

New wild diploids in *Paspalum notatum* Flügge (Poaceae): potential accessions for use in breeding.

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Abstract: *Paspalum notatum* is a polymorphic species with two accepted varieties, according to the ploidy level. The ploidy level is closely related to the reproductive mode of this species. Usually, diploid cytotypes have sexual reproduction, and tetraploid cytotypes have an apomictic reproduction. Apomixis limits genetic recombination, hindering the development of new cultivars. The identification of sexual plants represents many possibilities in breeding programs. This study aimed to analyze the chromosome number in *P. notatum* accessions under agronomic evaluation. Ploidy level was determined in 25 accessions. Two ploidy levels were detected, resulting in 21 tetraploid and four diploid accessions. The four diploid accessions originate from Argentina and Uruguay, in a region close to the origin of Pensacola, which is a diploid sexual cultivar of *P. notatum*. These new diploid accessions may be useful in the development of breeding programs of *Paspalum* species.

Keywords: Cytogenetics, genetic breeding, ploidy level.

INTRODUCTION

Paspalum L. comprises about 350 species distributed in tropical and subtropical American natural pastures (Zuloaga and Morrone 2005). Among the species of this genus, *Paspalum notatum* Flügge is considered as one of the most promising forage plants in Southern Brazil due to its yield, quality, resistance, and growth (Canto-Dorow et al. 1996, Pozzobon and Valls 1997).

P. notatum is a polymorphic species, with two varieties, according to the ploidy level (Canto-Dorow et al. 1996). *P. notatum* var. *saurae* is diploid ($2n = 2x = 20$ chromosomes), native to eastern Argentina, distributed in the provinces of Santa Fe, Corrientes, and Entre Rios (Burton 1967). Brazil has no native diploid varieties of this species, only tetraploids ($2n=2x=40$ chromosomes), which occur in pastures in the Americas, from Central Mexico to Uruguay (Quarin et al. 1984).

In the genus *Paspalum*, polyploidy is associated with apomixis (Moraes-Fernandes et al. 1974, Delgado et al. 2014), which is defined as an asexual mode of reproduction by seeds (Martinez et al. 2007). This reproduction mode is an obstacle to the genetic breeding because it hinders genetic recombination and consequently cultivars protection (Huber et al. 2016). Since apomixis prevents

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crosses, the genetic breeding in *Paspalum* is limited to the identification and selection of superior tetraploid accessions, without any breeding process (Aguilera et al. 2011).

The existence of sexual genotypes is an imperative prerequisite for any breeding program of polyploid apomictic grasses (Sartor et al. 2009). In other *Paspalum* species, cytogenetic analyses have revealed diploid accessions (Pozzobon et al. 2008, Pozzobon et al. 2013). In *P. notatum*, sexual tetraploids plant were obtained by the chromosome duplication of cultivar Pensacola (Quarin et al. 2001, Weiler et al. 2015, Machado et al. 2017). However, in some cases, the mode of reproduction changes after duplication, and tetraploidized plants become apomictic (Quarin et al. 2001, Krycki et al. 2016). 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 expression of apomixis in this duplicated plant is possibly a gene-dosage effect.

The search of diploids in germplasm banks associated with morphologic and molecular analyses could distinguish new diploid accessions of *P. notatum* Pensacola. Moreover, the analysis of their agronomic performance could indicate their use in breeding programs of this species. Based on these considerations, this study aimed to determine the ploidy level in a collection of *P. notatum* accessions subject to agronomic and morphologic evaluation.

