Euphytica **99:** 89–94, 1998.

Chromosome number in germplasm accessions of *Paspalum* (Plicatula group) from different regions in Brazil

S.Y. Takayama[1], P.M. Freitas[1], M.S. Pagliarini[1,*] & L.A.R. Batista[2]
[1] *Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, 87020-900, Maringá-Paraná, Brazil;* [2] *Centro de Pesquisa de Pecuária do Sudeste/EMBRAPA, 13560-970, São Carlos-São Paulo, Brazil; (*author for correspondence)*

Received 3 January 1997; accepted 22 August 1997

Key words: Paspalum, Brazilian accessions, chromosome number, polyploidy

Summary

We determined the chromosome number of 52 accessions of *Paspalum* from the germplasm collection of the Centro de Pesquisa de Pecuária do Sudeste (CPPSE/EMBRAPA) originating from different regions in Brazil. All accessions belonged to the Plicatula group, 13 of them being *P. plicatulum*, three *P. guenoarum*, two *P. yaguaronense*, two *P. compressifolium*, one *P. atratum*, and 31 still unidentified *Paspalum* sp. Except for a *P. yaguaronense* accession that presented 2n=60, all the remaining ones presented 2n=40 chromosomes. This is the first report of the chromosome number of *P. atratum*. Considering that the basic number for most species in the genus *Paspalum* is $\times$=10, the accessions analyzed are tetraploids (2n=4$\times$=40) and hexaploids (2n=6$\times$=60).

Introduction

The genus *Paspalum* comprises more than 400 tropical and subtropical species, most of them native to South America. Many species are abundant in natural pastures of Paraguay, Uruguay, southern Brazil and northeastern Argentina, where they represent some of the best forage grasses. Although some improved varieties of this genus are currently being used in the United States and Australia, there still is a high potential for improvement among wild species (Quarín & Hanna, 1980).

In Brazil, despite the enormous genetic variability existent, few experimentally tested forage options are available. In general, the few varieties on the market were obtained by selection of introduced or even native germplasm. In order to diversify the forage options for the country, several projects are currently underway in different regions. Particularly important among them is the improvement of *Paspalum*, a genus that comprises approximately 220 species in the Brazilian flora (Valls, 1987). Species belonging to this genus occur in virtually all herbaceous communities in the various ecosystems of the country. Species of the Notata and Dilatata groups develop well in the southern region, whereas species of the Plicatula group occur throughout the Brazilian territory, presenting wide morphological variation (Pozzobon & Valls, 1987).

The gradual realization of the importance of *Paspalum* species in natural Brazilian pastures has determined a growing interest in their use in culture. In 1992, the Centro Nacional de Recursos Genéticos (CENARGEN/EMBRAPA) already had available a germplasm collection with more than 1500 accessions of different *Paspalum* species registered (Valls, 1992). In view of the operational need of distribution of available germplasm among various research units in the country, CENARGEN allocated part of its collection to the Centro de Pesquisa de Pecuária do Sudeste (CPPSE/EMBRAPA), located in São Carlos, SP. Accessions from the Plicatula and Virgata groups are currently being evaluated at this center in terms of agronomic and zootechnic aspects (Batista et al., 1994, 1995).

According to Killen (1990), the greatest current difficulty for the botanical recognition of *Paspalum* species in Brazil and in neighboring tropical countries is the complicated circumscription of species in the

Table 1. Species analyzed, accession codes, collection sites, chromosome numbers detected and previously reported chromosome numbers

