

Cytogenetic analyses in *Paspalum* L. reveal new diploid species and accessions

Análises citogenéticas em *Paspalum* L. revelam novas espécies e acessos diplóides

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ABSTRACT

Chromosome numbers were counted in 126 new accessions of 50 *Paspalum* species from Brazil, Argentina, Paraguay and Bolivia. The chromosome numbers 2n=12, 20, 24, 30, 40, 50, 60, 80 were confirmed. Chromosome numbers for *P. arenarium* (2n=20), *P. barretoi* (2n=20), *P. aff. cereisia* (2n=40), *P. corcovadense* (2n=20), *P. crispulum* (2n=20), *P. flaccidum* (2n=40), *P. nummularium* (2n=20), *P. scalare* (2n=20), *P. vescum* (2n=20) and *P. rectum* (2n=20) and a diploid cytotype of *P. malacophyllum* are reported for the first time. The predominance of tetraploid accessions (43.6%) was confirmed, but an unusually high number of diploid species (44%) and accessions (35.7%) was found. These results open new perspectives for breeding programs, phylogenetic studies, and for research on apomixis control, since diploids of *Paspalum* are typically sexual.

Key words: chromosome, diploid, polyploid, ploidy level, hybrid, genetic resources.

RESUMO

O número cromossômico foi determinado para 126 novos acessos de 50 espécies de *Paspalum* do Brasil, Argentina, Paraguai e Bolívia. Foram verificados os números somáticos 2n=12, 20, 24, 30, 40, 50, 60 e 80. Estas são as primeiras contagens para *P. arenarium* (2n=20), *P. barretoi* (2n=20), *P. aff. cereisia* (2n=40), *P. corcovadense* (2n=20), *P. crispulum* (2n=20), *P. flaccidum* (2n=40), *P. nummularium* (2n=20), *P. scalare* (2n=20), *P. vescum* (2n=20) e *P. rectum* (2n=20). O nível diplóide (2n=20) é reportado pela primeira vez para *P. malacophyllum*. Os dados confirmam a predominância de acessos tetraplóides (43,6%) no gênero e mostram um número incomumente elevado de espécies (44%) e acessos diplóides (35,7%). Estes resultados trazem novas

perspectivas para programas de melhoramento, para estudos filogenéticos e para pesquisa orientada ao controle da apomixia, já que em *Paspalum* as plantas diplóides são tipicamente sexuais.

Palavras-chave: cromossomo, diplóide, poliplóide, híbrido, nível de ploidia, recursos genéticos.

INTRODUCTION

The genus *Paspalum* L., with about 350 species (ZULOAGA et al., 2003; DENHAM, 2005), is an important component of South American biodiversity. Its species inhabit areas ecologically diverse throughout North-, Meso-, and South America, with centers of highest diversity in the Brazilian cerrados and the 'campos' of Argentina, Uruguay and Southern Brazil (CHASE, 1929; BARRETO, 1974; NICORA & RÚGOLO DE AGRASAR, 1987; JUDZIEWICZ, 1990), where several *Paspalum* species are dominant (VALLS, 1994). Despite its importance and the volume of published data on many different aspects, genetic improvement is only incipient in the genus. Few species have been included into selection programs, and are currently cultivated as forage plants, mostly restricted to the informal taxonomic groups described by CHASE (1929) as Dilatata, Notata and Plicatula. They are *P. notatum*, *P. dilatatum*, *P. plicatulum* and *P. guenoarum* (VALLS, 1992), and most

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recently, *P. atratum* Swallen (KRETSCHMER et al., 1994). Also *P. vaginatum* was improved to some extent, and is currently cultivated as turfgrass (DUNCAN & CARROW, 2000).

