

Chromosome numbers, meiotic behavior and pollen fertility in a collection of *Paspalum nicorae* Parodi accessions

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ABSTRACT – Chromosome number, meiotic behavior and pollen viability were evaluated in a collection of 53 *Paspalum nicorae* Parodi accessions, which are part of a breeding project of the species. All accessions are tetraploid, with $2n=4x=40$. Despite the invariable chromosome numbers, there was variation among accessions in the frequencies of different chromosome configurations at diakinesis and metaphase I, such as univalents, trivalents and quadrivalents. Other abnormalities as bridges and laggards were also observed at anaphase and telophase I. Meiotic indexes ranged from 82.00 to 99.50% and pollen viability from 88.99 to 95.06%. As the species is pseudogamous apomictic, fertile pollen is necessary for endosperm formation. Results show that all plants are meiotically stable and have enough fertile pollen to be used as male parents in controlled crosses.

Key words: apomixis, meiotic behavior, chromosome number, pollen viability, *Paspalum nicorae*.

INTRODUCTION

The genus *Paspalum* L. (*Paniceae*, *Panicoideae*) contains more than 400 tropical and subtropical species, adapted to a wide range of ecosystems with high genetic diversity (Strapasson et al. 2000). Around 220 of the known *Paspalum* species occur in Brazil, as part of varying plant communities in different ecological conditions (Barreto 1974, Valls and Pozzobon 1987). Many species are part of the native pastures of Paraguay, Uruguay, southern Brazil and northeastern Argentina (Takayama et al. 1998).

Diploid and polyploidy levels, normally related to sexual an apomictic reproduction are common in the genus (Quarín and Norrmann 1990).

Paspalum nicorae Parodi (“grama cinzenta”, brunswickgrass) is a perennial apomictic species, highly tolerant to grazing and is included in the *Plicatula* group (Barreto 1974). Several authors stress the importance of *P. nicorae* as an important forage with high potential to be used not only for animal feeding but also for the recovery and conservation of degraded soils (Burson and Bennett 1970, Boldrini 2006, Dall’Agnol et al. 2006).

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Although the chromosome number of *P. nicorae* (a tetraploid with $2n=4x=40$) is known from literature (Burson and Bennett 1970, Moraes-Fernandes et al. 1974, Pagliarini et al. 2001), previous studies have analyzed one or few plants only. Moreover, for breeding projects, especially when crosses are involved, the chromosome number of the specific genotypes is extremely important (Burson 1975). Since intraspecific variability in chromosome number is rather common in *Paspalum* (Moraes-Fernandes et al. 1968a,b) any breeding project with species from this genus must include chromosome number determinations and, as far as possible, further information such as meiotic behavior and pollen fertility.

The objective of this study was to analyze the chromosome number, meiotic behavior and pollen fertility of a collection of 53 *P. nicorae* accessions that are part of a breeding project and that were morphologically analyzed by Reis (2008).

MATERIAL AND METHODS

Plant material

The original plants were collected as tillers at different locations in Rio Grande do Sul, southern Brazil (Table 1), transferred to pots filled with soil and were maintained as part of a germplasm collection at the Departamento de Plantas Forrageiras e Agrometeorologia, Universidade Federal do Rio Grande do Sul. The accessions (each population represents an accession) were identified in the collection by the species name plus the collector's number and collection site (Table 1) and numbered for practical reasons. After the plants had flowered, their taxonomic status was confirmed by a botanist. As the species is apomictic, each tiller, and consequently the plant grown from this tiller, represented an accession.

Cytogenetic studies

To determine the somatic chromosome number ($2n$) root-tip cells were pre-treated with a saturated paradichlorobenzene solution for 24 h at 4°C, fixed in Carnoy II 6:3:1 (chloroform:ethanol:acetic acid) for 24 h at room temperature, hydrolyzed with 1N HCl for 10 min at 60°C, stained with Schiff's reagent (Feulgen procedure) for 1h and squashed in propionic carmine 0.6%. At least 10 well-spread cells with no chromosome overlapping were counted per plant.

