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Cytogenetic relationships, polyploid origin and taxonomic issues in *Paspalum* species: inter- and intraspecific hybrids between a sexual synthetic autotetraploid and five wild apomictic tetraploid species

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ABSTRACT

- *Paspalum* is a noteworthy grass genus due to the forage quality of most species, with approximately 330 species, and the high proportion of those that reproduce *via* apomixis. Harnessing apomictic reproduction and widening knowledge about the cytogenetic relationships among species are essential tools for plant breeding.
- We conducted cytogenetic analyses of inter- and intraspecific hybridisations involving a sexual, colchicine-induced autotetraploid plant of *P. plicatulum* Michx. and five indigenous apomictic tetraploid (2n = 40) species: *P. compressifolium* Swallen, *P. lenticulare* Kunth, two accessions of *P. nicorae* Parodi, *P. rojasii* Hack. and two accessions of *P. plicatulum*. Fertility of the hybrids was investigated and their reproductive system was analysed considering the relative embryo:endosperm DNA content from flow cytometry. Morphological, nomenclatural and taxonomic issues were also analysed.
- Cytogenetic analysis suggested that all indigenous tetraploid accessions of five apomictic species are autotetraploid or segmental allotetraploid. If segmental allotetraploids, they probably originated through autoploidy followed by diploidisation processes. Autosyndetic male chromosome pairing observed in all hybrid families supported this assertion. Allosyndetic chromosome associations were also observed in all hybrid families. In the hybrids, the proportion of male parent chromosomes involved in allosyndesis per pollen mother cell varied from 5.5% to 35.0% and the maximum was between 25% and 60%. The apomictic condition of the indigenous male parents segregated in the hybrids.
- These results confirm a strong association between autoploidy and apomixis in *Paspalum*, and the existence of cytogenetic relationships between different species of the Plicatula group. Allosyndetic chromosome pairing and seed fertility of the hybrids suggest the feasibility of gene transfer among species.

INTRODUCTION

Paspalum is one of the most noteworthy grass genera due to its large number of species, their importance as forage constituents in native grasslands all over the Americas from temperate to warm-tropical regions and the high proportion of apomictic species. Due to the chronic lack of a global taxonomic revision of the genus, it is difficult to group the species into different sub-generic categories. Considering the large morphological variation observed among the approximately 330 species estimated for the genus by Zuloaga & Morrone (2005), there are very few officially accepted subgenera. This historical lack was first compensated by Chase (1929), in her revision of the North American species of *Paspalum*, where she accepted the well-marked subgenus *Ceresia*, with eight species, and created more than a dozen unofficial taxonomic 'groups' to indicate the sub-generic affinities among nearly all the 140 species that she recognised for North America. Zuloaga & Morrone (2005) reported 129 species in their taxonomic revision for southern South America (Argentina, Chile, Bolivia, Paraguay and the state of Rio Grande do Sul in Brazil). These authors accepted the subgenera *Anachyris* and *Ceresia* with five and 13 species, respectively, while the vast majority (111) was arranged into 28 groups following mainly Chase's criteria, and four species remained ungrouped.

Plicatula is the name of the group that contains the largest number of species (16) mentioned for southern South America (Zuloaga & Morrone 2005). Chase (1929) reported eight species of Plicatula for North America, four of which are among the 16 reported for southern South America. Despite the lack of a comprehensive taxonomic revision of the genus for the tropical regions of Central and South America, we estimate that the group Plicatula might contain up to 30 species. The main morphological characteristics of the group are plain-convex spikelets, usually with transverse wrinkles on the sterile lemma, drab green at young stages, turning brown or olivaceous at maturity, with dark brown and shining anthoecia (fertile lemma and palea).