Polyploidy is frequent in the genus, with predominance of the tetraploid level (QUARÍN, 1992). Cytological studies show that many *Paspalum* 'species' consist of both sexual, self-incompatible diploid cytotypes, and apomictic, pseudogamous, and self-fertile polyploids (QUARÍN & NORRMANN, 1990; QUARÍN, 1992; VALLS, 2000). Thus, they can originate agamic complexes, in which the very concept of 'species' becomes doubtful. According to SAVIDAN (1987), the units breeders have to deal with, in the case of agamic complexes, override the notion of botanical species and include more than one species or even several genera. By fixing interspecific forms that would have been eliminated in a sexual regimen, apomixis exceeds the boundaries of species and creates a continuous variability, making the botanical work difficult. On the other hand, breeders depend on a core collection, which must represent the natural variability of the species to be improved. The occurrence of apomixis and a wide range of ploidy levels make the knowledge on cytology and reproductive behavior of all available accessions an imperative issue for *Paspalum* improvement (NORRMANN et al., 1989). Routinely, crossing programs require knowing at least chromosome numbers and mode of reproduction of the potential parents.

In this paper, we report chromosome numbers for a range of new accessions of *Paspalum*, collected in West-central and Southern Brazil, Northeastern Argentina, Paraguay and Bolivia. This contribution significantly enlarges the known variability of South American *Paspalum* species, and contributes to future breeding programs through new available sexual-diploid accessions.

MATERIAL AND METHODS

All the accessions analysed are part of the germplasm collections of *Paspalum* maintained at EMBRAPA Genetic Resources and Biotechnology (EMBRAPA/CENARGEN), Brasília, DF, Brazil, and/or at 'Lucien Hauman' Botanical Garden, University of Buenos Aires, Argentina, and have voucher specimens deposited at their respective institutional herbaria (CEN and BAA). The collections were made in locations of the West-central and Southern regions of Brazil, Northeastern Argentina, Paraguay and Bolivia (Tables 1 to 4).

Somatic chromosome numbers were determined in root-tip cells, following the protocols of

POZZOBON & VALLS (1997), with minor modifications. Meiotic chromosomes were analysed in pollen mother cells from anthers fixed in ethanol-acetic acid 3:1 and squashed in 1% propionic carmine. At least five cells with good chromosome spreading and no overlapping were analysed per plant. Semi-permanent slides were examined using a light microscope and recorded by photomicrography.

RESULTS

The chromosome numbers for 126 new germplasm accessions of *Paspalum*, including 50 species, one of them with two varieties, are listed in tables 1 to 4. For a better understanding and further discussion of these results, the number of chromosomes is represented as $2n$ for all plants, even when obtained from meiotic pollen-mother-cells. The species are referred to the informal groups, as proposed by CHASE (1929) and revised by other authors (PARODI & NICORA, 1966; BARRETO, 1974; ZULOAGA & MORRONE, 2005). Nevertheless, such grouping has to be reviewed, since preliminary molecular studies (SPERANZA & RUA, unpubl.) suggest they are not consistent with the phylogeny of the genus. This is pointed out in the discussion, as relevant.

With the exception of *Paspalum alnum* ($2n=12, 24$), all the species studied here have chromosome numbers which are multiples of 10, varying from diploids to octoploids. The results show a surprisingly high frequency of diploid species (44%), as well as diploid accessions (35.7%), when compared to the predominant tetraploids (43.6 %) and the remaining ploidy levels (20.7% altogether).

Chromosome numbers are reported for the first time for the following species: *Paspalum arenarium*, *P. barretoi*, *P. corcovadense*, *P. crispulum*, *P. nummularium*, *P. scalare*, *P. vescum* and *P. rectum* 3, with $2n=20$ (Table 1, 3 and 4), and *P. aff. cerealia* and *P. flaccidum* with $2n=40$. Moreover, two accessions of *P. malacophyllum* (subg. Anachyris) showed $2n=20$, and are the first diploid accessions reported for this species. Pentaploid ($2n=50$) accessions of *P. mandiocanum* var. subaequiglume are also reported for the first time. All remaining chromosome counts reported here (Table 1, 3 and 4) confirm previous data (see Discussion below). Special mention is made to *P. conduplicatum*, of the Notata group, of which only two previous counts of $2n=60$ were mentioned in the literature (RODRIGUES et al., 2001; SOUZA-CHIES et al., 2006), and is here confirmed as an hexaploid, with 14 novel counts sampling its whole area of occurrence

Table 1 - *Paspalum* species of subgenus *Paspalum*, informal group Notata and allies (in bold), and accessions analysed (identified by collector's abbreviation and number), with respective origin, chromosome number and informal grouping.

