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REGULAR PAPER

Relative DNA content in diploid, polyploid, and multiploid species of *Paspalum* (Poaceae) with relation to reproductive mode and taxonomy

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Abstract It is generally accepted that polyploids have downsized basic genomes rather than additive values with respect to their related diploids. Changes in genome size have been reported in correlation with several biological characteristics. About 75 % of around 350 species recognized for *Paspalum* (Poaceae) are polyploid and most polyploids are apomictic. Multiploid species are common with most of them bearing sexual diploid and apomictic tetraploid or other ploidy levels. DNA content in the embryo and the endosperm was measured by flow cytometry in a seed-by-seed analysis of 47 species including 77 different entities. The relative DNA content of the embryo informed the genome size of the accession while the embryo:endosperm ratio of DNA content revealed its reproductive mode. The genome sizes (2C-value) varied from 0.5 to 6.5 pg and for 29 species were measured for the first time. Flow cytometry provided new information on the reproductive mode for 12 species and one botanical variety and supplied new data for 10 species concerning cytotypes reported for the first time. There was no significant difference between the mean basic genome sizes (1Cx-values) of 32 sexual and 45 apomictic entities. Seventeen entities were diploid and 60 were polyploids with different degrees. There were no clear patterns of changes in 1Cx-values due to polyploidy or reproductive systems, and the existing variations are in concordance with subgeneric taxonomical grouping.

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Introduction

The use of flow cytometry to estimate the DNA (deoxyribonucleic acid) content in plant nuclei has facilitated analysis in relation to fundamental aspects of plant biology. Genome size is an expression to specify the DNA content of the whole chromosome complement characteristic of any given individual in terms of 1C-value (Swift 1950). In order to clarify the term, Greilhuber et al. (2005) suggested that when the genome size is based on the whole unreplicated reduced chromosome complement, the abbreviated term is 1C-value, equal to half of the unreplicated non-reduced (zygotic diplophasic) complement, which has a 2C-value. The 1Cx-value is applied when genome size is referred to the monoploid chromosome set and represents the basic genome size. Correlations between genome size and specific biological traits have been documented repeatedly in higher plants. For example, annuals usually have lower genome size than related perennials, especially when annual life history is associated with selfing, and higher genome size is positively associated with high elevation habitat in the genus *Veronica* (Albach and Greilhuber, 2004). Significant differences have been found in 1Cx-values between diploid and triploid genotypes and between triploids and tetraploids, indicating downsizing of genomes in polyploids of *Hieracium*, a genus in which the genome size is strongly correlated with major phylogenetic groups but is not correlated with altitude, latitude and other ecological characters (Chrtek et al. 2009). Considering the tendency toward an increase in genome size during the evolution of angiosperm, Bennetzen and Kellog (1997) considered that the angiosperm genomes have

a "one-way ticket to obesity". However, Enke et al. (2011) demonstrated that there is a shrinking trend toward genome contraction within 30 diploid species of *Crepis*. Correlation of DNA content, phylogenetic characteristics and reproductive mode has been documented in the genus *Hypericum* (Matzk et al. 2003). The apomictic species of the modern section Hypericum increases the DNA content solely by polyploidy, while the apomicts of the earlier evolving section Ascyreia have significantly larger genomes than all other species due to polyploidization and also by higher DNA content per chromosome.

With approximately 330–350 species, *Paspalum* is one of largest genus of the Panicoid grass subfamily (Rua et al. 2010; Zuloaga and Morrone 2005). The great majority of its species are native of the Americas, distributed throughout the tropics, subtropics and temperate regions from northern Unites States to southern Chile and southwestern Argentina, at approximately 38° south latitude. A few species are native to Africa, Asia, or Oceania, and only three or four are cosmopolitan but probably of American origin (Rua et al. 2010). Several species of *Paspalum* are the main constituents of natural extensive pasturelands in South America, especially in Brazil, Paraguay, Argentina and Uruguay. Some species have been brought into cultivation due to their recognized forage quality. Because a great proportion of polyploid *Paspalum* species are apomictic (asexual reproduction by seed) the main breeding approach to obtain cultivated forage species was the simple process of collection, evaluation, and selection of those biotypes with better agronomic qualities (Blount and Acuña 2009). With this breeding approach, apomictic polyploids such as *P. dilatatum* (dallisgrass), *P. plicatulum, P. guenoarum, P. nicorae, P. atratum* and *P. notatum* (bahiagrass) have been used in southern Unites States, Australia, New Zealand, Japan, Uruguay, Argentina, Brazil, Paraguay, Zimbabwe, and Thailand to start breeding a promising species for forage purpose, or to develop a new cultivar (Blount and Acuña 2009; Evers and Burson 2004). The most important cultivated *Paspalum* species is Pensacola, *P. notatum* var. *saurae*, which belong to the sexual diploid ($2n = 20$) cytotype of bahiagrass. It is one of the most popular perennial pasture grasses in Florida and the southern part of the Gulf States. Its persistence, ability to grow on poor soils, excellent seeding habits, and ease of establishment are responsible for much of its popularity (Burton and Forbes 1970). It was accidentally introduced to Unites States probably before 1926 (Burton 1967) from its native distribution, a small area in central-eastern Argentina (Daurelio et al. 2004). There are actually several millions of hectares of naturalized or cultivated Pensacola bahiagrass or genetically improved cultivars in the United States and, in addition, it was sold and cultivated for forage or turf purposes in many temperate and warm regions around the world.

Two main biological characteristics are well represented in *Paspalum*: apomixis is a common feature in many species of the genus and multiploidy occurs in a large proportion of it species. According to Savidan (2007), over 500 species have been reported to reproduce apomictically in about 40 higher plant families, while in the genus *Paspalum* at least 45 species are apomictic (Ortiz et al. 2013), which represent 9 % of the total number of apomictic plant species known at present. Multiploids, species with different ploidy levels or in other words different cytotypes for the same species, constitute 40 % of the known polyploids. The current cytological data indicate that about 75 % of *Paspalum* species are polyploid or multiploid. These species frequently have a diploid cytotype and one or more polyploid cytotypes, usually a tetraploid. Diploid cytotypes reproduce sexually, while polyploid counterparts reproduce by apomixis (Ortiz et al. 2013). Multivalent chromosome associations at meiosis are the rule in polyploid cytotypes of multiploid species (reviewed in Quarin 1992). Thus, cytological results suggest that polyploid cytotypes, particularly tetraploids and triploids, originated by autoploidy or segmental alloploidy in *P. notatum* (Forbes and Burton 1961), *P. simplex* (Pupilli et al. 1997) and *P. rufum* (Quarin et al. 1998). Moreover tetrasomic inheritance was observed through molecular markers analyses in the 4× cytotypes of the multiploids species of *P. simplex* (Pupilli et al. 1997) and *P. notatum* (Stein et al. 2004). Therefore, these previous results have strongly suggested that the existence of multiploid species is a biological characteristic feature of the genus *Paspalum*, and sustained that the polyploid cytotypes likely originated by autoploidy from diploid counterparts, though a segmental alloploid origin would not be discarded in some cases.