Species	Accession code	Site	Chromosome no. detected (2n)	Chromosome no. reported per species (2n)	Country/Author
P. plicatulum	BRA-008869	Minas do Butiá-RS	40	20	USA (Brown, 1950)
	BRA-008877	Encruz. do Sul-RS	40		Mexico (Reeder, 1967)
	BRA-008893	São Gabriel-RS	40		Costa Rica (Davidse & Pohl, 1972)
	BRA-008907	São Gabriel-RS	40		Brazil (Moraes-Fernandes et al., 1974)
	BRA-008982	Lages-SC	40	40	Argentina (Saura, 1941)
	BRA-009032	Itaqui-RS	40		Uruguay (Nuñez, 1952)
	BRA-009211	Guaíba-RS	40		Honduras (Davidse & Pohl, 1972)
	BRA-010383	Corumbá-MS	40		Brazil (Pozzobon & Valls, 1987)
	BRA-012483	Pereira Barreto-SP	40	60	Australia (Pritchard & Gould, 1964)
	BRA-012912	Aral Moreira-MS	40		
	BRA-013048	Campo Largo-PR	40		
	BRA-013307	Quatro Barras-PR	40		
	BRA-013111	Balsa Nova-PR	40		
P. guenoarum	BRA-003824	Bela Vista-MS	40	40	Australia (Pritchard & Gould, 1964)
	BRA-006572	São Borja-RS	40		Australia (Pritchard, 1970)
	BRA-014851	João Pinheiro-MG	40		USA (Burson & Bennet, 1971)
					Brazil (Moraes-Fernandes et al., 1974)
					Brazil (Pozzobon &Valls, 1987)
					Brazil (Hickenbick et al., 1987)
P. compressifolium	BRA-008524	Vacaria-RS	40	20, 40, 60	Brazil (Vasconcellos et al., 1989)
	BRA-014907	Ponta Grossa-PR	40		
P. yaguaronense	BRA-011002	Santo Ângelo-RS	60	20	Australia (Pritchard, 1962)
					Brazil (Pinheiro et al., 1989)
	BRA-011401	Lagoa Vermelha-RS	40	40	Brazil (Moraes-Fernandes et al., 1974)
					Brazil (Valls & Pozzobon, 1987)
					Brazil (Pinheiro et al., 1989)
P. atratum	BRA-009610	Terenos-MS	40		

Plicatula group. The natural variation in morphological types observed among accessions has impaired their taxonomic interpretation. The characterization and agronomic evaluation studies of these germplasms currently underway at CPPSE are directed at the choice of accessions for intra- and interspecific crosses in order to increase genetic variability and to obtain new foraging varieties by selection. Considering that in this genus polyploidy is frequent and is correlated with apomixis, the determination of chromosome number in these accessions becomes imperative for the planning of crosses since, as mentioned by Pozzobon & Valls (1987), the genetic breeding of *Paspalum* has been limited to diploid sexual individuals that might serve as pollen recipients.

Material and methods

The cytogenetic studies were carried out on accessions from the *Paspalum* collection of CPPSE/EMBRAPA located in São Carlos, SP, which comprises approximately 215 accessions, especially of the Plicatula ad Virgata groups. This germplasm collection contains accessions obtained from collections made in different regions in the country, especially the South. Despite the large number of accessions existing, only those of the Plicatula group that presented potential for genetic improvement were evaluated.

Inflorescences in the ideal stage for meiotic study were collected and fixed in Carnoy (3:1 ethyl alcohol:acetic acid) for 24 hours and then transferred

Table 1. continued

Species	Accession code	Site	Chromosome no. detected (2n)	Chromosome no. reported per species (2n)	Country/Author
Paspalum sp.	BRA-003913	Miranda-MS	40		
	BRA-006700	Ciriaco-RS	40		
	BRA-008311	Corumbá-MS	40		
	BRA-008851	Arroio dos Ratos-RS	40		
	BRA-008923	São Gabriel-RS	40		
	BRA-008834	Porto Alegre-RS	40		
	BRA-009016	Santiago-RS	40		
	BRA-009059	Itaqui-RS	40		
	BRA-009075	Uruguaiana-RS	40		
	BRA-009083	São Sepé-RS	40		
	BRA-009113	Porto Alegre-RS	40		
	BRA-009130	Porto Lucena-RS	40		
	BRA-009202	Guaíba-RS	40		
	BRA-009628	Anastácio-MS	40		
	BRA-009644	Miranda-MS	40		
	BRA-009679	Aquidauana-MS	40		
	BRA-009741	Camaquan-RS	40		
	BRA-010464	Aquidauana-MS	40		
	BRA-010511	Coxim-MS	40		
	BRA-010537	Cáceres-MT	40		
	BRA-011134	Cruz Alta-RS	40		
	BRA-011479	Bom Jesus-RS	40		
	BRA-012424	Acreuna-GO	40		
	BRA-012742	Caarapó-MS	40		
	BRA-013293	Quatro Barras-PR	40		
	BRA-013391	Curitiba-PR	40		
	BRA-012718	Dourados-MS	40		
	BRA-014729	Mandirituba-PR	40		
	BRA-014885	Cach. de Goiás-GO	40		
	BRA-009652	Miranda-MS	40		
	BRA-015075	Quaraí-RS	40		