Species / Collector's number (Place of collection)	2n	Informal group
<i>P. alnum</i> Chase		Notata, Alma
Gh 582 (ARG, Corrientes, Mercedes)	12	
V 14440 (RS, São Gabriel)	24	
<i>P. barretoi</i> Canto-Dorow, Valls & Longhi-Wagner		Notata
Gh 731, V 14395*, V 14397, V 14846 (SC, São Joaquim); Gh 736, V 14853 (SC, Bom Jardim da Serra)	20*	
<i>P. conduplicatum</i> Canto-Dorow, Valls & Longhi-Wagner		Notata
V 8097 (SC, Bom Retiro); V 8180, V 14854 (SC, São Joaquim); V 10553 (SC, Irani); V 11094 (PR, Balsa Nova); V 11143 (PR, Quatro Barras); V 11250 (PR, Castro); V 11374 (PR, Guarapuava); V 11386 (SC, São Lourenço d'Oeste); V 11453 (PR, Palmas); V 14271*** (RS, São Gabriel); V 14816, V 14817 (PR, Mangueirinha); V 14838 (PR, Prudentópolis); V 14859 (RS, Ipê)	60	
<i>P. crispulum</i> Swallen		Linearia, Notata
Gh 631* (GO, Niquelândia)	20*	
<i>P. equitans</i> Mez		Fasciculata, Notata
V 14377 (RS, Caseiros)	20	
<i>P. ionanthum</i> Chase		Notata
V 14274 (RS, Cachoeira do Sul); V 14288*** (RS, Capão da Canoa); V 14791 (RS, São Pedro do Sul); V 14894 (RS, Sertão de Santana)	40	
V 14343 (RS, Triunfo)	80	
<i>P. lineare</i> Trin.		Linearia, Notata
Gh 665 (DF, APA da Cafuringa)	40	
<i>P. maculosum</i> Trin.		Notata, Maculosa
Gh 487 (ARG, Corrientes, Santo Tomé); V 14345*** (RS, Triunfo)	20	
V 14360 (RS, São José do Herval); V 14375 (RS, Caseiros); V 14790 (RS, São Pedro do Sul); V 14848 (SC, Bom Jardim da Serra)	40	
<i>P. minus</i> E. Fourn.		Notata
V 14573*** (MS, Ponta Porã)	50	
<i>P. nummularium</i> Chase ex Send. & A.G. Burman		Notata
Gh 737, V 14850* (SC, Bom Jardim da Serra)	20*	
<i>P. pumilum</i> Nees		Notata
V 14272 (RS, São Gabriel); V 14280*** (RS, Xangri-lá); V 14325 (RS, Capivari do Sul); V 14792 (RS, São Pedro do Sul); V 14847 (SC, São Joaquim); V 14889 (SC, Florianópolis)	20	

Collectors: V: J.F.M. Valls and collaborators, Gh: G.H. Rua.

Brazilian States: DF Distrito Federal; GO: Goiás; MS: Mato Grosso do Sul; PR: Paraná; RS: Rio Grande do Sul; SC: Santa Catarina.

Other countries: ARG: Argentina.

*First chromosome count for the species; *** Chromosome numbers previously mentioned in SOUZA-CHIES et al. (2006) as "unpublished [data] informed by J. F. M. Valls".

(Table 1). In fact, this species had its regular chromosome pairing (30 bivalents) described long ago (FERNANDES, 1971; FERNANDES et al., 1974), under the tentative name ***P. aff. minus*** (CANTO-DOROW et al., 1995).

DISCUSSION

Species counted for the first time

All accessions of ***P. barretoi*** and ***P. nummularium*** proved to be diploid. These species are placed in the Notata group, and are closely related to ***P. pumilum***, another species for which only sexual, autogamous diploids are known (BURSON &

BENNETT, 1971). Although a great variation in ploidy levels occurs within the Notata group, which ranges from 2n=20 to 80, this it seems, is not the case for the three species mentioned above. It would be interesting to study if they may be included in a monophyletic group. On the other hand, ***P. barretoi*** has been considered elsewhere (ZULOAGA et al., 2003, 2004) as a synonym of ***P. minus***, on the basis of morphological similarity. Nevertheless, with the only exception of a count of 2n=40 for a Columbian accession (DAVIDSE & POHL, 1978), all reported counts for ***P. minus*** in South- and Mesoamerican materials, including that presented here, were 2n=50 (BONILLA & QUARÍN, 1997). This fact, together with some morphological,

Table 2 - *Paspalum* species of subgenus *Paspalum*, informal groups Plicatula and allies, and Virgata (in bold), and accessions analysed (identified by collector's abbreviation and number), with respective origin, chromosome number and informal grouping.