The genome size has been established for 43 of the approximately 350 species of the genus *Paspalum* (Jarret et al. 1995; Vaio et al. 2007). The amount varied roughly fourfold (1.02–3.86 pg/2C nucleus) and was considered a useful descriptor for characterization of plant genetic resources. Nevertheless, a question arises as to whether genome size and some biological traits are correlated in the genus *Paspalum*, especially the reproductive mode, the ploidy level, and the current concepts on infrageneric taxonomical classification. The main task to elucidate this question is the identification of the actual reproductive pathway of individual plants in multiploid species, a condition which tends to be the rule for several taxonomic groups of the genus as new data on chromosome numbers are achieved for different accessions of the same species.

Fortunately, the development of flow cytometric seed screen (FCSS) facilitates the simultaneous analysis of the relative DNA content and the mode of reproduction for large sample numbers. By using dry seeds from a target entity it is possible to know the relative DNA content of the

embryos and to assess the reproductive mode by the ratio of DNA content between the embryo and the endosperm of the seeds (Matzk et al. 2000). Thus, in a FCSS the DNA content of the embryo informs the 2C-value of a given individual and the embryo:endosperm DNA content ratio reveals its reproductive mode. The method is especially suitable to the analysis in *Paspalum* since apomixis in the vast majority of *Paspalum* species belongs to the aposporous type. Normaly, the sexual plants develop cytologically reduced megagametophytes with an egg apparatus (egg cell and two synergids), a large bi-nucleated central cell, and many antipodals. Thus, after double fertilization, a seed is formed with a 2n embryo and a 3n endosperm, or respectively 2C and 3C in terms of DNA content. In aposporous embryo sacs of the apomictic plants, the nuclear constitution may be very variable though usually there is one unreduced egg cell (2n), accompanied or not by one or two synergids, and a large central cell containing two unreduced polar nuclei, while antipodals are usually absent. Following parthenogenesis of the unreduced egg cell (2n) and fertilization of the two unreduced polar nuclei (2n each) by a reduced sperm cell (n) (pseudogamy), a seed is formed bearing a 2n embryo (2C) and a 5n endosperm (5C). The FCSS analysis provides the 2C-value of the embryo and simultaneously the DNA content of the endosperm. The 2:3 or 2:5 embryo:endosperm ratios indicate the sexual or the apomictic (apospory + parthenogenesis + pseudogamy) origin of the seed, respectively (Matzk et al. 2000; Ortiz et al. 2013). However, occasional deviations from the normal sexual or apomictic pathways may occur and should be considered. For example, occasional double fertilization may occur in aposporous embryo sacs (instead of parthenogenesis) and in that case a seed with a 3:5 embryo:endosperm ratio is formed.

We have tested in a large sample of *Paspalum* species whether the genome size (1Cx-value) is correlated to the reproductive mode (sexual or apomictic), to different ploidy levels, and to the current infrageneric taxonomic grouping of the species according to morphological characteristics. Simultaneously, we have determined the reproduction mode for all these species through the specific ratio of embryo:endosperm DNA contents in their seeds. In most cases the results obtained through flow cytometry were compared with previous determinations by means of embryological analyses, and in the remaining species the reproduction mode was established for the first time.

Materials and methods

The mode of reproduction and the relative DNA content were analysed for 47 species, i.e. 47 botanical taxa at the species rank. In *Paspalum*, below the species ranking it is possible to separate different categories: from the officially recognized subspecies or botanical varieties to different cytotypes (organisms that belong to the same species but have different ploidy levels) or very special biotypes (e.g. experimental hybrids, colchicine-induced polyploids, or botanical collections included in a given species but differing in some morphological or cytological characteristics from the species type). Consequently, a total of 77 different entities were examined, ranking from species (47), subspecies (1), botanical varieties (2), and different intraspecific cytotypes or biotypes (27). In most entities only one individual plant was analysed. Exceptionally, more than one accession (individuals of the same species collected at different localities) was considered. Thus, a total of 91 individuals were examined. The 47 species examined in this study are currently assigned to three different subgenera (Zuloaga and Morrone 2005): *Anachyris* for which three species were measured, including 7 entities; *Ceresia* with one species analysed; and subgenus *Paspalum* with 43 species, 39 of which are classified among 16 informal taxonomic categories (groups), while *P. durifolium, P. equitans, P. redondense,* and *P. rufum* have not been assigned to any group. The use of "group" as informal secondary taxonomic category between genus and species ranks was established by Chase (1929) and widely used in grass taxonomic literature since then (see the Article 4 of The International Code of Nomenclature for algae, fungi, and plants—Melbourne Code—http://www.iapt-taxon.org/nomen/main.php for officially accepted secondary ranks in descending sequence between genus and species, and below species rank).

Seed samples were originated from living collections in greenhouses or in the experimental *Paspalum* nursery on campus of the Faculty of Agricultural Sciences, Corrientes, Argentina (FCA UNNE). In most cases the name of the accession was composed of letter(s) and numbers: letters stand for some of the collector's name initials and the numbers for the herbarium voucher number. Most herbarium vouchers are deposited in the CTES herbarium. The main exceptions are those accessions identified with a number preceded by the letters V or TK, which have been collected by J.F.M. Valls or T. Killeen and the vouchers are deposited in the CEN or the US herbaria, respectively (for herbarium codes see: http://sweetgum.nybg.org/ih/). Exceptionally, the name of the accession reflects just the collection site.

The ploidy levels and the specific chromosome numbers were already known for several accessions because they had been previously reported in different publications of the *Paspalum* research team at FCA-UNNE. Chromosome numbers for the remaining accessions were determined through chromosome counts in root tips according to the procedures described in Norrmann et al. (1994).

For the flow cytometry seed screen (FCSS), a Partec Flow Cytometer CA and a Partec PA II were used at IPK, Gatersleben, Germany, and at Botanical Institute of Northeast, Corrientes, Argentina, respectively. Preparation, measurement and interpretations of the histograms were made as described by Matzk et al. (2000). The nuclei were stained with DAPI (4′, 6-diamidino-2-phenylindole) using the CyStain UV Precise P kit (05-5002) from Partec. The measurements of relative fluorescence intensity of stained nuclei were performed on a linear scale, with at least 3 000 nuclei per sample and CV ≤8 % (Fig. 1). In most cases 15–25 seeds were analysed for each entity in order to assess the reproductive mode and to establish the relative DNA content of each target entity. The protocol of Matzk et al. (2000) is suitable either for bulked or for single seed analysis. We used the seed-by-seed analysis, a procedure that reveals more clear evidence for facultative apomixis than the bulked analysis. Facultative apomicts form seeds not only through apomictic pathways (apospory or diplospory, parthenogenesis, and usually pseudogamy) but also by sexual means. In Germany, the relative DNA content was registered by DAPI measurement, using *Hypericum maculatum* subsp. *maculatum* as internal standard (relative 1C-value = 2.0 pg; Matzk et al. 2001 and 2003). In Argentina diploid *Paspalum notatum* var. *saurae* (relative 1C-value = 1.2 pg in comparison to *Hypericum maculatum* subsp. *maculatum*) was used as internal standard.