Most species of Plicatula are among the outstanding members of the grass family in native pasturelands in tropical and subtropical South America. Some of these species like *Paspalum plicatulum* Michx., *P. guenoarum* Arechav., *P. nicorae* Parodi and *P. atratum* Swallen have been introduced to cultivation and used for forage, not only in the Americas but also in other warm regions around the world (Evers & Burson 2004). Some species are tetraploid (2n = 4x = 40) and others are multiploid, *i.e.* species including a diploid (2x) as well as polyploid co-specific cytotypes, usually tetraploid (4x), and rarely triploid (3x) or hexaploid (6x). Diploids reproduce sexually, while all polyploids (cytotypes or species) investigated so far in the group are apomictic. Apomixis causes serious inconvenience in any programme of genetic improvement based on hybridisation and gene transfer. Fortunately, two sexual synthetic tetraploid plants were created by doubling the chromosome number of the sexual diploid cytotype of *P. plicatulum* through colchicine treatments (Sartor *et al.* 2009). Using these synthetic autotetraploids as female parents, identified as 4PT and 7PT, a genetic improvement programme was recently initiated for forage purpose. It includes *P. guenoarum*, *P. chaseanum* Parodi and *P. oteroi* Swallen, which belong to the same group. Their interspecific hybrids with sexual 4x *P. plicatulum* showed satisfactory levels of seed set, and demonstrated that all these species shared genomes bearing chromosomes with important degrees of homology or homeology (Sartor *et al.* 2009; Aguilera *et al.* 2011; Novo *et al.* 2013, 2016). Such features allow the use of gene exchange among these species through hybridisation and backcrossing. In a more extensive analysis (Novo *et al.* 2017), the genetic distance for 22 accessions of 12 different species of the Plicatula group was analysed in correlation with inter- or intraspecific crossability, fertility of the hybrids and possible heterosis for agronomic traits expressed in the hybrids. In view of these previous results, we undertook further research to elucidate how much further the gene exchange might be possible among *P. plicatulum* and other tetraploid species of the group. Taking advantage of the already obtained hybrids, we investigated the cytogenetic relationships among these species and, in addition, we analysed genome homologies in connection with taxonomic issues of this group.

Our first objective was to analyse the meiotic chromosome behaviour in: (i) five indigenous apomictic 4x species: *P. compressifolium* Swallen, *P. lenticulare* Kunth, two accessions of *P. nicorae*, two accessions of *P. plicatulum* and *P. rojasii* Hack.; and (ii) the interspecific and intraspecific hybrids recovered from their crosses with synthetic 4x *P. plicatulum*. The aim of cytogenetic analysis was addressed to better understand the genome homologies between parents, which constitutes the genetic basis for gene transfer between species. A second objective was investigating the reproductive mode of *P. lenticulare*, the two accessions of *P. nicorae* and the indigenous accession ML5 of *P. plicatulum*, since the reproductive mode was unknown for these particular accessions used as male parents in crosses. The method of reproduction was also analysed in individual plants of the hybrid families to examine whether apomixis was transmitted to some individuals of the progeny. Knowledge of the reproductive mode of the parents and the segregation of the trait in the progeny provide crucial information for a programme dealing with genetic plant improvement.

The third objective was to describe the current situation regarding taxonomic issues, nomenclatural status and morphological particularities of the species and accessions used herein for crossing, in order to contribute to the still lacking general taxonomic revision of the genus.

A summary of previous information about cytology, reproduction and geographic distribution of each species follows:

Paspalum compressifolium inhabits low wet areas and is distributed naturally from Bolivia, eastern Paraguay, central and southern Brazil to northeast Argentina. It is a multiploid species with chromosome numbers of 2n = 20, 40 and 60 for diploid, tetraploid and hexaploid cytotypes (Quarin *et al.* 1996). However, tetraploidy seems to be the most common ploidy level since only one accession was diploid, one was hexaploid and the other 16 were tetraploid, taking into account and summing up the results of four previous reports (Honfi *et al.* 1990; Quarin *et al.* 1996; Takayama *et al.* 1998; and Pagliarini *et al.* 2001).

Paspalum lenticulare, originally described from Venezuela, has a wide natural distribution in Central America, Caribbean Islands, vast areas of Brazil, eastern Bolivia to eastern Paraguay (Oliveira & Valls 2008). It is a multiploid species. Espinoza *et al.* (2001) reported two diploid (2n = 20, sub *P. limbatum*) and five tetraploid (2n = 40) accessions. These ploidy levels were then confirmed by Galdeano *et al.* (2016), who added a hexaploid (2n = 60) cytotype, indicating that the diploids reproduced sexually while polyploids (tetraploids and hexaploids) were apomictic.

Paspalum nicorae, native to South America, naturally occurs in eastern Paraguay, southern Brazil, northeast Argentina and Uruguay. It was introduced to the United States and naturalised in the Gulf Coast States and the Atlantic Coast of Georgia (Evers & Burson 2004). It was reported that it reproduces by obligate aposporous apomixis (Burson & Bennett 1970) or by facultative apomixis with a low degree of residual sexuality (Sartor *et al.* 2011).