Species / Collector's number (Place of collection)	2n	Informal group
<i>P. compressifolium</i> Swallen		Plicatula
Gh 734 (SC, São Joaquim); V14359 (RS, São José do Herval)	40	
<i>P. glaucescens</i> Hack.		Plicatula
V 14207 (RS, Passo Fundo); V 14287 (RS, Capão da Canoa)	20	
V 14419 (RS, Correia Pinto, RS)	40	
<i>P. guenoarum</i> Arechav.		Plicatula
Gh 720 (MG, Presidente Kubitschek)	40	
<i>P. lepton</i> Schult.		Plicatula
V 14281 (RS, Xangri-lá); V 14305 (RS, Rosário do Sul); V 14322 (RS, Capivari do Sul); V 14344 (RS, Triunfo); V 14436 (RS, São Gabriel)	40	
<i>P. limbatum</i> Henrard		Plicatula
Gh 45 (PRY, Amambay)	40	
Gh 372 (BOL, Santa Cruz, Ñuflo de Chávez)	20	
<i>P. modestum</i> Mez		Modesta, Plicatula
Gh 146 (ARG, Corrientes, Gral. San Martín); V 14269 (RS, São Gabriel); V 14320 (RS, Capivari do Sul)	20	
<i>P. plicatulum</i> Michx.		Plicatula
V 14196 (RS, Vila Flores); V 14203, V 14204, V 14206, V 14373 (RS, Passo Fundo); V 14229 (RS, São Luiz Gonzaga); V 14315 (RS, Barra do Quarai); V 14341 (RS, Montenegro); V 14346 (RS, Triunfo); V 14351 (RS, São José do Herval)	40	
<i>P. rhodopedum</i> L.B. Sm. & Wassh.		Plicatula
Gh 729 (RS, Campestre da Serra); V 14355, V 14356, V 14358 (RS, São José do Herval)	40	
<i>P. aff. rojasii</i> Hack.		Plicatula
V 14386 (RS, Capão Alto)	20	
<i>Paspalum</i> sp. indet. 1		Plicatula
V 14197 – (RS, Passo Fundo)	40	
<i>Paspalum</i> sp. indet. 2		Plicatula
Gh 818 (DF, Parque Nacional de Brasília)	40	
<i>P. commune</i> Lillo		Virgata
Gh 325 (BOL, Santa Cruz, Valle Grande)	40	
<i>P. conspersum</i> Schrad.		Virgata
Gh 572 (PRY, Cordillera)	60	
<i>P. regnellii</i> Mez		Virgata
V 14410 (SC, Lages); V 14348 (RS, Bom Retiro do Sul)	40	
<i>P. rufum</i> Nees ex Steud.		Virgata
V 14262 (RS, Uruguiana)	40	

Collectors: V: J.F.M. Valls and collaborators; Gh: G.H. Rua

Brazilian States: DF: Distrito Federal; MG: Minas Gerais; RS: Rio Grande do Sul; SC: Santa Catarina.

Other countries: ARG: Argentina; BOL: Bolívia; PRY: Paraguay

ecological, and biogeographical evidences, suggests that the specific status of *P. barretoi* has to be reassessed.

The limits of the Notata group were recently expanded, through the fusion with the former Linearia group (ZULOAGA et al., 2004). A recent molecular study (SOUZA-CHIES et al., 2006) was coherent with this approach, since the Linearia group was shown to be paraphyletic. An accession of *P. crispulum* with 2n=20, a member of the former Linearia group, represents the first cytological report for the species. It is a quite rare

species restricted to areas of serpentine soils (PRASAD, 2005) near Niquelândia, Goiás State, in the Brazilian central plateau (OLIVEIRA & VALLS, 2002; ZULOAGA et al., 2004).