In the FCSS we follow the short forms used by Matzk et al. (2000) for the 1C-values of the embryo and the endosperm nuclei of the tested seed samples. Thus, the formula 2C + (3C) stands for a seed developed from an ovule bearing a reduced embryo sac through a double fertilization process by reduced sperms. An apomictically formed seed shows a 2C + (5C) formula corresponding to parthenogenetic embryo development from the unreduced egg cell and the pseudogamous formation of the endosperm (syngamy of two unreduced polar nuclei of the aposporous embryo sac and one reduced sperm). Though sexual species developing seeds with 2C + (3C) histogram peaks, or apomictic species showing 2C + (5C) represent the two most common combinations in the embryo:endosperm ratios of DNA content in *Paspalum* species, some exceptions have to be considered. The array of possible combinations in the DNA content of embryo and endosperm nuclei in monocots and dicots with respect to reproductive events are summarized in Matzk et al. (2000). According to previous research (e.g. Sartor et al. 2011), and our current expertise in cytology, embryology and flow cytometry to deal with reproductive mode in this grass material, the main exceptions for the rule that could be found in *Paspalum* are: (1) Entities

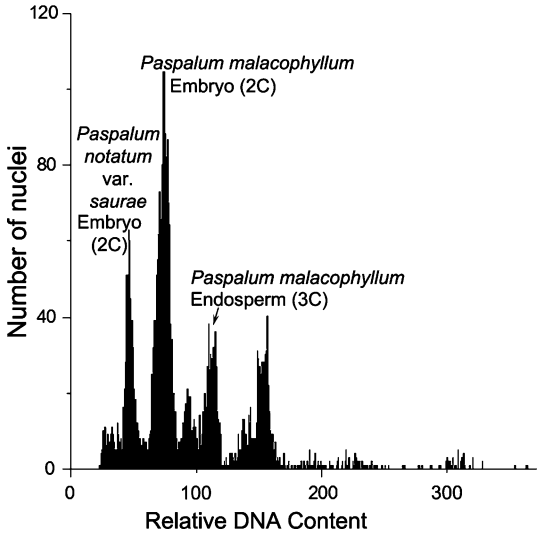

Fig. 1 Histogram illustrating the relative DNA content of 2C for diploid *Paspalum notatum* var. *saurae* (standard) and (2C) + (3C) of diploid *P. malacophyllum* (V14855) obtained by flow cytometric analysis of DAPI stained nuclei isolated from mature seeds. The (3C) peak corresponding to endosperm of the standard is overlapped by the (2C) peak of *P. malacophyllum.* Other peaks correspond to G2 of each respective embryo

for which most seeds show 2C + (5C) contents, though some seeds with 2C + (3C) peaks are also observed. These entities are interpreted as facultative apomictic (fap). (2) Apomictic entities showing mainly 2C + (5C) peaks, but with some occasional 3C + (5C) histograms, which imply double fertilization of an aposporous embryo sac, forming occasional $B_{III}(2n + n)$ embryos. (3) Entities forming histograms with 2C + (6C) peaks or occasional 4C + (6C) or even 2C + (5C to 6C). The interpretation of a 6C value in the endosperm is that pseudogamy has been accomplished by unreduced male gametes (umg), while the variation in the endosperm DNA content from more or less 5C to about 6C (~5C) is attributed to chromosomically variable male gametes (vmg) due to irregular meiotic chromosome behaviour. The occasional 4C value in the embryo nuclei reveals the formation of 2n + 2n embryos involving both female and male unreduced gametes. (4) Variable DNA contents among embryos of some entities with odd ploidy levels may be observed when sexual pathways occur. It should be attributed to chromosomically variable female gametes (vfg) and/or vmg due to unbalanced chromosome distribution during meiotic divisions. (5) Entities showing 2C + (4C) peaks, attributable to autonomous apomixis (aap) with parthenogenesis but not pseudogamy (autonomous endosperm development). InfoStat version 2014 was used for statistical analyses (Di Rienzo et al. 2014).

Results

Chromosome numbers and relative DNA contents

The chromosome numbers of the 47 sampled species varied from 2n = 12 in diploid *P. almum* to 2n = 80 in octoploid *P. erianthum*, *P. humboldtianum*, *P. ionanthum*, and *P. ovale*. Chromosome counts in root tips were established for 56 accessions, while the chromosome numbers of the remaining 35 accessions have already been known from our previous works (Table 1). With the exception of *P. almum* which has a basic (monoploid) chromosome set of x = 6 all other species showed x = 10. The 2C-values of the embryos varied from 0.5 pg in diploid *P. fimbriatum* to 6.5 pg in octoploid *P. erianthum* (Table 1). The relative mean DNA content per chromosome observed in species with x = 10 varied from 0.026 pg in *P. fimbriatum* (1Cx-value = 0.257 pg) to 0.100 pg in diploid cytotypes of *P. malacophyllum* and *P. simplex* (1Cx-value = 1.0 pg); while the diploid cytotype of *P. almum* showed a mean of 0.125 pg and the tetraploid 0.129 pg per chromosome with 1Cx-values of 0.750 and 0.775 pg, respectively.

Our records of genome size are new for 29 out of the 47 species that were analysed. Twenty of the 29 species are represented by only one cytotype, 5 by two cytotypes, and 4 include three ploidy levels (see Table 1).

Assessment of reproductive mode through FCSS

In general terms, absolute coincidence was observed between the mode of reproduction previously reported according embryological studies and the present analysis of relative embryo: endosperm DNA contents (Table 1). Each species, subspecies, cytotype, or strain previously classified as sexual or apomictic by embryological studies was confirmed by FCSS, though particular variations were detected mainly in the expressed degree of the apomictic character. Some taxa that had been classified as apomictic are now classified as facultative apomictic, or vice versa. This is a meaningless difference because both categories (apomictic or facultative apomictic) have the genetic constitution for apomictic reproduction in an opposite way to those taxa that were classified as sexual, independently of the method used for classification. In addition, the relative embryo:endosperm DNA contents provided new original information on the reproductive mode for 12 species and one botanical variety of *Paspalum*. Furthermore, the embryo:endosperm DNA content supplied new information on the reproductive mode of 10 species concerning cytotypes different from those previously reported (Table 1).

Mode of reproduction and genome size

Seventy-seven entities were analysed taking into account species, botanical varieties, biotypes, and different ploidies: 32 reproduced by sexuality and 45 by means of apomixis (obligate or facultative). The relative DNA content of the monoploid chromosome set (1Cx-value) was considered rather than the 1C-value in order to eliminate the effect of different ploidy levels. Sexuals averaged 1Cx = 0.680 pg, while the mean 1Cx-value was 0.700 pg for apomictic entities. Thus, no significant difference was observed between the mean basic genome size of sexual and apomictic entities ($P = 0.05$). 1Cx-values of sexual entities varied from 0.257 pg in diploid *P. fimbriatum* to 1 pg in diploid cytotypes of *P. malacophyllum* and *P. plicatulum*. In apomicts, the 1Cx-value ranged from 0.475 pg in octoploid *P. humboldtianum* to 0.925 pg in the tetraploid cytotype of *P. chaseanum*.