MATERIAL AND METHODS

A cytogenetic study was carried out on 25 *P. notatum* accessions. These materials belong to the Germplasm Bank of the United States Department of Agriculture (USDA) and were collected from the years of 1950 to 1970 in Southern Brazil, Argentina, Uruguay, and Paraguay (Fig. 1). For this study, plants were obtained from an experiment for the agronomic evaluation conducted by Fachinetto et al. (2012). Ploidy level was determined from the gametic chromosome number and pollen mother cells (PMC) analysis. During the summer of 2010, young inflorescences of two individuals randomly chosen from each accession were collected and fixed in Carnoy 3:1 (ethanol: acetic acid) for 24 hours, at room temperature, and stored in ethanol 70% under refrigeration (Pereira et al. 2014, Moreira et al. 2017). Slides were prepared by isolating the anthers, squashing, and staining with 1% propionic carmine (Pagliarini et al. 2002, Simioni and Vale 2009). Cells were analyzed in an optical microscope, totaling ten cells per individual. Gametic chromosome numbers were determined in diakinesis/metaphase I and anaphase I when the chromosomes showed visible and adequate spread. The ploidy level was determined considering $x = 10$ chromosomes, described as the basic number for the species (Dahmer et al. 2008).

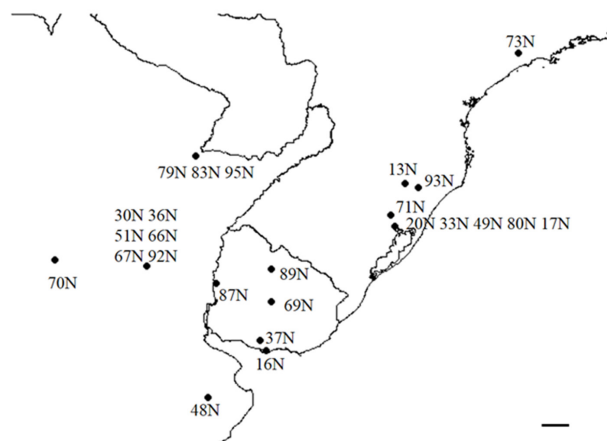


Figure 1. Location of *P. notatum* accessions. Scale: 100 km.

Table 1. Ploidy level of *P. notatum* accessions

Accessions	Identification	Ploidy
13N	PI404473	4x
16N	PI276251	4x
17N	PI214118	4x
20N	PI508826	4x
30N	PI508833	4x
33N	PI310169	4x
36N	PI508834	4x
37N	PI404861	4x
48N	PI508838	4x
49N	PI310168	4x
51N	PI337566	4x
66N	PI508832	2x
67N	PI337573	2x
69N	PI404866	4x
70N	PI508828	4x
71N	PI310145	4x
73N	PI241878	4x
79N	PI508840	4x
80N	PI310170	4x
83N	PI508827	4x
87N	PI404863	2x
89N	PI404865	4x
92N	PI508831	2x
93N	PI404476	4x
95N	PI424652	4x

RESULTS AND DISCUSSION

The chromosome number of the 25 *P. notatum* accessions was determined. In this study, 21 accessions were tetraploid ($2n = 4x = 40$ chromosomes) and four accessions were diploid ($2n = 2x = 20$ chromosomes) (Table 1, Figure 2). These data corroborated the findings of other authors, indicating that tetraploids are more common than diploids in *Paspalum*. Adamowski et al (2005) analyzed 36 *Paspalum* accessions, revealing one diploid ($2n = 2x = 20$), 34 tetraploids ($2n = 4x = 40$), and one hexaploid ($2n = 6x = 60$). In a study with *P. leptan*, all the 53 accessions analyzed were tetraploid (Reis et al. 2008). In *P. notatum*, Pozzobon and Valls (1997) demonstrated that 90% of the accessions were tetraploid and 10% were diploid. Similar results were obtained in a study with 92 accessions, of which 83 were tetraploid, one was hexaploid ($2n = 6x = 60$), and eight were diploid, of which seven corresponded to Pensacola, used as control, and one was considered as an escape of Pensacola (Dahmer et al. 2008).

In this study, the diploid accessions were not considered as an escape of Pensacola due to their origin. Accessions 66N, 67N, and 92N were collected in Santa Fe, Argentina, while 87N was collected in Paysandú, Uruguay (Figure 1, Table 1). These four accessions were considered as wild diploids since they were collected in the origin region of Pensacola (Burton 1967).