Gametic chromosome numbers (n) as well as meiotic behavior analysis were performed in pollen-

mother-cells, from young inflorescences fixed in Carnoy II 6:3:1 at room temperature and squashed in propionic carmine 0.6%. Special attention was paid to chromosome configurations at diakinesis and metaphase I and when possible (i.e. when the cells allowed a reliable interpretation) to chromosome segregation at anaphase and telophase I. As very few cells were found at meiosis II, they were not included in the analysis.

Meiotic indexes were estimated from 200 pollen tetrads per plant following Löve (1949). Pollen tetrads with four equal-sized microspores were considered as normal and all others as abnormal.

Pollen analysis

Pollen viability was estimated from 1200 mature pollen grains per plant, stained with propionic carmine 0.6%. Full, well-stained grains were considered as potentially viable and empty or poorly stained grains as non-viable.

RESULTS AND DISCUSSION

This is the first study to report on cytogenetic information for a large number (53) of *P. nicorae* populations.

The somatic chromosome number (Figure 1 D) was determined in 19 accessions and the gametic chromosome number (Figure 1C) for all 53 accessions (Table 1). All accessions are tetraploid ($n=20$ or $2n=4x=40$). These results agree with those of Burson and Bennet (1970) who analyzed ten *P. nicorae* accessions, Moraes-Fernandes et al. (1974) two, and Pagliarini et al. (2001) one *P. nicorae* accession.

Despite the absence of variability in chromosome numbers, the frequencies of different chromosome associations varied among accessions. Besides the normal pairing in bivalents (II) at diakinesis and metaphase I (Figure 1E), univalents (I), trivalents(III) and quadrivalents (IV) were also observed in the cell configurations of all accessions (Table 1). The most frequent configuration was 18II + 1IV (Figure 1A). In the accessions 6B, 12B, 20B, 27 B, 30B, 31A, and 31B no cells with 20 II were detected and exclusive bivalent formation was not observed in any accession. Bridges, laggards and abnormal chromosome segregation were also found at anaphase and telophase I, (Table 1, Figure 1B). In accession 6B, chromosome segregation at anaphase and telophase I was irregular in 61.12% of the cells.

Table 1. Accession number, collector and collection site, chromosome numbers, chromosome associations and segregation (meiosis I), meiotic index and pollen viability in the 53 *Paspalum nicorae* accessions studied