* Accessions never analyzed.

to 70% alcohol and stored under refrigeration until the time for use. Microsporocytes were prepared by squashing and stained with 1% propionic carmine. Chromosome number was determined in five plants per accession in diakinesis and the number of cells scored ranged from 50 to 150.

Results and discussion

In the first stage of the study, 52 accessions of germplasm belonging to the Plicatula group were analyzed. Twenty four were from the State of Rio Grande do Sul, one from Santa Catarina, seven from Paraná, one from São Paulo, 14 from Mato Grosso do Sul, two from Goiás, one from Minas Gerais, and one from Mato Grosso. Table 1 lists the collection sites for each accession and their coding at the germplasm collection.

In the material analyzed, 13 accessions were *P. plicatulum*, three *P. guenoarum*, two *P. compressifolium,* two *P. yaguaronense*, one *P. atratum*, and 31 still unidentified *Paspalum* sp. Table 1 shows the chromosome number detected for each accession and compares it with previous reports for the same species.

Except for the BRA-011002 accession of *P. yaguaronense* which presented 2n=60 chromosomes, all other accessions presented 2n=40 chromosomes. Previous determinations of chromosome number in different accessions from Brazil or from other countries for most of the species investigated here demonstrated some divergences with respect to the results obtained. Accessions with 2n=20 (Brown, 1950; Reeder, 1967; Davidse & Pohl, 1972; Moraes-Fernandes et al., 1974) and 2n=40 chromosomes (Saura, 1941; Nuñez, 1952; Davidse & Pohl, 1972; Pozzobon & Valls, 1987) have been reported for *P. plicatulum*. The chromosome number detected for the BRA-011002 accession of *P. yaguaronense* (2n=60) differs from previous reports for this species, where 2n=20 (Pritchard, 1962; Pinheiro et al., 1989) and 2n=40 (Moraes-Fernandes et al., 1974; Pozzobon & Valls, 1987; Pinheiro et al. 1989) have been reported. For *P. guenoarum* there was no discrepancy between the chromosome number detected here (2n=40) and that previously reported (Pritchard & Gould, 1964; Pritchard, 1970; Burson & Bennet, 1971; Moraes-Fernandes et al., 1974; Pozzobon & Valls, 1987; Hickenbick et al., 1987), whereas 2n=20, 40 and 60 chromosomes have been reported by Vasconcellos et al. (1989) for other Brazilian accessions of *P. compressifolium*. A single accession of *P. atratum* was analyzed, presenting 2n=40 chromosomes. No previous reports are available about this species.

A basic number of x=6 and ×=10 has been generally accepted for the genus *Paspalum* (Burson, 1975), with most species believed to have chromosome numbers that are multiple of 10. Only two species, *P. hexastachyum* and *P. almum*, are assumed to have 6 as the basic number. The origin of such different basic numbers is controversial (Pitman et al., 1987). Quarín & Hanna (1980) proposed ×=5 as the basic number for the genus *Paspalum*, from which ×=6 may have originated. Although this is an interesting proposition, the absence of 2n=10 cytotypes in nature and the chromosomal pairing of 2n=20 diploid cytotypes forming only bivalents exclude the possibility of a basic number of ×=5. Studies of the phylogeny of *Paspalum* species with different basic chromosome numbers carried out by Pitman et al., (1987) have suggested that the original basic number is 10, from which × = 6 originated.

In this genus, many of the species are polyploid, although there is a prevalence of tetraploid and hexaploid levels. The presence of hexadecaploids (2n=160) in the genus indicates a possible presence of hybridization (Pi & Chao, 1974). In the accessions analyzed in the present study, the chromosome numbers detected were 2n=40 and 2n=60 for a single accession of *P. yaguaronense*, respectively corresponding to a tetraploid (2n=4×=40) and hexaploid (2n=6×=60). The polyploid condition of these accessions was demonstrated by the multivalent associations, tetravalent in particular, frequently observed at diakinesis (Figure 1).