Paspalum rojasii is naturally distributed in eastern Paraguay, southern Brazil and northeast Argentina. There is some controversy about its species concept and its taxonomic status (see Oliveira & Valls 2008). Therefore, the reports on cytology and reproductive mode (Bashaw *et al.* 1970; Burson & Bennett 1971) might refer to a different species. These authors made no reference to herbarium vouchers, so it is not possible to affirm whether we are dealing with the same species or not. We used in the present study the same plant material reported as tetraploid apomictic by Galdeano *et al.* (2016) whose herbarium-collected sample is now identified as *C.L.Quarin no. 4363*.

Two indigenous apomictic tetraploid accessions of *P. plicatulum* were used as pollen donors in the intraspecific crosses: the accession ML5 is a natural tetraploid (Novo *et al.* 2017) native to Uruguay, and the accession Hojs388 is an apomictic tetraploid (Galdeano *et al.* 2016) from Corrientes, Argentina, which represents a particular biotype of the species.

MATERIAL AND METHODS

Plant material

One sexual autotetraploid plant of *P. plicatulum* was used as female parent and seven accessions belonging to five apomictic tetraploid species were used as male parents in hybridisation experiments as detailed in Novo *et al.* (2017), except for *P. lenticulare*, which is detailed below. The female autotetraploid

parent was previously generated by doubling the chromosome number of a sexually reproducing diploid (2n = 2x = 20) plant of *P. plicatulum*. It is self-incompatible but cross-compatible and reproduces by sexuality. Originally identified as the '4c-4x' plant (Sartor *et al.* 2009), this genotype was then named 4PT (Aguilera *et al.* 2011; Novo *et al.* 2013), and herbarium specimens (*C.L.Quarin & P.E.Novo no. 4367*) are deposited in CTES, K, US, MO, SI, CEN, NY, BR, MA, UEC.

The identification of accessions, name of the collector and its respective number (in italics), and official acronyms of the herbaria (as referred in the Index Herbariorum) housing each specimen and their duplicates are as follow (the first Herbarium holds the original specimen and the subsequent Herbaria contain the duplicates): *P. plicatulum* accession ML5, *C.L.Quarin no. 4367* (CTES, MO); accession Hojs388, *D.Hojsgaard no. 388* (CTES, SI, BAB, SPF); *P. compressifolium* accession AK40811, *A.Krapovickas no. 40811* (CTES, MBM, GH); *P. lenticulare* accession V11893, *C.L.Quarin no. 4024* (CTES, US, K, BAA) cultivated at Corrientes, Argentina, from rhizomes collected 16 km south of Ponta Porá, Serra de Amambai, Brazil (22°48′10″ S 55°39′17″ W) in 1988; *P. nicorae* accession PI508821, *C.L.Quarin & P.E.Novo no. 4366* (CTES, K, US, MO, SI, CEN); accession CPI27707, *C.L.Quarin no. 4365* (CTES, K, US, MO, SI, CEN, NY, BR, MA, UEC); and *P. rojasii* accession AK40732, *C.L.Quarin no. 4363* (CTES, US, SI). Some duplicate specimens have been sent recently to the above-mentioned herbaria.

Cytogenetic and analysis of chromosome homologies

Young inflorescences of plants growing in the field or in a greenhouse were fixed in a 5:1 solution of absolute alcohol and lactic acid for 24 h and conserved in 70% ethyl alcohol at 4 °C. Meiotic chromosome behaviour was examined in microsporocytes by the aceto-carmine anther squash technique with a light-transmitted or phase contrast microscope.

The concept of homology used in this work implies that homologous chromosomes have similar length, gene position and centromere location. The position of the genes on each homologous chromosome is the same, however the genes may contain different alleles. Homologous chromosomes pair at meiosis. Homologous genomes are basic chromosome sets that share homologous chromosomes.