Accession	Collector ^a	Collection site (township)	2n	n	Associations at diakinesis and metaphase I ^b	Anaphase/ Telophase ^c	Meiotic index (%)	Pollen viability (%)
2	MD, CN s/n	Cristal	-	20	20II (7); 32I + 2IV (10)	N(9); L(1)	99.5	90.75
2A	MD, CN s/n	Cristal	40	20	20II (3); 10-18II; 4-14I; 1-5IV (19)	N(11); L(2)	87.5	93.54
3	MD, CN s/n	São Lourenço	40	20	20II (3); 18II + 1IV (5); 12II + 4IV (4)	N(5); L(4)	-	91.11
4	MD, CN s/n	Capão do Leão	40	20	20II (1); 13-19II; 2-4I; 1-3IV (14)	N(16); L(4)	94.0	91.50
4A	MD, CN s/n	Capão do Leão	40	20	20II (3); 18-19II; 2-36I; 1-5IV (22)	N(20); L(5)	-	91.07
5	MD, CN s/n	Capão do Leão	-	20	20II (3); 14II + 4I + 2IV (3); 12II + 4IV (2)	N(18); L(6)	-	92.50
6A	MD, CN s/n	Pinheiro Machado	-	20	20II (1); 5-19II; 2-25I; 1III; 1-3IV (12)	N(5); L(2)	-	92.22
6B	MD, CN s/n	Pinheiro Machado	-	20	2-18II; 34-36I; 1III; 1-2IV (6)	N(7); L(11)	98.0	88.99
8A	MD, CN s/n	Pinheiro Machado	40	20	20II (12); 19II + 2I (4); 18II + 4I (3)	N(6); L(9)	-	92.11
8B	MD, CN s/n	Pinheiro Machado	-	20	20II (17); 14II + 3IV (5)	N(4); L(6)	-	92.35
9	MD, CN s/n	Pinheiro Machado/ Candiota	-	20	20II (7); 7-18II; 2-32I; 1-6IV (10)	N(1); L(3)	-	91.88
9A	MD, CN s/n	Pinheiro Machado/ Candiota	-	20	20II (3); 10-18II; 2-6I; 1III; 1-4IV (13)	N(6); L(6)	-	92.89
10	MD, CN s/n	Hulha Negra/ Candiota	-	20	20II (2); 12-18II; 1-2I; 1III; 1-4IV (9)	N(6); L(10)	-	91.99
11A	MD, CN s/n	Bagé	40	20	20II (8); 10-19II; 2-20I; 1-3IV (19)	N(15); L(13)	99.5	95.06
11B	MD, CN s/n	Bagé	40	20	20II (8); 14-19II; 2-4I; 1-4 (15)	N(31); L(15)	87.5	91.10
12A	MD, CN s/n	Dom Pedrito	40	20	20II (3); 16-18II; 1I; 1III; 1-2IV (3)	N(28); L(2)	82.0	91.90
12B	MD, CN s/n	Dom Pedrito	40	20	10-18II; 1-20I; 1III; 1-3IV (16)	N(8); L(3)	91.0	93.21
13A	MD, CN s/n	Dom Pedrito	-	20	20II (9); 8-16II; 3-20I; 1III, 1-5IV (13)	N(44)	96.5	91.18
13B	MD, CN s/n	Dom Pedrito	-	20	20II (13); 2-19II; 2-24I; 1-4IV (44)	N(40); L(19)	96.0	91.90
14	MD, CN s/n	Dom Pedrito/ Santana do Livramento	-	20	20II (15); 16II + 2IV (6)	-	-	92.62
14A	MD, CN s/n	Dom Pedrito/ Santana do Livramento	-	20	20II (9); 18II + 1IV (6); 14II + 3IV (2)	N(10); L(2)	-	90.06
17	MD, CN s/n	Santana do Livramento	40	20	20II (5); 16-18II; 1I; 1III; 1-2IV (12)	N(6); L(1)	-	91.18
18	MD, CN s/n	Santana do Livramento	40	20	20II (8); 18II + 1IV (5) 10II + 5IV (2)	N(22); L(13)	-	91.99
19	MD, CN s/n	Santana do Livramento	40	20	20II (8) 12-19II, 2-16I; 1-4IV (21)	N(9); L(17)	-	91.71