Studies of geographic distribution conducted on different *Paspalum* species in Argentina, Uruguay and Brazil (Cabrera & Willink, 1973; Quarín and Lombardo, 1986; Norrmann et al., 1989) have revealed a relationship between ploidy level and environmental conditions. Diploid cytotypes of *P. quadrifarium*, for example, are limited to a subtropical area in northeastern Argentina between latitudes 30° and 32° (Quarín & Lombardo, 1986), whereas triploids were distributed from humid temperate environments to dry and warm environments and tetraploids were detected only in southwestern Brazil and northeastern Uruguay (Norrmann et al., 1989). In the present study, however, this relationship was not observed. The area of collection of the accessions analyzed for the various species extended from the South Region to the Center-West Region of the country, whose climatic conditions are extremely contrastant. However, except for a *P. yaguaronense* accession that presented 2n=60, all others were found to be tetraploid. Both the tetraploid and hexaploid accessions of *P. yaguaronense* were from the State of Rio Grande do Sul. The coexistence of cytotypes with different ploidy levels in the same area has also been reported for other *Paspalum* species (Norrmann et al. 1989).

Information about the cytology and reproductive mode of a species is essential for breeding programs when interspecific hybridization is planned. In the specific case of the genus *Paspalum*, which has high ploidy levels, knowing the chromosome number is indispensable. A correlation between chromosome number and reproductive mode has been observed in this genus. In general, the diploid forms (2n=20) have sexual reproduction, are highly self-pollinating and cross with difficulty with other species (Burson & Quarín, 1992), whereas the reproduction of polyploids is apomictic, with apomixis being obligate or facultative (Quarín, 1986). Considering that the accessions analyzed here were tetraploids or hexaploids, thus probably being apomictic, the breeding program currently underway at CPPSE/EMBRAPA, which intends to perform not only interspecific crosses but also crosses between different accessions of the same species, will not be very successful with this methodology since it will be lim-