DNA content and the infrageneric taxonomic grouping

Since we have analysed a wide range of specific variation within the genus, most infrageneric grouping are represented by few species. In consequence, we took into account only the four infrageneric categories that were represented in our study with at least 7 species in order to correlate taxonomic grouping and DNA content. The selected categories and the number of entities for each one were: subgenus *Anachyris* (7), the group Dilatata (9), the group Notata (11), and the group Plicatula (16). The mean DNA content (1Cx-value) varied among these four infrageneric categories (Fig. 2).

DNA content and ploidy levels

The relation between basic genome size and the ploidy levels was analysed in two different ways: first, the mean 1Cx-value of all diploid entities against the mean basic genome sizes of polyploids at different ploidy levels, and then the diploid cytotype of each multiploid species against the polyploid cytotypes of the same species.

Paspalum, unlike many other genera of angiosperms, offers the possibility to address the analysis of DNA content at different conspecific ploidy levels because many species are multiploid. Eleven of the 47 species that were analysed in this study were represented by diploid as well as polyploid cytotypes (usually a tetraploid). This circumstance allowed the comparison of the intraspecific 1Cx-values among diploids and their conspecific polyploid counterparts (Table 2). As expected, differences in 2C values among conspecific cytotypes showed an increase in concordance with ploidy levels. We observed no variation or

Table 1 Chromosome numbers, relative DNA content in the embryonic nuclei (2C- and 1Cx-values in pg), observed ratios between 1C values of embryo and endosperm nuclei, reproductive modes as inferred from FCSS and reported in previous embryological studies for 47 species of *Paspalum* including 77 entities with a total of 91 accessions

Entities and ploidy levels (species, subspecies, varieties, cytotypes, and morphotypes)		Accessions	2n	Embryo relative DNA content[a]		Observed C-values of embryo + (endosperm)	Reproduction by FCSS[b]	Reproduction according previous embryological studies		Infrageneric taxonomic groups or subgenus[d]
				2C (SE)	1Cx			Mode[c]	References	
P. acuminatum Raddi[e]	4x	Q4164	40[f]	2.2 ± 0.065	0.550	2C + (3C)	sex[g]	–	–	Dissecta
P. almum Chase	2x	Perichón	12[f]	1.5 ± 0.054	0.750	2C + (3C)	sex	sex	Quarin and Hanna (1980a) sub *P. hexastachyum*	Alma
	4x	Q4165	24[f]	3.1 ± 0.077	0.775		ap	ap	Bashaw et al. (1970)	
P. arundinellum Mez[e]	4x	D&H479	40[f]	2.6 ± 0.063	0.650	2C + (6C); 4C + (6C)	ap + umg; B_{III} +umg	ap	Bashaw et al. (1970)	Quadrifaria
	5x	Q4154	50[f]	3.1 ± 0.074	0.620	2C + (6C)	ap + umg	–	–	
P. atratum Swallen[e]	4x	V9880	40	3.2 ± 0.057	0.800	2C + (5C)	ap	ap	Quarin et al. (1997)	Plicatula
P. buckleyanum Vasey[e]	5x	Hojs 355	50[f]	2.4 ± 0.085	0.480	2C + (5C); 2C + (3C)	fap	ap	Burson (1997), Sartor et al. (2011)	Livida
	6x	Hojs 354	60[f]	3.0 ± 0.083	0.500	2C + (5C); 2C + (3C)	fap	ap	Sartor et al. (2011)	
P. chaseanum Parodi[e]	2x	AK45457	20[f]	1.8 ± 0.062	0.900	2C + (3C)	sex	sex	Espinoza and Quarin (1997)	Plicatula
	4x	ST13894	40[f]	3.7 ± 0.072	0.920	2C + (5C)	ap	ap	Novo et al. (2013)	
P. commune Lillo	4x	IC139	40[f]	2.4 ± 0.067	0.600	2C + (5C)	ap[g]	–	–	Virgata
P. conjugatum Bergius	6x	FCA N185	60[f] 60[f]	3.2 ± 0.057	0.530	2C + (5C)	ap[g]	–	–	Conjugata
P. conspersum Schrad.	6x	M. Mboi	60[f]	3.9 ± 0.058	0.650	2C + (3C)	sex	sex	Quarin and Hanna (1980b)	Virgata
P. cromyorrhizon Trin.	2x	Q3635	20	1.4 ± 0.054	0.700	2C + (3C)	sex	sex + res apy	Quarin et al. (1982)	Notata
	3x	Q3635nt	30[f]	2.0 ± 0.063	0.667	2C + (~ 5C); ~ 2.6C + (~ 5C); 1.3C + (2C) to 2C + (3C);	fap (ap + vmg; B_{III} + vmg; sex + vfg + vmg	fap	Quarin et al. (1984)	
	4x	Q4039	40[f]	2.7 ± 0.075	0.675	2C + (5C); 2C + (3C)	fap	fap	Quarin et al. (1982)	
P. dasypleurum Kunze ex Desv.	4x	V12112	40[f]	1.9 ± 0.076	0.475	2C + (3C)	sex	sex	Quarin and Caponio (1995)	Dilatata
P. dedeccae Quarín[e]	2x	V11796	20[f]	1.5 ± 0.057	0.750	2C + (3C)	sex[g]	–	–	Linearia
	4x	Q3807	40	3.0 ± 0.079	0.750	2C + (5C); 2C + (3C)	fap	ap	Quarin and Burson (1991)	
P. densum Poir.[e]	4x	Q4298	40[f]	2.6 ± 0.076	0.650	2C + (3C)	sex[g]	–	–	Quadrifaria
P. dilatatum Poir. Common type	5x	B20 AH163	50[f] 50[f]	2.6 ± 0.056	0.520	2C + (~ 5C)	ap	ap	Smith (1948), Hayman (1956), Bashaw and Holt (1958)	Dilatata
Torres type	6x	Q4259	60[f]	3.0 ± 0.065	0.500	2C + (6x); 2C + (~ 5C)	ap + umg or vmg	ap	Burson et al. (1991)	
Uruguayan type	6x	Q4081	60[f]	3.4 ± 0.057	0.567	2C + (5C); 2C + (3C)	fap	fap	Burson et al. (1991)	