The criteria for cytogenetic analysis were as follow: since the female parent 4PT *P. plicatulum* is an autotetraploid plant obtained experimentally by chromosome doubling of a diploid cytotype, all hybrids received two homologous genomes (chromosome sets). Therefore, during the first meiotic division, each homologous chromosome of the two sets contributed by the female parent would primarily pair by autosyndesis. In this way, it is assumed that the 20 chromosomes contributed by the mother plant would join in ten autosyndetic associations. Any other bivalent (II) association observed in PMCs beyond those expected ten bivalents should be attributed to autosyndetic pairing between chromosomes of the two chromosome sets provided by the male parent. Theoretically, a cross between the induced autotetraploid 4PT parent with other natural autotetraploid species would generate an allotetraploid hybrid forming 20 II at meiosis I provided they have no homology. However, the formation of multivalent associations in the hybrids would imply homology between chromosomes of both parents by allosyndetic pairing and would be an association between two maternal chromosomes sharing homology with two homologous chromosomes from the male parent. Considering these theoretical assumptions, we estimated (I) the number of male parent chromosomes per PMC involved, and (II) in autosyndetic pairing (MPCau); (III) the number of male-parent chromosomes per PMC involved in allosyndetic pairing. For the first estimation, it was considered that each II and each III had two chromosomes joined by autosyndesis, and that each IV is formed by four chromosomes involved in autosyndetic associations (two from each parent); extremely rare hexavalent (VI) was also considered as three synapsed couples of chromosomes primarily associated by autosyndesis. The induced autotetraploid female parent (4PT *P. plicatulum*) never showed multivalent associations higher than IVs (Sartor *et al.* 2009). Therefore, the presence of hexavalents in the hybrids would only be expected when a male parent has multivalent associations higher than IVs. The number of male parent chromosomes synapsed by autosyndesis (MPCau) was estimated as follows: $MPCau = [(b.2) + (t.2) + (q.4) + (h.6)] - 20$, where the number of chromosome associations per pollen mother cell is represented by b, bivalents; t, trivalents; q, quadrivalents; and h, hexavalents. The estimated number of male parent chromosomes involved in allosyndetic associations (MPCal) was: $MPCal = t + [(q.2) + (h.4)]$.

Reproductive mode

The reproductive mode was determined for the native accessions of male parents that had not been studied previously. All hybrids that survived and set seed during their first growing season were also investigated for reproductive mode. We followed the flow-cytometric seed screening (FCSS) method developed by Matzk *et al.* (2000), according to the procedures previously described by Aguilera *et al.* (2015). Briefly, gametophytic apomixis in the genus *Paspalum* occurs through apospory followed by parthenogenesis and pseudogamy, with rare exceptions (Ortiz *et al.* 2013). Any new seed formed *via* apomixis has an embryo with a DNA content of 2C as a result of apospory + parthenogenesis (2n + 0), and an endosperm with a DNA content of 5C as a result of pseudogamy and fertilisation of the two unreduced polar nuclei (2n) of the aposporous embryo sac with a reduced sperm nucleus (n). This 2C:5C ratio between embryo and endosperm of apomictically formed seed contrasts with the usual 2C:3C DNA content ratio usual in sexual seeds of angiosperms. In a sexually formed seed, the embryo has a value of 2C and the endosperm of 3C due to the double fertilisation process: embryo (n + n) and endosperm [(n + n) + n]. A total of 20 seeds were examined for each male parent accession and 20 for each hybrid. When all seed from a plant developed *via* apospory + parthenogenesis + pseudogamy (embryo:endosperm ratio 2C:5C), the plant was classified as apomictic. When a plant produced most seeds with 2C:5C embryo:endosperm ratio, and some with 2C:3C ratio, it was classified as apomictic with residual sexuality. If all analysed seeds showed 2C:3C ratio, the plant was considered to be sexual.

RESULTS

Cytogenetics

The accession V11893 of *P. lenticulare* was tetraploid (2n = 40), determined in meiotically dividing meiocytes