These accessions are part of a larger *P. notatum* collection and were evaluated for agronomic, morphological, and molecular aspects. Dry matter yield was higher in three diploid accessions (66N, 67N and 92N); however, these accessions did not differ from 87N when compared with Pensacola. 66N and 67N produced about four times more, and 92N produced about seven times more than Pensacola. Furthermore, these accessions showed superior persistence to winter conditions than Pensacola (Fachinetto et al. 2012). Moreover, morphological analyses indicated that the four diploid accessions grouped separately from each other and Pensacola (Fachinetto et al. 2017).

This separation into different morphological and molecular groups may be due to the higher variability since these accessions are probably of sexual reproduction. *P. notatum* var. *saurae* has narrower leaves, small spikelets, and more racemes per inflorescence (Quarin et al. 1984, Canto-Dorow et al. 1996), being classified together with plants from sexual reproduction. So far, all the diploid plants of this species (wild or cultivated) are typically sexual (Espinoza and Quarin 1997, Daurelio et al. 2004, Pozzobon et al. 2008). Delgado et al. (2014) stated that genetic determinants of apomixis found in diploids are not sufficient for a considerable expression of the trait, as found in *P. rufum*, and that the gene expression of this trait is related to other factors.

Apomixis is a type of asexual reproduction in which seeds formation occurs without fertilization. The resulting individuals are genetically identical to the plant that originated them. Asexual reproduction allows the genotypes to be fixed, and the desired traits to be maintained. Due to the absence of recombination, this phenomenon maintains the presence of gene blocks and linked genes, allowing the continuous exploration of heterosis, besides eliminating the need for plant isolation during seed production. However, plants that reproduce exclusively by apomixis hinder breeding processes for preventing genetic recombination between individuals, making them highly dependent on mutations to evidence genetic variability (Carvalho et al. 2008). These diploids, which probably present sexual reproduction, could be used for crossing between each other or even with Pensacola, without chromosome doubling. These findings would ensure

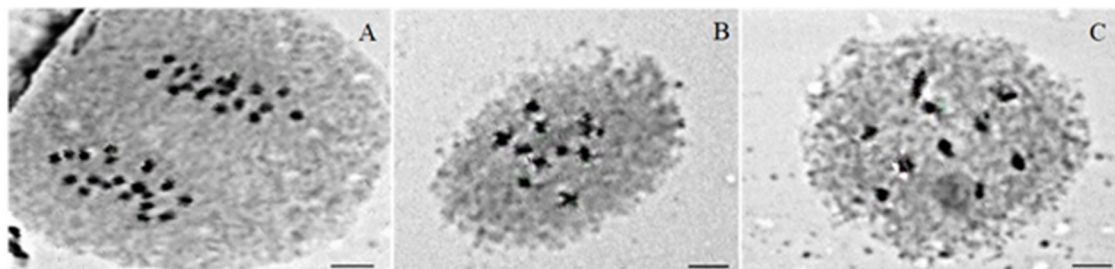


Figure 2. Pollen mother cells (PMC) in *P. notatum*. A – Accession 36N, anaphase I, $n = 2x = 20$ chromosomes in each pole. B – Accession 66N, diakinesis, $n = x = 10$ bivalent chromosomes. C – Accession 92N, diakinesis, $n = x = 10$ bivalent chromosomes. Scale: 10 μm .

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sexual accessions for crossing since chromosome doubling can lead to apomixis (Quarin et al. 2001, Krycki et al. 2016).

Sexual plants with superior traits could be used in new recombinations within the breeding program, in the formation of new individuals, maximizing the benefits of hybrid vigor (Burton et al. 1973, Jank et al. 2011, Lopes et al. 2018).

After the crossing between the diploids and the obtainment of plants with favorable traits and heterosis, chromosome doubling could lead to apomictic tetraploid plants to be used to keep desired traits. The findings of this work may contribute to the *Paspalum* species, especially *P. notatum*, conferring new possibilities of crossing and insertion of genetic variability in breeding programs.

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