Table 1 continued

Entities and ploidy levels (species, subspecies, varieties, cytotypes, and morphotypes)		Accessions	2n	Embryo relative DNA content[a]		Observed C-values of embryo + (endosperm)	Reproduction by FCSS[b]	Reproduction according previous embryological studies		Infrageneric taxonomic groups or subgenus[d]
				2C (SE)	1Cx			Mode[c]	References	
Uruguaiana type	6x	V12388	60[f]	3.4 ± 0.075	0.567	2C + (~ 5C); 3C + (5C)	ap + vmg; B_{III}	ap	Burson et al. (1991)	
Virasoro type	4x	Q2960	40	2.1 ± 0.073	0.520	2C + (3C)	sex	sex	Caponio and Quarin (1987)	
subsp. *flavescens* Rosen., B.R. Arrill. & Izag.	4x	Q3952 Q4209	40 40[f]	2.1 ± 0.075	0.520		sex	sex	Bashaw and Holt (1958)	
P. durifolium Mez[e]	4x	Q3947	40	2.9 ± 0.067	0.725	2C + (3C)	sex	sex + res apy	Quarin (1994)	Ungrouped
	5x	V12282	50[f]	3.4 ± 0.054	0.680	>2C + (~ 3C); 2C + (~ 5C)	fap (sex + vfg + vmg; ap + vmg)[g]	–	–	
	6x	Q4056	60	4.2 ± 0.064	0.700	2C + (5C)	ap	ap	Burson (1985)	
P. equitans Mez[e]	2x	Q3683	20	1.4 ± 0.054	0.700	2C + (3C)	sex	sex + res apy	Quarin and Norrmann (1987)	Ungrouped
P. erianthum Nees ex Trin.[e]	8x	TK1194	80	6.5 ± 0.053	0.812	2C + (5C); 2C + (3C)	fap[g]	–	–	Eriantha
P. falcatum Nees ex Steud.[e]	2x	AH882A	20	1.6 ± 0.074	0.800	2C + (3C)	sex[g]	–	–	Falcata
P. fimbriatum Kunth[e]	2x	W37098 W36875	20[f] 20[f]	0.5 ± 0.063	0.257	2C + (3C)	sex[g]	–	–	(Fimbriata)
P. guenoarum Arechav.	4x	TK2390	40	3.6 ± 0.054	0.900	2C + (5C)	ap	ap	Burson and Bennett (1971a), Espinoza et al. (2001)	Plicatula
P. humboldtianum Flüggé	8x	SV2640	80[f]	3.8 ± 0.057	0.475	2C + (4C)	aap[g]	–	–	Subg. Ceresia
P. ionanthum Chase	4x	Q3726 Q3777	40[f] 40[f]	2.7 ± 0.068	0.675	2C + (3C)	sex	sex + res apy	Quarin and Norrmann (1987), Burson and Bennett (1970b) sub *P. guaraniticum*	Notata
	8x	V4294	80[f]	5.2 ± 0.066	0.650	2C + (5C); 2C + (3C)	fap	ap	Burson and Bennett (1970b)	
P. kempfii Killeen[e]	4x	TK2272	40	3.9 ± 0.064	0.975	2C + (5C)	ap[g]	–	–	(Plicatula)
P. lenticulare Kunth[e]	2x	N188	20	1.5 ± 0.078	0.750	2C + (3C)	sex	sex	Espinoza et al. (2001) *sub P. limbatum* Henr.	(Plicatula)
	4x	TK2417 U57 V11724	40[f] 40[f] 40[f]	3.0 ± 0.74	0.750	2C + (5C); 3C + (5C)	ap, B_{III}	ap; fap	Espinoza et al. (2001)	
	6x	U54	60[f]	4.5 ± 0.069	0.750	2C + (5C); 2C + (3C); 3C + (5C)	$fap_{III}B$[g]	–	–	
P. lividum Trin.	4x	Hojs 365	40[f]	2.7 ± 0.084	0.675	2C + (5C); 2C + (3C)	fap	fap	Burson and Bennett (1971a), Sartor et al. (2011)	(Livida)
P. macedoi Swallen[e]	4x	TK2323	40	3.1 ± 0.076	0.775	2C + (5C)	ap[g]	–	–	(Plicatula)

Table 1 continued

Entities and ploidy levels (species, subspecies, varieties, cytotypes, and morphotypes)		Accessions	2n	Embryo relative DNA content[a]		Observed C-values of embryo + (endosperm)	Reproduction by FCSS[b]	Reproduction according previous embryological studies		Infrageneric taxonomic groups or subgenus[d]
				2C (SE)	1Cx			Mode[c]	References	
P. malacophyllum Trin.	2x	V14855 V14411	20 20	2.0 ± 0.078	1.000	2C + (3C)	sex	sex + res apy	Hojsgaard et al. (2008)	Subg. Anachyris
	4x	Q4080 Q4112 V11813 TK2449	40 40 40[f] 40	3.4 ± 0.075	0.850	2C + (5C); 2C + (3C)	fap	fap	Burson and Hussey (1998), Hojsgaard et al. (2008)	
P. mandiocanum Trin.	5x	V12010	50[f]	2.6 ± 0.075	0.520	2C + (6C); 4C + (6C)	ap + umg; B_{III} + umg[g]	–	–	Corcovadensia
P. millegrana Schrad.[e]	4x	Q4297	40[f]	3.1 ± 0.057	0.775	2C + (3C)	sex[g]	–	–	(Quadrifaria)
P. minus E.Fourn.[e]	5x	V14573	50[f]	3.2 ± 0.078	0.640	2C + (6C)	ap + umg	ap (dy + apy)	Bonilla and Quarin (1997)	Notata
P. nicorae Parodi	4x	Sartor 10	40	3.3 ± 0.055	0.825	2C + (5C); 2C + (3C)	fap	ap	Burson and Bennett (1970a), Sartor et al. (2011)	Plicatula
P. notatum Flüggé var. *notatum*	4x	Q4117	40	2.2 ± 0.056	0.550	2C + (5C)	ap	ap; fap	Burton (1948), Martínez et al. (2001)	Notata
Experimental hybrid	4x	Q4188	40	2.1 ± 0.057	0.525	2C + (3C)	sex	sex	Martínez et al. (2001)	
var. *saurae* Parodi	2x	Q4084	20	1.2 ± 0.082	0.600	2C + (3C)	sex	sex	Burton (1948)	
Colchicine-treated diploid	2x	C4-2x	20	1.2 ± 0.075	0.600	2C + (3C)	sex	sex	Quarin et al. (2001)	
Colchicine-induced tetraploid	4x	C4-4x	40	2.4 ± 0.069	0.600	2C + (3C)	sex	sex	Quarin et al. (2001)	
P. oteroi Swallen[e]	4x	Q4095	40[f]	3.0 ± 0.067	0.750	2C + (5C)	fap[g]	–	–	(Plicatula)
P. ovale Nees ex Steud.[e]	8x	Q4083	80[f]	5.5 ± 0.058	0.687	2C + (5C)	ap[g]	–	–	Linearia
P. palustre Mez[e]	4x	Bord 110	40[f]	2.4 ± 0.075	0.600	2C + (5C)	ap[g]	–	–	Plicatula
P. plicatulum Michx.	2x	Honfi 14	20	2.0 ± 0.078	1.000	2C + (3C)	sex	sex	Espinoza and Quarin (1997)	Plicatula
	4x	Hojs 388	40[f]	2.8 ± 0.075	0.700	2C + (5C)	ap	ap	Pritchard (1970), Bashaw et al. (1970), Burson and Bennett (1971b)	
var. *intumescens* Döll	4x	Q4087	40[f]	3.4 ± 0.074	0.850	2C + (5C); 2C + (3C)	fap[g]	–	–	
P. procurrens Quarín[e]	4x	Q4094	40	3.2 ± 0.064		2C + (5C)	ap	fap	Hojsgaard et al. (2008)	Subg. Anachyris
P. redondense Swallen[e]	2x	V11370R	20[f]	1.2 ± 0.054	0.600	2C + (3C)	sex[g]	–	–	Ungrouped
P. regnellii Mez[e]	4x	V10121 V4040 V11900 AH130	40[f] 40[f] 40[f] 40[f]	2.5 ± 0.063	0.625	2C + (3C)	sex	sex	Norrmann (1981)	Virgata