(Fig. 1A, B). All other male parent accessions were previously reported to be tetraploid (see Table 1 in Novo *et al.* 2017) and their meiotic chromosome behaviour was determined in the present study (Table 1). The meiotic behaviour of the female parent, 4PT *P. plicatulum*, was analysed by Sartor *et al.* (2009). All pollen donor accessions, used for intraspecific as well as for interspecific crosses, showed mainly II and IV chromosome associations with occasional III plus some unpaired chromosomes (univalents, I). In addition, a very occasional VI or an octovalent (VIII) were observed in *P. rojasii* (Table 1). There was a clear prevalence of chromosomes synapsed forming II and IV in all male parents, as illustrated as an example in Fig. 1C for *P. nicorae* accession CPI27707, although important variations in the number and average of different chromosome associations were observed (Table 1). The maximum number of II per cell (mean 18.9, range 10–20) and the minimum number of IV (mean 0.3, range 0–2) were observed in the accession Hojs388 of *P. plicatulum* (Fig. 1D). On the other hand, the individual plant AK40811 of *P. compressifolium* had the minimum number of II per cell (mean 9.8, range 4–15) and maximum number of IV (mean 4.8, range 2–7) among all male parent plants, according to cytological data reported previously by Quarin *et al.* (1996). Another male parent having a high number of chromosomes associated as quadrivalents was *P. rojasii* (Fig. 1E), which in addition had some rare VI or VIII. The frequency of IV in chromosome configurations at meiosis indicated that none of these tetraploid species could have been originated by a typical allopolyploid process. Moreover, the number of II chromosome associations observed in intraspecific as well as in interspecific hybrids was consistently high (Fig. 1F, G). With the exception of 4PT × Hojs388 intraspecific *P. plicatulum* hybrids and the interspecific 4PT × *P. rojasii* for which we observed 18 II as a maximum per PMC, all other hybrids showed some PMC with 20 II. In all intra- and interspecific hybrids, it was estimated that most of the 20 chromosomes afforded by their male parents were associated by autosyndesis (Table 1). On average, the number of male-parent chromosomes associated primarily by autosyndesis (MPCau) varied from 14.6 in 4PT × Hojs388 *P. plicatulum* hybrids to 19.5 in *P. plicatulum* × *P. rojasii.* There was a wide range of MPCau when the male parent was accession Hojs388 of *P. plicatulum* (8–20 per PMC), while the range observed in hybrids of *P. rojasii* was quite narrow (18–20 per PMC). The total amount of 20 chromosomes contributed by each male parent was involved in autosyndetic pairing at least in some PMC of all hybrids (Fig. 1H), suggesting homology or different degrees of homeology between both chromosome sets (genomes) provided by male parents. The maximum possible of 20 MPCau was observed in all different hybrids (Table 1).

In addition to or in spite of the number of MPCau, several male parent chromosomes associated by allosyndesis (MPCal) with a couple of female parent chromosomes to form trivalents, or more frequently quadrivalents, and also some unusual hexavalents in the particular case of 4PT *P. plicatulum* × *P. rojasii.* On average, the number of male parent chromosomes involved in allosyndetic associations varied from 1.1 per PMC (range 0–6) in the hybrid 4PT *P. plicatulum* × PI508821 *P. nicorae* (Table 1, Fig. 1I) to 7.0 per PMC (range 2–12) in 4PT *P. plicatulum* × *P. rojasii* (Table 1).

Reproductive mode

The results of our analysis regarding the reproductive mode of four male parent accessions are presented in Table 2, together with previous reported results for the remaining three male parents. The ML5 accession of *P. plicatulum* showed a relative DNA content that fit the 2C:5C ratio in the embryo and the endosperm. The accession V11893 of *P. lenticulare* and the accessions CPI27707 and PI50881 of *P. nicorae* also produced seeds with an embryo:endosperm DNA content ratio of 2C:5C, although some seeds showed a 2C:3C ratio. The results suggest that accessions of *P. nicorae* and *P. lenticulare* have some residual genetic capacity for occasional sexual reproduction. If residual sexuality exists in ML5 accession of *P. plicatulum*, it could not be detected because all 20 seeds developed *via* apomixis.

A brief analysis of the reproductive mode conducted in hybrids from crosses between the sexual 4x plant and seven apomictic 4x accessions demonstrated that a proportion of individual plants of the hybrid progenies inherited apomixis from the male parent (Table 3), with the exception of the cross *P. plicatulum* 4PT × *P. compressifolium* AK40811. The inheritance of apomixis could not be proven in this cross because only one hybrid survived, and the analysis indicated that it was sexual. Because it is expected that apomixis and sexuality segregate in the progeny, it is necessary to analyse several individuals in the progeny to assess whether or not apomixis can be transmitted to the progeny of this cross. Most apomictic hybrids showed some degree of residual sexuality.

