erg. Indeed, 'A16' and '437' are facultative apomictic and sexual plants but 'C18' is a sexual plant and had low incidence of the disease. The genetics mechanisms responsible for ergot resistance are not elucidated, although enough variability is found for this trait indicating that plant breeding can enhance it (Rios et al., 2015).

The causes that impaired ‘A16’ seed production could be related to the seed filling phase and its response to the deferred date and to the incidence of ergot disease. This hybrid presented satisfactory reproductive structures to support seed development and high seed yield. For instance, NSR had the double of mean value among genotypes in the first year and RL reached 16.6 cm over the period. Although its NI was not among the highest, it was around the average in both years.

In addition, ‘A16’ SY was around the mean in the first year but showed low values in VSY and PSY, due to lows G and TZ. In the second year, SY was low but in both years, the values observed to FS were low, indicating a potential to increase the seed production. The WTS was the lowest among the genotypes excluding the 'Pensacola' because it is diploid. ‘Bagual’ presented the highest values for SY, TZ and WTS although the data stated the ecotype has potential to great seed production (Lopes et al., 2011; Steiner et al., 2017). The Erg incidence was low. Indeed, the defoliation time has a direct impact of seed yield (Adjei et al, 2000; Lopes et al., 2016).

All variables related to seed yield and quality were negatively or not affected from the first year to the second. The hybrid ‘336’ were the most stable over the period having a good SY in both years. In addition, ‘C18’ showed high quality of seeds and short flowering. Therefore, these hybrids could be used in the breeding program to improve seed production. On the other hand, the highly apomictic ‘Bagual’, ‘V4’ and ‘C15’ can be used in crosses as pollen donors, due its forage value or be released as cultivars. Although, RL, FS, NRI and NSR were not highly correlated with SY, there are indications that they are closely related to genetic factors and could be used to discriminate varieties and be target in breeding programs.

Nevertheless, the production reduction in the second year was not equal for all genotypes. PSY allowed identifying the potential of viable seed production, since the five most productive genotypes in the first year produced more than 350 kg.ha^{-1} (‘Bagual’, ‘C18’, ‘336’, ‘V4’ and ‘C15’). This value is larger than that found for other warm grasses from *Urochloa spp.* and *Megathirsus spp.* (Canto et al., 2012; Catuchi et al., 2013; Monteiro et al., 2016).

3.5 CONCLUSION

The genotypes presented different patterns of seed yield components. The differences found for seed yield, thousand seed weight, percentage of full seeds, germination and dormancy rate among the genotypes, are valuable information to guide future crosses. The seed yield component most related to seed production were the number of inflorescences per area. The hybrids ‘C18’ and ‘336’ had a good combination between production and quality of seeds and it is suggested to use them in new crosses as mothers, because their sexual reproduction mode. Among the apomictic hybrids, ‘C15’ was the most promising, but it was negatively affected by deferment management. Hence, more studies must be carried out to provide knowledge about the best hybrids aiming to improve seed production with high quality.

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4. CHAPTER IV

4.1 FINAL CONSIDERATIONS

The adoption of improved forage species adapted to local environments is important to the progress of livestock farming and to the southern field's conservation of the country. Since many ranchers give up the activity or change for other agricultural crops.

Several studies were developed by the Forage Department of UFRGS to identify the best genotypes adapted to Brazil southern. The answers generated in the current studies provocate more questions that be asked for the next students. The seed production experiment provided some information about *P. notatum* and helped in selection for the new candidates to cultivar. However, the use of seed as vehicle to disseminate and guarantee adoption by ranchers is huge as seen for the "Brachiarias" (*Urochloa spp.*) and more studies to improve quality and quantity are necessary. Among the attributes, dormancy has highlight because is an obstacle to establish new pasture because need some attenuation or remove to be ready to germinate. This characteristic is variable among genotypes of *P. notatum* and can be attenuated by breeding.