Table 1 continued

Entities and ploidy levels (species, subspecies, varieties, cytotypes, and morphotypes)		Accessions	2n	Embryo relative DNA content[a]		Observed C-values of embryo + (endosperm)	Reproduction by FCSS[b]	Reproduction according previous embryological studies		Infrageneric taxonomic groups or subgenus[d]
				2C (SE)	1Cx			Mode[c]	References	
P. rojasii Hackel[e]	4x	AK40732	40[f]	3.5 ± 0.078	0.875	2C + (5C)	ap	ap	Bashaw et al. (1970),Burson and Bennett (1971b)	(Plicatula)
P. rufum Nees[e]	2x	Q3754	20	1.5 ± 0.068	0.750	2C + (3C)	sex	sex + res apy	Norrmann et al. (1989)	Ungrouped
	4x	Q3785	40	3.0 ± 0.084	0.750	2C + (5C)	ap	ap; fap	Burson (1975) Norrmann et al. (1989)	
P. simplex Morong[e]	2x	Q4109	20	1.5 ± 0.081	0.750	2C + (3C)	sex	sex	Espinoza and Quarin (1997)	Subg. Anachyris
	3x	U36	30	2.5 ± 0.067	0.833	1.3C + (2C) to 2C + (3C)	sex + vfg + vmg	sex	Urbani et al. (2002)	
	3x	U45	30	2.6 ± 0.082	0.867	2C + (~ 5C)	ap + vmg	ap	Urbani et al. (2002)	
	4x	Q4126	40	3.0 ± 0.072	0.750	2C + (5C); 2C + (3C)	fap	ap	Caponio and Quarin (1987)	
P. umbrosum Trin.[e]	2x	V11828	20[f]	1.0 ± 0.063	0.500	2C + (3C)	sex	sex	Bashaw et al. (1970)	Paniculata
P. unispicatum (Scribn. and Merr.) Nash	2x	U15	20[f]	1.1 ± 0.076	0.550	2C + (3C)	sex[g]	–	–	Decumbentes
	3x	Q4211	30[f]	1.7 ± 0.075	0.567	2C + (5C)	ap[g]	–	–	
	4x	Q4265	40[f]	2.3 ± 0.052	0.575	2C + (5C)	ap	fap	Burson (1997)	
P. urvillei Steud.	4x	Q4111	40[f]	2.1 ± 0.054	0.525	2C + (3C)	sex	sex	Brown and Emery (1958)	Dilatata
P. sp.nov. (*P. urvillei* × *P. mandiocanum*?)[e]	5x	V8009	50[f]	2.5 ± 0.064	0.500	2C + (6x); 4C + (6C)	ap + umg; B_{III} + umg[g]	–	–	(Dilatata)
P. virgatum L.	4x	BAA23248	40[f]	2.3 ± 0.072	0.575	2C + (3C)	sex	sex	Burson and Quarin (1982)	Virgata

[a] DNA content relative to DNA of *Hypericum maculatum* ssp. *maculatum* or diploid *Paspalum notatum* var. *saurae*. Peak position of *H. maculatum* 2× = 2.0 pg, and *P. notatum* var. *saurae* 2× = 1.2 pg (internal standards)

[b] Abbreviations relative to the array of possibilities observed by FCSS in the reproductive processes: *aap* autonomous apomixis (parthenogenetic embryo and autonomous endosperm development), *ap* apomict with exclusively 2C + (5C) embryo + (endosperm) DNA contents, B_{III} some seeds with 3C + (5C) or 4C + (6C) embryo:endosperm peaks due to double fertilization of an apomicic (aposporous or diplosporous) embryo sac by a reduced or unreduced male gamete, respectively, *fap* facultative apomictic with predominantly 2C + (5C), but also 2C + (3C) DNA contents; *sex* sexual with exclusively 2C + (3C) embryo + (endosperm) peaks, *umg* unreduced male gametes with a 2C value in species with failure of chromosome pairing during meiotic division followed by restitution nucleus in all meiocytes, *vfg* variable female gametes with gain or loss of DNA content around the C-value due to irregular meiosis behaviour in sexual polyploids with odd ploidy levels, *vmg* variable male gametes due to irregular male meiosis in some sexual or apomictic polyploids

[c] Additional abbreviations regarding reproductive processes according embryological literature: *apy* apospory, *dy* diplospory, *res apy* residual apospory (ability of an usually sexual reproducing species to occasionally develop an aposporous embryo sac beside the regular sexual one)

[d] According to the taxonomic revision of the genus for austral South America (Zuloaga and Morrone 2005). Those species that had not been considered in the revision were assigned to the corresponding group by CL Quarin and are given in brackets. Likewise, the group Linearia is considered in its original perspective proposed by Chase (1929, 1939)

[e] Relative DNA content of the species is determined for the first time

[f] Chromosome counts specifically determined in root tips for this research

[g] Reproductive modes reported for the first time

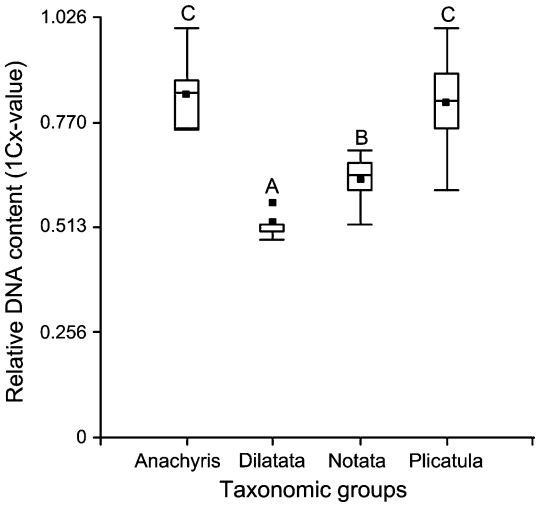

Fig. 2 Box plot with basic relative DNA Content (pg) of *Paspalum* species of the Subgenus *Anachyris* and three groups of the subgenus *Paspalum*. Means followed by the same letter are not significantly different. Tukey test ($P = 0.05$)