Chromosome counts for the three species of the Recta group (OLIVEIRA & VALLS, 2002) are provided for the first time (*P. flaccidum*, 2n=40; *P. rectum*, 2n=20; *P. vescum*, 2n=20). This is a group of doubtful relationships within the genus, possibly related to the Cuban *P. rocanum* Léon (CHASE, 1929). ZULOAGA & MORRONE (2005) consider *P. vescum* a

Table 3 - *Paspalum* species of subgenus ***Paspalum***, of assorted informal groups (in bold), and accessions analysed (identified by collector's abbreviation and number), with respective origin, chromosome number and informal grouping.

Species / Collector's number (Place of collection)	2n	Informal group
<i>P. acuminatum</i> Raddi V 14248, V 14256 (RS, Uruguaiana)	40	Dissecta
<i>P. arenarium</i> Schrad. V 14279* (RS, Xangri-lá)	20*	Caespitosa, Setacea
<i>P. conjugatum</i> P. J. Bergius V 14284 (RS, Xangri-lá)	40	Conjugata
<i>P. dilatatum</i> Poir. "Comum" V 14312 (RS, Barra do Quarai)	50	Dilatata
<i>P. distichum</i> L. V 14350 (RS, São José do Herval)	40	Disticha
<i>P. falcatum</i> Nees ex Steud. V 14362 (RS, Passo Fundo); V 14376 (RS, Caseiros)	20	Falcata, Lachnea
<i>P. flaccidum</i> Ness Gh 753* (PR, Balsa Nova)	40*	Recta
<i>P. indecorum</i> Mez Gh 490 (ARG, Corrientes, Santo Tomé); V 14226 (RS, São Luiz Gonzaga); V 14236 (RS, Santo Antônio das Missões)	20	Caespitosa
<i>P. juergensii</i> Hack. V 14209 (RS, Passo Fundo); V 14212 (RS, Carazinho); V 14223 (RS, São Luiz Gonzaga); V 14425 (SC, Capão Alto)	20	Paniculata
<i>P. lividum</i> Trin. ex Schldl. Gh 466 (ARG, Corrientes, Paso de los Libres); V 14254 (RS, Uruguaiana)	40	Livida, Denticulata
<i>P. maritimum</i> Trin. Gh 712 (ES, Guarapari); Gh 715 (ES, Conceição da Barra)	40	Caespitosa, Paniculata
<i>P. pauciciliatum</i> (Parodi) Herter V 14319 (RS, Capivari do Sul)	40	Dilatata, Livida
<i>P. paucifolium</i> Swallen Gh 313 (ARG, Corrientes, Mercedes)	40	Eriantha
<i>P. rectum</i> Nees Gh 832* (GO, Teresina de Goiás)	20*	Recta
<i>P. scalare</i> Trin. Gh 622* (GO, Alto Paraíso)	20*	Parviflora
<i>P. vaginatum</i> Sw. V 14340 (RS, Arroio do Sal)	20	Disticha
<i>P. vescum</i> Swallen Gh 652* (DF, APA da Cafuringa)	20*	Recta

Collectors: V: J.F.M. Valls and collaborators; Gh: G.H. Rue

Brazilian States: DF: Distrito Federal; ES: Espírito Santo; GO: Goiás; PR: Paraná; RS: Rio Grande do Sul; SC: Santa Catarina.

Other countries: ARG: Argentina.

*First chromosome count for the species.

synonym of ***P. flaccidum***. We believe the materials we analysed represent two distinctive taxa, although we recognize that parallel occurrence of the diploid and tetraploid levels is common in *Paspalum* species.

The chromosome number of Brazilian ***P. aff. ceresia***, $2n=40$ (subgenus ***Ceresia*** (Pers.) Rchb.) is reported for the first time. Available counts for Andean ***P. ceresia*** are $2n=40$ (MORRONE et al., 2006) and $2n=60$ (HOJSGAARD et al., 2005). Thus, chromosome

numbers do not correlate with morphological evidences supporting the segregation of the Brazilian material as a different species (RUA et al., unpubl.).