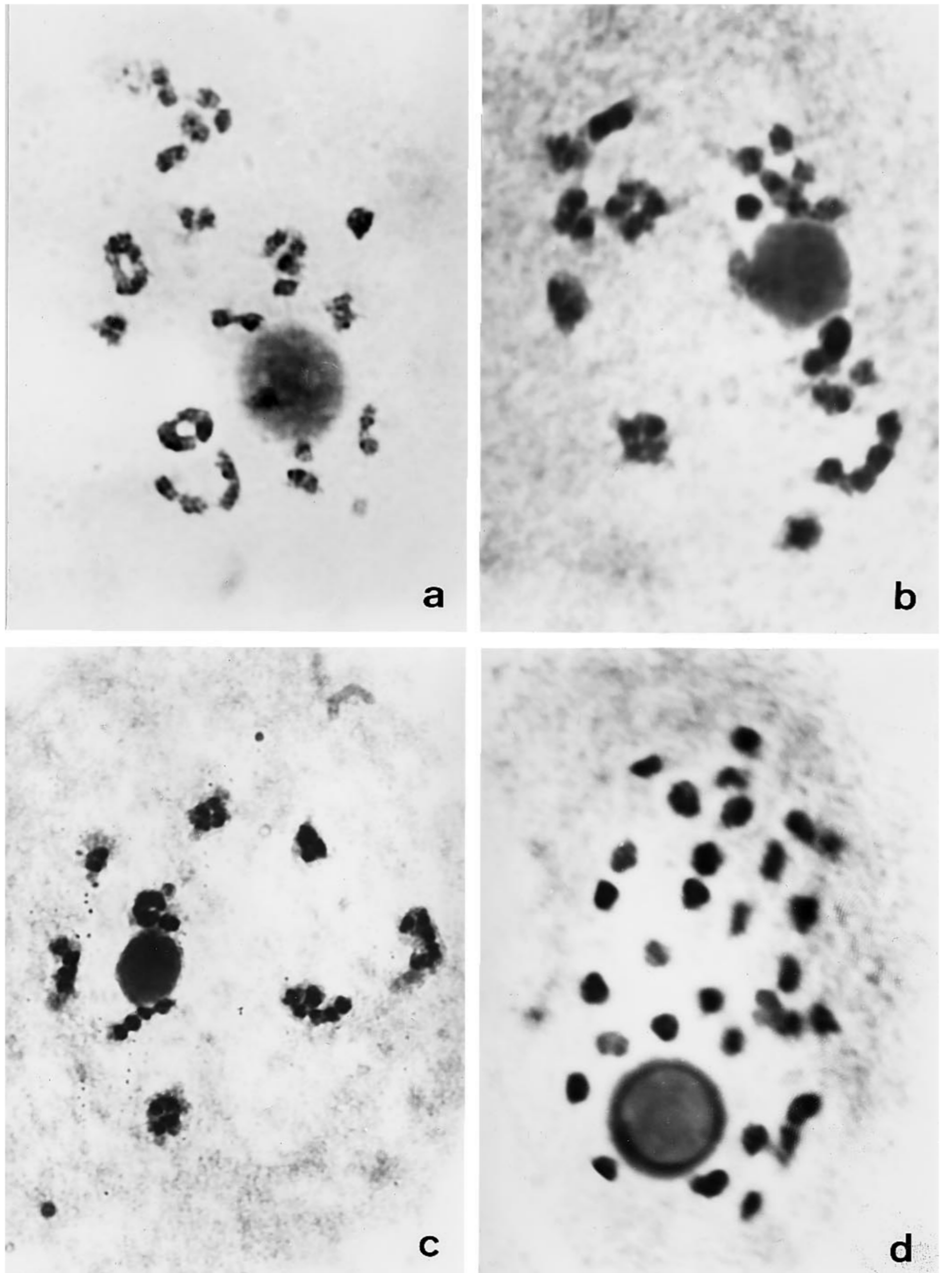

Figure 1. Some aspects of the chromosomal behavior at diakinesis related to the form of pairing in the accessions analyzed. a) Diakinesis with 16 bivalents and two tetravalents. b and c) Diakinesis showing many multivalent associations. d) Diakinesis with 30 bivalents in accession BRA-011002 of *P. yaguaronense* with 2n=6×=60.

ited to the use of these accessions as pollen donors for other sexual individuals. Considering also that hybrid production in *Paspalum* results in less than 1% viable crosses in some cases (Burson & Quarín, 1992), the genetic breeding program for these accessions, whose potential for pasture formation is high, will have to be based on other methodologies.

References

Batista, L.A.R., R.E. Godoy & J.M. Pereira, 1994. Avaliação do potencial forrageiro de espécies do gênero *Paspalum*. Ensaio 1992/93. In: XXXI Reunião Anual da Sociedade Brasileira de Zootecnia, Anais, Maringá-PR pp 641.

Batista, L.A.R., R.E. Godoy & J.M. Pereira, 1995. Potencial forrageiro de acessos de germoplasma de *Paspalum*. Ensaio 1993/94. In: XXXII Reunião Anual da Sociedade Brasileira de Zootecnia, Anais, Brasília-DF, pp 231–233.

Brown, W.V., 1950. Cytological study of some Texas gramineae. Bull Torrey Bot Club 77: 63-76.

Burson, B.L., 1975 Cytology of some apomitic *Paspalum* species. Crop Sci 15: 229–232.

Burson, B.L. & H.W. Bennett, 1971. Chromosome numbers, microsporogenesis and mode of reproduction of seven *Paspalum* species. Crop Sci 11: 292–294.

Burson, B.L. & C.L. Quarín, 1992. Cytological relationship between *Paspalum dilatatum* and diploid cytotypes of *P. brunneum* and *P. rufum*. Genome 35: 332–336.

Cabrera, A.L. & A. Willink, 1973. Biogeografia de América Latina. OEA. Monografias Científicas, Série Biología 13, Washington, DC pp 75.

Davidse, G. & R.W. Pohl, 1972. Chromosome numbers and notes on some Central American Grasses. Can J Bot 50: 273–283.

Hickenbick, M.C.M., M. Dall'Agnol & G.E. Gomes, 1987. Estudos citogenéticos em espécies do gênero *Paspalum* (Gramineae). In: Encontro Internacional sobre Melhoramento Genético de *Paspalum*. Anais, Nova Odessa, Instituto de Zootecnia, pp 57–63.