Specific observations and comments on morphological, phenological and biogeographic particularities: Nomenclatural and taxonomic issues

All accessions used in this research show transverse wrinkles across sterile lemma, which is one of the most typical morphological characteristics of the species belonging to the Plicatula group of *Paspalum* (Fig. 2A).

The Uruguayan accession ML5 of *P. plicatulum* flowers early in the spring. When cultivated in Corrientes, Argentina; full flowering occurred around mid-October. The general morphological characteristics of this accession represent the common concept of the species, although it has the green-bluish foliage, which is a distinguishing property of most collections of the species from the central region of Uruguay.

The accession Hojs388 of *P. plicatulum* has a caespitose growth habit, typical of this species, which is characterised by a variable number of erect shoots bunched together in the plant base due to their intravaginal origin from a dense clump of very short vertical rhizomes. Although the accession Hojs388 shares this specific habit with the common type of the species, it is distinguished by some occasional extravaginal cataphyllous rhizomes, usually not longer than 5 cm, bent upward to build a new shoot emerging from the ground just a little beyond the clumped intravaginal shoots (Fig. 2B). This accession belongs to an special biotype of *P. plicatulum*, inhabiting rice fields and neighbouring areas, where it is usually regarded as a weed. This biotype has some variation in the leaf-blades shape and width.

Paspalum compressifolium is morphologically related to *P. plicatulum*, characterised by flattened culms, covered by