Paspalum corcovadense, $2n=20$ (Corcovadensis group) and ***P. scalare***, $2n=20$ (Parviflora group) were also counted for the first time, as well as ***P. arenarium*** ($2n=20$), a species probably related to the North American ***P. setaceum*** Michx. (BANKS, 1964, 1966; ZULOAGA & MORRONE, 2005), an auto-

Table 4 - *Paspalum* species of subgenus *Paspalum*, informal groups Corcovadensis and Quadrifaria, and of subgenera *Ceresia*, *Anachyris*, and *Harpastachys* (in bold), and accessions analysed (identified by collector's abbreviation and number), with respective origin, chromosome number and informal grouping.

Species	2n	Informal group/ subgenus
Collector's number (Place of collection)		
<i>P. corcovadense</i> Raddi V 14283* (RS, Xangri-lá); V 14291 (RS, Dom Pedro de Alcântara)	20*	Corcovadensis
<i>P. inaequivalve</i> Raddi V 14261 (RS, Uruguaiana)	60	Corcovadensis, Inaequivivalvia
<i>P. mandiocanum</i> Trin. var. <i>mandiocanum</i> V 14292 (RS, Dom Pedro de Alcântara)	50	Corcovadensis
<i>P. mandiocanum</i> var. <i>subaequiglume</i> I.L. Barreto Gh 301 (PRY, Amambay); V 14800** (RS, Julio de Castilhos) Gh 746 (PR Balsa Nova); V 14222 (RS, São Luiz Gonzaga); V 14427 (RS, Capão Alto)	50 60	Corcovadensis
<i>P. quarinii</i> Morrone & Zuloaga V 14208 (RS, Passo Fundo) V 14220*** (RS, São Miguel das Missões)	40 20	Quadrifaria
<i>P. aff. cereia</i> (Kuntze) Chase Gh 632* (GO, Niquelândia); Gh 642(GO, Alto Paraíso)	40*	subg. <i>Ceresia</i>
<i>P. malacophyllum</i> Trin. V 14411**, V 14855 (RS, Correia Pinto)	20	subg. <i>Anachyris</i>
<i>P. unispicatum</i> (Scribn. & Merr.) Nash Gh 506 (ARG, Corrientes, Capital)	40	subg. <i>Harpastachys</i>

Collectors: V: J.F.M. Valls and collaborators; Gh: G.H. Rua

Brazilian States: GO: Goiás; PR: Paraná; RS: Rio Grande do Sul; SC: Santa Catarina.

Other countries: ARG: Argentina; BOL: Bolívia; PRY: Paraguay.

*First chromosome count for the species; ** First report of the ploidy level for the taxon; *** Chromosome numbers previously mentioned in SOUZA-CHIES et al. (2006) as "unpublished [data] informed by J. F. M. Valls".

compatible sexual diploid (BANKS, 1966; SPERANZA, unpubl.). Altogether, our counts reveal an unusual number of diploid species and accessions for the genus.

Species with new chromosome numbers

Two accessions of *P. malacophyllum* showed 2n=20, representing the first diploids of this species reported in the literature. This is a highly variable species belonging to the subg. *Anachyris* (MORRONE et al., 2000). Chromosome numbers of 2n=40 and 2n=60 were known for the species, the tetraploids being largely the most common (BURTON, 1940; GOULD, 1975; BURSON & HUSSEY, 1998; POZZOBON et al., 2000; PAGLIARINI et al., 2001).

Paspalum mandiocanum comprises two varieties: *P. mandiocanum* var. *mandiocanum* and *P. mandiocanum* var. *subaequiglume*. Previously reported chromosome counts were 2n=50 for var. *mandiocanum* and 2n=60 for var. *subaequiglume* (HONFI et al., 1990), both confirmed in this paper. Furthermore, two accessions with 2n=50 are here reported, which morphologically belong to var. *subaequiglume*. This finding establishes the first pentaploid record for this variety, and suggests that

the segregation of var. *subaequiglume* as a distinct species, as proposed by HONFI et al. (1990), requires further evidence, since correlation between morphology and chromosome number is not as direct as formerly thought.

CONCLUSIONS

The paper presents several new chromosome counts in South American species of *Paspalum*, revealing an unusually high number of diploid species and accessions. The identification of new diploid accessions renders new materials for genetic improvement, and increases the knowledge on the diversity of the genus. The finding of new diploid species is important for phylogenetic studies using molecular markers, as well as through crossings, to establish relationships between these taxa and to give further support to taxonomic treatments.

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