Similarly, the seed yield can be improved. The sexual hybrids showed high forage and seed yield demonstrating be valuable tools for the breeding program. During the summer of 2018, the group of forage breeding led by prof. Miguel Dall'Agnol made a new expedition collecting ecotypes of *P. notatum*, *P. guenoarum* and *P. lepton* to compound a new germplasm collection. This germplasm has been characterized to ecologic and agronomic aspect because besides aimed to select genotype to be used in the breeding program, also, has the motivation to preserve and identify plants to be used for restoration of degraded pastoral ecosystems.

For now, there are hybrids with great performance for forage production and seasonal distribution. Trials required by Ministério da Agricultura (MAPA) to prove value, cultivation and use (VCU) have been conducting since 2016 in three regions of Rio Grande do Sul (Brazil). The locations choose: Eldorado do Sul, Ijuí and Bagé, have different edaphic and climate conditions that allow recommending the best genotypes for the state. This is the exigence to allow release a cultivar according to Brazil's law. The information provided by the current and previous experiments, as well, the VCU is valuable to assure the success of the future cultivar. In addition, *Paspalum notatum* is a pasture well accepted by the whole world for different production systems.

By the other hand, there is a lack of information about animal production using these hybrids. And new research needs to be guided for this target. The high costs of land, fertilizer and labor hand, associated with low prices of commodities and a higher requirement of nutrients in the diet by animals of genetic for high performance ask for forage with bigger nutritive value and high efficiency in the use of resources besides high forage yield.

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6. AUTHOR BIOGRAPHY

Cleber Henrique Lopes de Souza, son of Letícia Lopes de Souza and José Cleber de Souza, was born on April 17th, 1989, in Gravataí-RS, Brazil. Studied at the São Mateus Lutheran School in Cachoeirinha, RS, wherein completed the Elementary School in 2002. In Gravataí, RS at Dom Feliciano School RS, complete the high school in 2005. In 2006, started the Agronomy course of the Federal University of Rio Grande do Sul. In 2007, started activities as a Scientific Initiation Scholar (CNPq) at the Rio Grandense do Arroz Institute (IRGA), under the guidance of researcher Élio Marcolin until 2008, when was located under the guidance of researcher Vera Mussoi Macedo and consultant Dr. Cláudio Mundstock by 2009. During the period at IRGA joined several kinds of research focus in developing technologies to the efficiency of resources and to improve yield in rice, as well, had the opportunity to participate in many trainings. In 2009, started in the department of crop plants of UFRGS under the guidance of Prof. Dr. Christian Bredemeier which for two years studied and searched nitrogen fertilization by different localized management techniques to improve yield and grain quality for Wheat and Barley. In 2012 carried out the curricular internship at EPAGRI at the São Joaquim experimental station in Santa Catarina (Brazil) helping in the evaluations and activities of the Apple breeding program and pre-breeding of Feijoa. Undergraduate as Agronomist Engineer in August 2012, started the graduation in April 2013 in Animal Science Program under the guidance of Prof. Dr. Lúcia Brandão Franke, developing research in ecology, technology and production of forage seeds. In March 2015, submitted to master’s degree defense with a thesis entitled *Paspalum guenoarum* 'Azulão' seed production under cuts management in the second year. In the same year, started the Ph.D. degree under orientation of Prof. Dr. Miguel Dal’Agnoll developing research focus in *Paspalum* genus to improve forage and seed agronomic traits. During April to October 2018 had a “sandwich” period at the University of Florida, Gainesville, FL, USA, under the supervision of Dr. Esteban Fernando Rios. In March 2019, submitted the Ph.D. dissertation to the defence committee at the UFRGS, Porto Alegre, RS, Brazil.

7. APÊNDICE

5/8/2019 International Journal of Plant Production - Guide for Authors

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[2] Chettri, M.K., Sawidis, T., Zachariadis, G.A., Stratis, J.A., 1997. Uptake of heavy metals by living and dead Cladonia thalli. Environ. Exp. Bot.37, 39-42.
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[5] Rice, K., 1992. Theory and conceptual issues. In: Gall, G.A.E., Staton, M. (Eds.), Integrating Conservation Biology and Agricultural Production. Agric. Ecosyst. Env. 42, 9-26.

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Scientific Names: common names of organisms should always be accompanied, when first cited, by their complete scientific name in italics (genus, species, attribution and, if appropriate, cultivar).

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