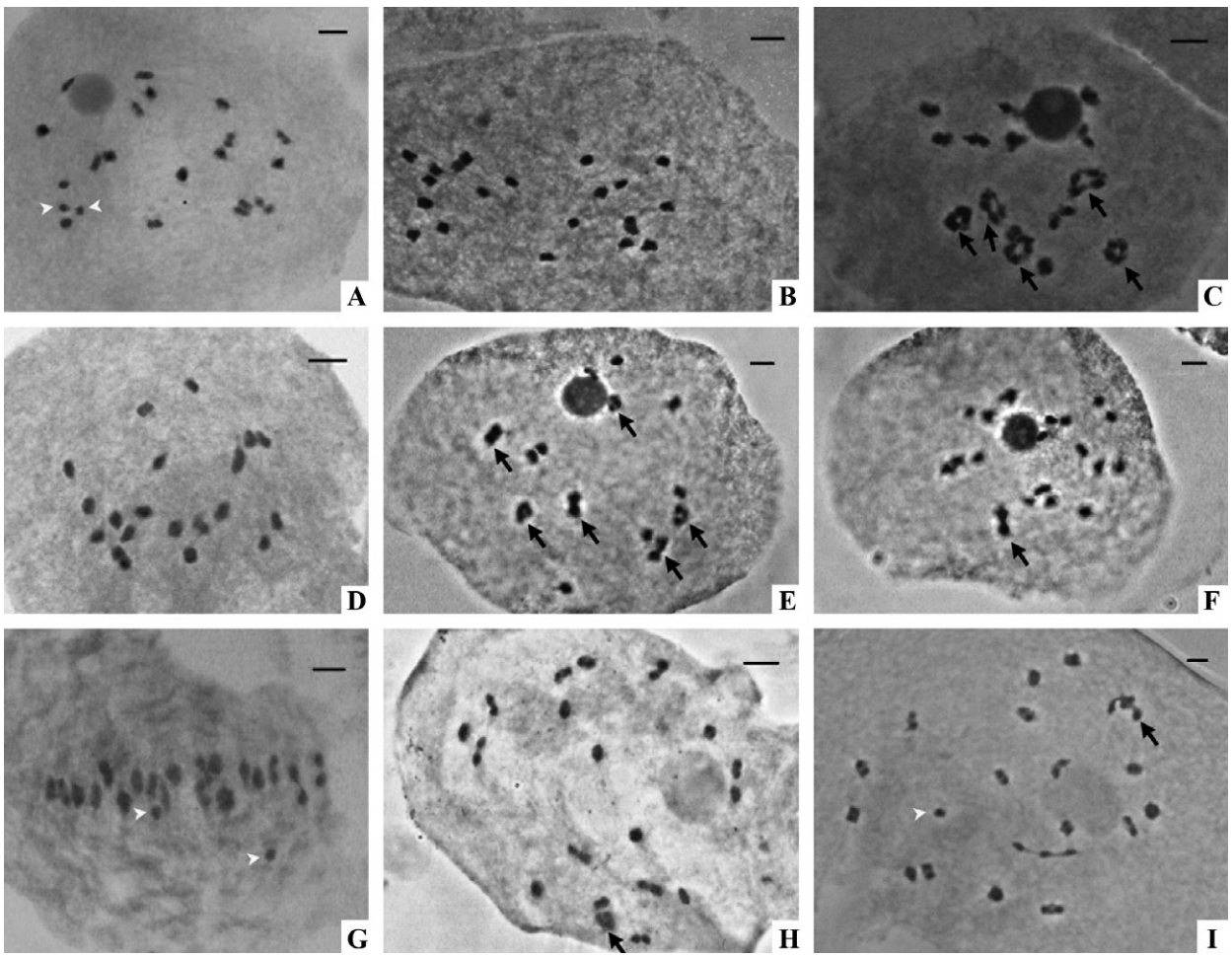

Fig. 1. Meiotic chromosomes of *Paspalum* species and hybrids. A–B: accession V11893 of *P. lenticulare*, diakinesis with 2 I + 19 II, and 20 chromosomes at second metaphase. C: *P. nicorae* accession CPI27707, diakinesis with10 II + 5 IV. D: *P. plicatulum* accession Hojs388, first prometaphase with 20 II. E: *P. rojasii*, diakinesis with 8 II + 6 IV. F: *P. plicatulum* 4PT × *P. rojasii* hybrid, diakinesis with 18 II + 1 IV. G: *P. plicatulum* 4PT × *P. nicorae* PI508821 hybrid, first metaphase with 2 I + 19 II. H: *P. plicatulum* 4PT × *P. compressifolium*, 18 II + 1 IV. I: 4PT *P. plicatulum* × PI508821 *P. nicorae* hybrid with a configuration of 1 I + 16 II + 1 III + 1 IV, which implies at least three male parent chromosomes involved in allosyndetic associations. All bars = 5 μm. White arrowheads point to univalents (I) and black arrows to quadrivalents (IV).

laterally compressed, equitant and conspicuously keeled sheaths (Fig. 2C–E).

The name *P. lenticulare* was largely considered to be a synonym of *P. plicatulum* as proposed by Chase (1929). It was accepted by Killeen (1990) as a good species occurring in the region of Chiquitanía, Bolivia. Oliveira & Valls (2008) also accepted the species, indicating that it differs from *P. plicatulum* mainly because it has multi-nodal culms, branched usually from the middle or upper nodes, a characteristic that become especially noticeable at flowering. Moreover, these authors included *P. limbatum* Henrard, *P. formosum* Swallen and *P. pontanale* Swallen in the synonymy of *P. lenticulare*. We accept and follow this criterion in the present study. We also observed differences in the flowering time: in *P. plicatulum* typically occurs during the morning, while in all tetraploid accessions of *P. lenticulare* cultivated at Corrientes, Argentina, including accession V11893, the full blooming of the spikelets occurs a few hours after midday. The species occurs in Central and South America (Colombia, Venezuela, Brazil, Bolivia, Paraguay and Argentina) in seasonally inundated and humid savannas. The accession V11893 of *P. lenticulare* shares most morphological and physiological characteristics with other accessions of the species introduced from Brazil, Paraguay and Bolivia to Corrientes, Argentina, except for the flowering season. Most accessions flower during a short period in mid-autumn, while accession V11893 starts flowering in early summer. This accession develops progressive flowering branches from culm nodes usually after seed maturity and spikelet shattering occurred in the terminal inflorescence. Thus, flowering lasts for the whole summer until early autumn.

Although the exact data of *P. nicorae* introduction into the USA is unknown, it became naturalised in sandy clay loam soils of the Gulf Coast states and the Atlantic Coast of Georgia (Evers & Burson 2004). It is known as Brunswick grass in the USA because it was first collected near Brunswick, Georgia, in 1945 and transplanted to the Soil Conservation Service nursery, Americus, Georgia, by Paul Tabor. Grown under experimental number SC 20-672, it was described as a rhizomatous species

Table 1. Meiotic chromosome pairing in a sexual tetraploid (2n = 4x = 40) *Paspalum plicatulum*, in several apomictic tetraploid species and their hybrids.