Killen, T.J., 1990. The grasses of Chiquitanía. Santa Cruz, Bolívia. Ann Missouri Bot Garden 77: 125–201.

Moraes-Fernandes, M.I.B., I.L. Barreto, F.M.G. Salzano & M.C. Freitas-Sacchet, 1974. Cytological and evolutionary relationships in Brazilian forms of *Paspalum* (Gramineae). Caryologia 27: 455–465.

Norrmann, G.A., C.L. Quarín & B.L. Burson, 1989. Cytogenetics and reproductive behavior of different chromosome races in six *Paspalum* species. J Hered 80: 24–28.

Nuñez, O., 1952. Las gramineas argentinas de la tribus 'Paniceae'. Rev Fac Agron 28: 229–255.

Pi, P. & C. Chao, 1974. Microsporogenesis in *Paspalum longifolium* and *P. commersonii* on two different polyploid levels. Cytologia 39: 453–465.

Pinheiro Jr, A., M.T. Pozzobon & J.F.M. Valls, 1989. Caracterização citogenética de acessos de germoplasma de *Paspalum yaguaronense* (grupo Plicatula). In: 26ª Reunião Anual da Sociedade Brasileira de Zootecnia, Anais, Porto Alegre, pp 2.

Pitman, M.W., B.L. Burson & E.C. Bashaw, 1987. Phylogenetic relationship among *Paspalum* species with different base chromosome numbers. Bot Gaz 148: 130–135.

Pozzobon, M.T. & J.F.M. Valls, 1987. Caracterização citogenética em acessos de germoplasma de espécies brasileiras de *Paspalum* (Gramineae). In: Encontro Internacional sobre Melhoramento de *Paspalum*, Anais, Nova Odessa, Instituto de Zootecnia, pp 73–78.

Pritchard, A.J., 1962. The cytology and reproduction of *Paspalum yaguaronense* (Henr) Aust J Agric Res 13: 206–211.

Pritchard, A.J., 1970. Meiosis and embryo sac development in *Urochloa mosambicensis* and three *Paspalum* species. Aust J Agric Res 21: 649–652.

Pritchard, A.J. & K.F. Gould, 1964. Chromosome numbers in some introduced and indigenous legumes and grasses, CSIRO (Australia) Div Trop Pastures Tech Paper No 2 pp 18.

Quarín, C.L., 1986. Seasonal changes in the incidence of apomixis of diploid, triploid and tetraploid plants of *Paspalum cromyorrhizon*. Euphytica 35: 515–522.

Quarin, C. & W.W. Hanna, 1980. Chromosome behavior, embryo sac development, and fertility of *Paspalum modestum, P. bosianum*, and *P. conspersum*. J Hered 71: 419–422.

Quarin, C.L. & E.P. Lombardo, 1986. Niveles de ploidia y distribución geográfica de *Paspalum quadrifarium* (Gramineae) Mendeliana 7: 101–107.

Reeder, J.R., 1967. Notes on Mexican grasses VI. Miscellaneous chromosome numbers. Bull Torrey Bot Club 94: 1–17.

Saura, R., 1941. Cariologia de algumas espécies del género *Paspalum*. Rev Fac Agron Veterin 2: 41–48.

Valls, J.F.M., 1987. Recursos genéticos de espécies de *Paspalum* no Brasil. In: Encontro Internacional sobre Melhoramento de *Paspalum*, Anais, Nova Odessa, Instituto de Zootecnia, pp 3–13.

Valls, J.F.M., 1992. Origem do germoplasma de *Paspalum* disponível no Brasil para a área tropical. In: Red Internacional de Evaluación de Pastos Tropicales/RIEPT, 1ª Reunión Sabanas, Brasília, EMBRAPA/CPAC/CIAT, pp 68–80.

Vasconcellos, A.M., M.T. Pozzobon & J.F.M. Valls, 1989. Caracterização citogenética de acessos de germoplasma de *Paspalum compressifolium* (grupo Plicatula). In: 26ª Reunião Anual da Sociedade Brasileira de Zootecnia, Anais, Porto Alegre, p 1.