to be continued

Accession	Collector ^a	Collection site (township)	2n	n	Associations at diakinesis and metaphase I ^b	Anaphase/Telophase ^c	Meiotic index (%)	Pollen viability (%)
20B	MD, CN s/n	Santana do Livramento	-	20	19II + 2I (3); 15II + 2III + 1IV (1)	N(12); L(4)	-	91.21
20C	MD, CN s/n	Santana do Livramento	-	20	20II (8); 18II + 1IV (5) 14II + 8I + 1IV (6)	N(2)	-	91.89
26A	MD, CN s/n	Alegrete	-	20	20II (11); 18II + 1IV (10) 18II + 1I + 1III (7)	-	-	92.96
26C	MD, CN s/n	Alegrete	40	20	20II (2); 1-10II; 1-3III; 1-38I; 1-5IV (31)	N(37); L(5)	93.0	90.16
26D	MD, CN s/n	Alegrete	-	20	20II (11); 9-18II; 1-22I; 1III; 1-3IV (20)	N(15); L(7)	97.0	92.54
26F	MD, CN s/n	Alegrete	-	20	20II (17); 18II + 1I + 1III (3)18II + 1IV (4)	N(24)	-	91.80
27A	MD, CN s/n	Alegrete	-	20	20II (21); 10-16II; 1I; 1III; 1-5IV (5)	N(71); L(4)	88.5	91.47
27B	MD, CN s/n	Alegrete	-	20	8-18II; 12I; 1-6IV (14)	N(14); L(1)	-	93.00
28A	MD, CN s/n	Rosário do Sul	-	20	20II (5); 15II + 2I + 2IV (10)	N(8); L(2)	-	91.47
28B	MD, CN s/n	Rosário do Sul	-	20	20II (6); 12-18II; 1-4IV (10)	N(14); L(3)	90.5	90.55
28C	MD, CN s/n	Rosário do Sul	40	20	20II (6); 18II + 1IV (6) 16II + 2IV (5)	N(14); L(3)	-	92.42
28D	MD, CN s/n	Rosário do Sul	-	20	20II (8); 10-19II; 1-2I; 1III; 1-5IV (11)	N(14); L(10)	-	91.16
28E	MD, CN s/n	Rosário do Sul	-	20	20II (5); 2-18II; 4-36I; 1III; 1-5IV (14)	N(20); L(10)	-	93.23
29	MD, CN s/n	Rosário do Sul	-	20	20II (9); 3-19II; 1-34I; 1-2III; 1-5IV (18)	N(7); L(4)	-	90.97
30A	MD, CN s/n	São Gabriel	-	20	20II (5); 18II + 1IV (10) 16II + 2IV (5)	N(15); L(12)	-	93.07
30B	MD, CN s/n	São Gabriel	-	20	18II + 1IV (12); 16II + 2IV (4)	N(18); L(10)	-	91.72
31A	MD, CN s/n	São Gabriel	-	20	18II + 1IV (13); 16II + 2IV (6)	N(19); L(19)	-	92.08
31B	MD, CN s/n	São Gabriel	-	20	18II + 1IV (10); 16II + 2IV (6)	N(6)	-	90.16
32A	MD, CN s/n	Santa Margarida do Sul	-	20	20II (15); 18II + 1IV (2)	N(25); L(10)	-	92.24
32B	MD, CN s/n	Santa Margarida do Sul	40	20	20II (24); 18II + 1IV (9) 16II + 2IV (2)	N(26)	-	89.18
32C	MD, CN s/n	Santa Margarida do Sul	40	20	20II (12); 18II + 1IV (6) 16II + 4I + 1IV (3)	N(21); L(16)	-	91.64
33A	MD, CN s/n	Vila Nova do Sul	40	20	20II (3); 14II + 3IV (6) 16II + 2IV (2)	N(15); L(11)	-	92.13
33B	MD, CN s/n	Vila Nova do Sul	-	20	20II (6); 14-18II; 1-3IV (7)	N(18); L(1)	-	91.92
34	MD, CN s/n	Vila Nova do Sul	-	20	20II (3); 8-9II; 1-4I; 1III; 1-6IV (31)	N(1)	-	91.78
35	MD, CN s/n	Eldorado do Sul	40	20	18II + 1IV (6); 16II + 2IV (2)	N(20); L(8)	-	91.81
36	MD, CN s/n	Eldorado do Sul	-	20	20II (9); 18II + 1IV (4); 16II + 2IV (8)	N(12)	-	93.06
37A	DN s/n	Viamão	40	20	20II (9); 16-19II; 2-4I; 1IV (8)	N(10); L(3)	-	90.85
37C	DN s/n	Viamão	-	20	20II (3); 16-18II; 4I; 1-2IV (13)	N(15)	-	91.50
37D	DN s/n	Viamão	-	20	20II (11); 12-18II; 1-4IV (13)	N(16); L(1)	-	91.26

^a MD—Miguel Dall'Agnol; CN—Carlos Nabinger; DN—Danilo Menezes Sant'Anna; ^b Associations at diakinesis and metaphase I; I univalents, II bivalents, III trivalents, IV quadrivalents; in brackets the number of cells analyzed; ^c N – normal disjunction; L – bridges and/or lagging chromosomes.