not significant variation in the 1Cx-values between conspecific cytotypes of some species; in other species there was an increase of the 1Cx-values in polyploids, and in some cases the 1Cx-values decreased in polyploids with respect to their diploid counterparts. Namely, the same intraspecific DNA 1Cx-values were observed for diploid and tetraploid cytotypes of *P. dedeccae*, *P. rufum*, *P.simplex*, *P. notatum* (diploid accessions Q4084 and C4-2x, and tetraploid accession C4-4x); for diploid and triploid accessions of *P. unispicatum*; and for diploid, tetraploid and hexaploid cytotypes of *P. lenticulare*. In addition, the basic genome size was slightly higher or lower in polyploid cytotypes than in conspecific diploids, but not significantly different, in tetraploid *P.almum* (+3.33 %), tetraploid *P. chaseanum* (+2.2 %), tetraploid *P. unispicatum* (+1.7 %) and triploid and tetraploid *P. cromyorrhizon* (−4.28 %). Otherwise, important increases were observed in triploids of *P. simplex*: 12 % in the sexual plant U36 and 16 % in apomictic plant U45, while as indicated above the tetraploid accession of this species showed the same 1Cx-value than the diploid accession. A drastic reduction of basic genome size was observed in tetraploid *P. malacophyllum* (−15 %) and tetraploid *P. plicatulum* (−29 %) in relation to their conspecific diploid cytotypes. A special case was *P. notatum*: natural tetraploid Q4117 had a basic genome size smaller (−8.3 %) than the natural diploid Q4084 while the colchicine-induced tetraploid C4-4x had the same 1Cx-value than the diploid C4-2x from which C4-4x was induced. In addition, an important genome downsizing was observed in the tetraploid Q4188 (−13 %), a particular genotype obtained through experimental means including chromosome doubling, hybridization, cycles of outcrossing and selection.

Table 2 Relative DNA contents in different cytotypes of 11 multiploid species of *Paspalum*

Species	Accession	Ploidy	Cx-value (pg)	Percentage of downsized (−) or upsized (+) DNA content with respect to 2x level
P. almum	Perichón	2x	0.750a	
	Q4165	4x	0.775a	+3.33
P. chaseanum	AK45457	2x	0.900a	
	ST13894	4x	0.920a	+2.20
P. cromyorrhizon	Q3635	2x	0.700a	
	Q3635nt	3x	0.670a	–4.28
	Q4039	4x	0.670a	–4.28
P. dedeccae	V11796	2x	0.750a	
	Q3807	4x	0.750a	0
P. lenticulare	N188	2x	0.750a	
	TK2417	4x	0.750a	0
	U54	6x	0.750a	0
P. malacophyllum	V14855	2x	1.000b	
	TK2449	4x	0.850a	–15.00
P. notatum	Q4084	2x	0.600b	
	C4-2x	2x	0.600b	
	C4-4x	4x	0.600b	0
	Q4117	4x	0.550a	–8.30
	Q4188	4x	0.520a	–13.00
P. plicatulum	AH14	2x	1.000b	
	Hoj388	4x	0.710a	–29.00
P. rufum	Q3754	2x	0.750a	
	Q3785	4x	0.750a	0
P. simplex	Q4109	2x	0.750a	
	U36	3x	0.840ab	+12.00
	U45	3x	0.870b	+16.00
	Q4126	4x	0.750a	0
P. unispicatum	U15	2x	0.570a	
	Q4211	3x	0.570a	0
	Q4265	4x	0.580a	+1.70

Means followed by the same letter are not significantly different. Tukey test ($P = 0.05$)

Discussion

We are recording the genome size of 47 species of *Paspalum* from which 29 are new records. The remaining 18 species have been recorded before (Bennett and Leitch 2010; Jarret et al. 1995). The DNA contents of a large list of 35 species of *Paspalum* published by Jarret et al. (1995) could be comparable with our results, because in both studies the DNA contents were measured with DAPI. Although the chromosome numbers are not indicated in Jarret`s and co-workers list, now it would be possible to extrapolate

their ploidies, providing that aneuploidy is not a common feature among *Paspalum* species. However, the range of variation in DNA content values that they have observed among accessions of the same species makes it difficult to ascertain what was the real ploidy level of some accessions. The information regarding the chromosome numbers of the analysed plants is crucial because in the genus *Paspalum,* multiploid species are the rule, i.e. species with different ploidy levels or in other words different cytotypes for the same species (Ortiz et al. 2013). The results indicated that *P. almum*, the species with a rare base chromosome number of x = 6, has a mean DNA content per chromosome higher than in any other species with x = 10.

Vaio et al. (2007) published another list of DNA contents for *Paspalum* species. Most of these species were allopolyploid and belong to the Dilatata group, while some putative diploid ancestors were also analysed. The DNA content was estimated by using flow cytometry and propidium iodide (PI) as fluorochrome, and the chromosome number for each species was informed. Some of the species of our list are coincident with the list of Vaio et al. (2007). In general, we observed somewhat lower 2C-values for the same species. This may be due to the different stain reactions of the fluorochromes: PI binds base pairs of double-stranded DNA with very little or no base specificity while DAPI binds DNA preferentially to A-T base regions (Johnston et al., 1999). However, the scores that we obtained with DAPI should not be very far from the real values in picograms. In fact, our determination with DAPI of the biotype Virasoro of *P. dilatatum* was 2C = 2.10 pg, while Vaio et al. (2007) reported a 2C-value 9 % higher (2.29 pg) for the same plant material, i.e. accessions of a sexual autogamous species collected in the same wild population; or in a similar way, we observed 3.4 pg in the apomictic hexaploid Uruguaiana biotype of *P. dilatatum* while Vaio et al. (2007) measured 3.69 pg (8.5 % higher) for plant material collected in the same wild population.

We are reporting for the first time the reproductive mode of 12 species and one botanical variety. In addition, new information on reproductive mode is provided for 10 species concerning cytotypes that are different from the previous reported cytotype. The FCSS proved to be a reliable and very simple method to determine the reproductive mode in species of the genus *Paspalum.* We observed histograms with 2C + (3C) peaks for all those species and cytotypes that have been reported to reproduce by sexual means. Likewise, histograms with 2C + (5C) or other peak constitution characteristic of apomictic development were observed for all species or cytotypes that have been reported to be apomictic. Nonetheless, quantitative differences were found in reproductive mode of some species, as for example *P. nicorae*, a species considered earlier an obligate apomict according to cytoembryological studies (Burson and Bennett 1970a). In fact, we confirmed the recent observation of Sartor et al. (2011) that most seeds of *P. nicorae* showed the 2C + (5C) peaks characteristic of apomictic development, but a small proportion exhibited the 2C + (3C) peaks indicating presence of sexual reproduction. Therefore, the species was considered to be facultative apomictic because it reproduced mainly by parthenogenesis and pseudogamy with some residual potentiality for sexual reproduction.