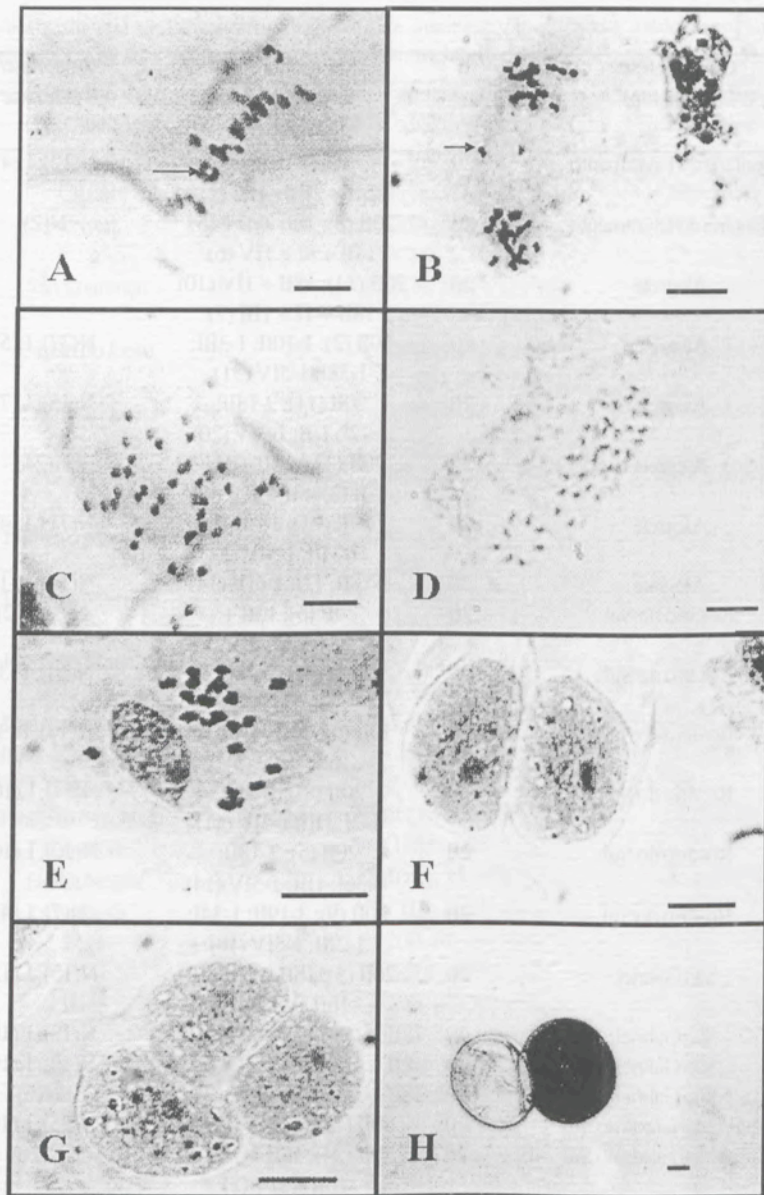


Figure 1. *P. nicorae* A) accession 11A, metaphase I (18II + 1IV, arrow) B) accession 29, anaphase I with lagging chromosomes (arrow); C) *P.* accession 28B, early anaphase I, 40 chromosomes; D) accession 19, somatic metaphase (2n = 40); E) accession 29, late diakinesis (20II); F) accession 26C, dyad; G) accession 26C, triad; H) accession 26A, viable and non-viable pollen grains. Scale bar = 10 μ m