We observed exclusive 2C + (3C) peaks in all sampled diploid species. However, previous embryological studies have demonstrated that the diploid cytotypes of several multiploid species usually developed not only a meiotic (cytologically reduced) embryo sac per ovule, but also an aposporous embryo sac (Caponio and Quarin 1993; Delgado et al. 2014; Hojsgaard et al. 2008; Norrmann et al. 1989; Quarin et al. 1982). The absence of seeds with 2C:5C embryo:endosperm ratio in diploid cytotypes was expected in our study because seeds came from open pollination. These diploids of multiploid species are highly outbreeders due to a self-incompatibility system (Quarin 1992). The functioning of the occasional aposporous embryo sacs was demonstrated when selfing was induced by mixed loads of self and heterospecific pollen (mentor effect) or by self-pollen combined with pollen of conspecific tetraploids; however, when pollination involved another genotype of the same diploid species, the progeny was formed exclusively by sexuality (Siena et al. 2008). In consequence, seeds bearing 2C:3C embryo: endosperm ratios were primarily expected in our FCSS analysis of diploids because seeds had originated from open pollination.

Our data indicate that no correlation exist between the genome size and the reproduction mode of *Paspalum* material evaluated here. The results show a different scenario for coevolution of genome size and the reproduction mode than the situation pictured by Matzk et al. (2003) in the genus *Hypericum* (Clusiaceae). Several apomictic species have been identified in this genus, and with the exception of *H. scabrum*, all of them are facultative and pseudogamous apomicts that produce reduced male gametes, while *H. scabrum* is an obligate pseudogamous apomict, which produces unreduced male gametes. All facultative apomicts belong to two different sections of the genus: the phylogenetically younger section Hypericum and the evolutionarily older section Ascyreia. Within the section Hypericum the genome sizes of apomictic species are about twice those of the sexual ones, but the DNA increase is due exclusively to polyploidy because the 1Cx-values of sexuals do not differ significantly from the DNA values of apomictic ones. However, in the section Ascyreia the apomictic species have significantly higher 1Cx-values than the sexual species of the section and altogether, 85 % of the total variation of the relative DNA content of the genus is found within

this section. In a contrasting way, apomixis was found to occur in nearly all the infrageneric sections of the genus *Paspalum* analysed here, but there is not a direct correlation between the reproductive system and the variation of the 1Cx-value. Since important ranges of variation in 1Cx-value were observed among sexual as well as among apomictic entities, though the means were not significantly different between both categories, the evolutionary history of the reproductive mode in *Paspalum* may not be considered a parameter affecting the genome size in the genus.

Only four well represented categories were considered to analyse the relationship between the genome size and taxonomic grouping: the subgenus *Anachyris* and the groups Notata, Dilatata, and Plicatula of the subgenus *Paspalum.* The highest 1Cx-values were observed in species of subgenus *Anachyris* and those of the Plicatula group, which are native to tropical regions of America though some of them are also distributed in the subtropics. In contrast, the lowest 1Cx-values were observed in the analysed species of the Dilatata and the Notata groups, which are remarkably abundant throughout the humid subtropical and temperate regions of South America in southern Brazil, Uruguay and central and northeast of Argentina toward the Paraná River Delta and even further southeast in the Buenos Aires province. Notwithstanding, some species have been introduced and naturalized over many regions in the world including *P. dilatatum*, *P. urvillei* and *P. notatum*.

In general, we observed that the basic genome of *Paspalum* multiploid species was equal in size or very similar in different cytotypes of the same species. These results do not follow the general tendency observed by Leitch and Bennett (2004) concerning more than 3,000 species of angiosperms, which postulate that genome downsizing is a frequent phenomenon in polyploid angiosperms. Moreover, an opposite behaviour to this general tendency was observed in triploids of *P. simplex,* with a relative high value of mean basic genome size. Leitch and Bennett (2004) excluded all species with odd-ploids from their analysis, because data was available only for a few species. The odd-ploidy phenomenon and specially triploidy, though infrequent among most angiosperms, may play an important role as a bridge in the polyploidization processes and evolution of genera in which apomixes and autoploidy are so frequent like in *Paspalum* (Quarin 1992; Yamauchi et al. 2004). Considering this situation, we understand that the relation between triploidy and genome size deserves further research especially in multiploid species of *Paspalum*.

Finally, we had a very special opportunity to analyse the basic genome size in relation to polyploidization. The C4-2x and C4-4x accessions of *P. notatum* are the diploid and the tetraploid plants, both obtained from a callus originated by tissue culture of a piece of young inflorescence, followed by colchicine treatment (Quarin et al. 2001). We observed the same 1Cx-value for the induced autotetraploid plant C4-4x than for the diploid C4-2x plant. The results support the suggestion that polyploid cytotypes in multiploid species arose from diploids through autopolyploidization (Quarin 1992). Our analyses indicated that in most multiploid species the diploid and the polyploidy co-specific counterparts have the same or not significantly different 1Cx-values. However, there are some exceptions in which the tetraploids cytotypes had much lower 1Cx-values than their diploid counterparts, e.g. *P. notatum,* a species bearing different morphological biotypes at the tetraploid level. In this case, though the tetraploid cytotype likely originated by autoploidy, the tetraploid biotypes with lower 1Cx-value (taxonomically identified as co-specific variants) might have evolved through segmental allopolyploidy due to occasional hybridization with related species that have lower basic genome size. Interestingly, Dahmer et al. (2008) analysed the meiotic chromosome associations in 34 different accessions of tetraploid *P. notatum*; most of them showed a variable number of quadrivalent associations (suggesting autoploid origin), but some accessions showed exclusively 20 bivalent or with a very rare quadrivalent at first metaphase, a meiotic chromosome behaviour characteristic of alloploids or segmental allopolyploids. In fact, we observed the lowest 1Cx-value in a particular sexual tetraploid genotype (Q4188) of this species obtained experimentally through chromosome doubling of a diploid, hybridization with different wild biotypes, and cycles of open pollination and selection.

Besides the special case of *P. notatum*, we observed important decreases of DNA contents in only two tetraploids with respect to their currently intraspecific diploid counterparts: *P. malacophyllum* (−13 %) and *P. plicatulum* (−29 %). However, this can be attributed to a taxonomic issue as a result of doubtful identification. In fact, in a recent study of genetic diversity in subgenus *Anachyris* concerning microsatellite markers and morphological characteristics, it was suggested that diploid accession V14855 of *P. malacophyllum* might belong to a new different species, while the tetraploid accession TK2449 fell into the clade of *P. malacophyllum* (Zilli et al. 2014). In the same way, both the diploid AH14 and the tetraploid Hoj388 accessions were doubtfully joined under the name of *P. plicatulum*. The doubt arose because Hoj388 have scaly rhizomes larger than usual for the species, though the general morphological characteristics resembled *P. plicatulum*. In both species the differences in morphological characteristics and in DNA content between diploid and tetraploid cytotypes may suggest that the tetraploid plants had not originated exclusively by autoploidy, but also by some occasional interspecific hybridization event.

Considering that the expected mean basic genome size should be the same for different cytotypes of multiploid species (based on the assumption that polyploid cytotypes arose by autoploidy), most polyploids showed either

the expected genome size, in some cases a slight genome downsizing effect, or a slight increase in the size of the genome. Whether such genome size increases or decreases can be attributable to taxonomic issues like incorrect identification it remains to be supported.

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