Bashaw et al. (1970) reported the predominance of configuration 10II+5IV. Burson and Bennet (1970) also reported the predominance of bivalents and quadrivalents in the *P. nicorae* accessions analyzed: univalents ranged from 0 to 6 with an average of 0.49 per cell; bivalents ranged from 6 to 20 with an average of 16.67 per cell, and quadrivalents ranged from 0 to 7 with an average of 1.48. Some introductions had

laggards and micronuclei in 50 to 60% of the cells at anaphase and telophase I and in 70% of the cells during anaphase and telophase II. Pagliarini et al. (2001) found a rather high percentage of cells with meiotic irregularities in *P. nicorae*: 38.47% in anaphase and telophase I, 26.32% in metaphase I and 80.67% in meiosis II. Balbinot (2007) found a high variability in chromosome associations, as well as in irregularities

among 49 accessions of *P. notatum* Flüggé. According to Burson and Bennett (1970), *P. nicorae* has the genome constitution XX X₁X₁, indicating similar (genome with partial homology) but not identical genomes. The presence of quadrivalents and laggards would indicate that *P. nicorae* was originated by hybridization between two close species. Quadrivalents may be explained by heterozygosity for chromosome translocations or may reflect chromosome homology due to polyploid origin (Stebbins 1971). The quadrivalents found in the *P. nicorae* accessions examined here can be explained by both reasons.

Meiotic indexes ranged from 82.00 to 99.50% (Table 1) among the 12 accessions analyzed. Dyads (Figure 1F) and triads (Figure 1G) were found in frequencies ranging from 0.50 to 18.00%. The meiotic indexes of most of the plants were higher than 90%, indicating meiotic stability, according to Löve (1949). Pagliarini et al. (2001) found 19.80% irregularities in pollen tetrads of a *P. nicorae* accession.

Pollen viability (Table 1, Figure 1H) was high (mean 91.78%), ranging from 88.99% (accession 6B) to 95.06% (accession 11A). Burson and Bennet (1970) found a mean of 65.7% stainable pollen among the *P. nicorae* accessions. In 64 accessions of *P. notatum*, Balbinot (2007) found pollen viability ranging from 72.40 to 98.00%.

No direct correlation was found between frequency of meiotic abnormalities and meiotic indexes and pollen viability. The same was observed in other species such as *P. notatum* (Balbinot 2007). This may seem paradoxical at first sight but it should be

considered that meiotic indexes, as well as the determination of pollen viability by stainability are estimations and not accurate measurements.

As suggested for *P. notatum* by Balbinot (2007), the variability in chromosome associations and the relatively high pollen viability found here could be explained by the apomictic mode of reproduction of *P. nicorae*: remaining quadrivalents from the polyploidization process, as well as those caused by translocations, as well as trivalents, univalents etc., caused by chromosome alterations (such as inversions) may be maintained in the populations without much harm to population survival. At the same time some meiotic regularity should be maintained in order to assure enough pollen fertility for endosperm formation, essentially important in a pseudogamous species. All 53 accessions studied have enough pollen viability to be used as male parents in controlled crosses.

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Números cromossômicos, comportamento meiótico e fertilidade do pólen em uma coleção de acessos de *Paspalum nicorae* Parodi

RESUMO – Foram analisados o número cromossômico, o comportamento meiótico e viabilidade do pólen em uma coleção de 53 acessos de *Paspalum nicorae* Parodi, que fazem parte de um projeto mais amplo de melhoramento da espécie. Todos os acessos são tetraplóides, com $2n=4x=40$. Não houve variabilidade no número cromossômico mas foi observada variação entre os acessos nas frequências das diferentes configurações cromossômicas em diacinese e metáfase I, como univalentes, trivalentes e quadrivalentes. Outras anormalidades como pontes e cromossomos retardatários também foram observadas na anáfase e telófase I. O índice meiótico variou de 82,00 a 99,50% e a viabilidade do pólen de de 88,99 a 95,06%. Como a espécie é um apomítico pseudogâmico há necessidade de pólen fértil para formar o endosperma. Os resultados indicam que todas as plantas são meioticamente estáveis e poderiam ser usadas como progenitores masculinos em cruzamentos controlados.

Palavras-chave: apomixia, comportamento meiótico, número cromossômico, viabilidade de pólen, *Paspalum nicorae*.

REFERENCES

- Balbinot ND (2007) **Variabilidade citogenética em uma coleção de acessos de *Paspalum notatum* Flüge**. Msc Thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, 67p.
- Barreto IL (1974) **O gênero *Paspalum* (Gramineae) no Rio Grande do Sul**. Livre-Docência Thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, 258p.
- Bashaw EC, Hovin A and Holt EC (1970) Apomixis, its evolutionary significance and utilization in plant breeding. In: **Internacional Grassland Congress, II Proceedings**, Queensland, p. 245-247.
- Boldrini II (2006) Biodiversidade dos campos sulinos. In: Dall'Agnol M, Nabinger C, Rosa LM, Silva JLS, Santos DT and Santos RJ (eds.) **Anais do I Simpósio de Forrageiras e Produção Animal**. Faculdade de Agronomia-UFRGS, Porto Alegre, p. 11-24.
- Burson BL (1975) Cytology of some apomitic *Paspalum* species. **Crop Science** 15: 229-232.
- Burson BL and Bennett HW (1970) Cytology, method of reproduction, and fertility of Brunswickgrass, *Paspalum nicorae* Parodi. **Crop Science** 10: 184 -187.
- Dall'Agnol M, Steiner MG, Baréa K and Scheffer-Basso SM (2006) Perspectivas de lançamento de cultivares de espécies forrageiras nativas: o gênero *Paspalum*. In: Dall'Agnol M, Nabinger C, Rosa LM, Silva JLS, Santos DT and Santos RJ (eds.) **Anais do I Simpósio de Forrageiras e Produção Animal**. Faculdade de Agronomia-UFRGS, Porto Alegre, p. 149-162.
- Löve RM (1949) **Estudos citológicos preliminares de trigos rio-grandenses**. Secretaria de Estado dos Negócios da Agricultura, Indústria e Comércio, Porto Alegre, 23p. (Circular, 74)
- Moraes-Fernandes MIB, Barreto IL and Salzano FM (1968a) Cytogenetic, ecologic and morphologic studies in Brazilian forms of *Paspalum dilatatum*. **Canadian Journal of Genetics and Cytology** 10: 131-138.
- Moraes-Fernandes MIB, Barreto IL and Salzano FM (1968b) Cytogenetic, ecologic and morphologic studies in Brazilian forms of *Paspalum notatum*. **Canadian Journal of Genetics and Cytology** 15: 523-531.
- Moraes-Fernandes MIB, Barreto IL, Salzano FM and Sacchet AMOF (1974) Cytological and evolutionary relationships in Brazilian forms of *Paspalum* (Gramineae). **Caryologia** 27: 455-465.
- Pagliari MS, Carraro LR, Freitas PM, Adamowski EV, Batista LAR and Valls JFM (2001) Cytogenetic characterization of Brazilian *Paspalum* accessions. **Hereditas** 137: 27-34.
- Quarin CL and Norrmann GA (1990) Interspecific hybrids between five *Paspalum* species. **Botanical Gazette** 151: 366-369.
- Reis CAO (2008) **Caracterização citogenética e morfológica em uma coleção de acessos de *Paspalum nicorae* Parodi**. MSc Thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, 142p.
- Stebbins GL (1971) **Chromosomal evolution in higher plants**. Edward Arnold, London, 216p.
- Strapasson E, Vencovsky R and Batista LAR (2000) Seleção de descritores na caracterização de germoplasma de *Paspalum* sp. por meio de componentes principais. **Revista Brasileira de Zootecnia** 29: 373-381.
- Takayama SY, Freitas PM, Pagliarini MS and Batista LAR (1998) Chromosome number in germplasm accessions of *Paspalum* (Plicatula group) from different regions in Brazil. **Euphytica** 99: 89-94.
- Valls JFM and Pozzobon MT (1987) Variação apresentada pelos principais grupos taxonômicos de *Paspalum* com interesse forrageiros no Brasil. In: Savidan YH, Alcântara PB, Valls JFM, Quarin C and Almeida ARP (eds.) **Encontro Internacional sobre Melhoramento Genético de *Paspalum***. Anais, Nova Odessa, p. 15-21.