species or hybrids	no. of plants	no. of PMC	average and range per cell						MPCau[a]	MPCal[b]
			I	II	III	IV	VI	VIII		
P. plicatulum 4PT[c] (female parent)	1	53	0.1 (0–1)	14.2 (6–18)	0.1 (0–1)	2.8 (1–7)	–	–	–	–
Male parents and intraspecific crosses										
P. plicatulum Hojs388	1	51	1.1 (0–12)	18.9 (10–20)	–	0.3 (0–2)	–	–	–	–
4PT × Hojs388 hybrids	5	48	4.7 (0–11)	13.7 (0–18)	0.7 (0–2)	1.5 (0–4)	–	–	14.6 (8–20)	3.6 (0–8)
P. plicatulum ML5	1	61	1.7 (0–6)	16.7 (10–20)	0.2 (0–2)	1.1 (0–3)	–	–	–	–
4PT × ML5 hybrids	2	20	2.2 (0–6)	17.0 (12–20)	0.1 (0–1)	0.9 (0–3)	–	–	17.7 (14–20)	2.0 (0–6)
Male parents and interspecific crosses										
P. compressifolium AK40811[d]	1	26	0.7 (0–3)	9.8 (4–15)	0.2 (0–2)	4.8 (2–7)	–	–	–	–
4PT × AK40811 hybrids	1	28	1.3 (0–4)	15.9 (11–20)	0.4 (0–2)	1.4 (0–4)	–	–	18.3 (16–20)	3.2 (0–8)
P. lenticulare V11893	1	32	0.7 (0–4)	15.4 (11–20)	0.1 (0–1)	2.0 (0–4)	–	–	–	–
4PT × V11893 hybrids	1	44	1.4 (0–4)	17.4 (14–20)	0.1 (0–1)	0.8 (0–2)	–	–	18.5 (16–20)	1.8 (0–5)
P. nicorae PI508821	1	31	1.5 (0–6)	15.5 (7–20)	–	1.8 (0–5)	–	–	–	–
4PT × PI508821 hybrids	5	70	1.6 (0–6)	18.0 (13–20)	0.2 (0–2)	0.5 (0–3)	–	–	18.3 (14–20)	1.1 (0–6)
P. nicorae CPI27707	1	23	0.8 (0–4)	15.3 (10–20)	–	2.2 (0–5)	–	–	–	–
4PT × CPI27707 hybrids	1	16	1.1 (0–4)	17.6 (12–20)	–	0.9 (0–4)	–	–	18.9 (16–20)	1.9 (0–8)
P. rojasii AK40732	1	32	1.1 (0–5)	12.1 (4–18)	0.1 (0–1)	3.3 (0–6)	0.1 (0–1)	0.1 (0–1)	–	–
4PT × AK40732 hybrids	2	30	0.4 (0–2)	12.7 (8–18)	0.2 (0–1)	3.4 (1–6)	0.03 (0–1)	–	19.5 (18–20)	7.0 (2–12)

[a]Average number and range per pollen mother cell of male parent chromosomes associated primarily by autosyndesis.
[b]Average number and range per pollen mother cell of male parent chromosomes involved in multivalent associations by allosyndesis.
[c]From Sartor *et al.* 2009.
[d]From Quarin *et al.* 1996.

Table 2. Reproductive mode of five indigenous tetraploid *Paspalum* species.

species	accession	reproductive mode	references
P. plicatulum Michx	Hojs388	A	Galdeano *et al.* 2016
	ML5	A	This work
P. compressifolium Swallen	AK40811	A	Quarin *et al.* 1996
P. lenticulare Kunth	V11893	A/rS	This work
P. nicorae Parodi	CPI27707	A/rS	This work
	PI508821	A/rS	This work
P. rojasii Hackel	AK40732	A	Galdeano *et al.* 2016

A = apomictic; A/rS = apomictic with residual sexuality.

Table 3. Reproductive mode of individual plants of F_1 hybrid families derived from crosses between one sexual and seven apomictic genotypes of the Plicatula group of *Paspalum*.

crosses	number of analysed plants	reproductive mode		
		S	A	A/rS
P. plicatulum 4PT × *P. plicatulum* Hojs388[a]	18	7	1	10
× *P. plicatulum* ML5	6	–	1	5
× *P. compressifolium* AK40811	1	1	–	–
× *P. lenticulare* V11893	4	1	–	3
× *P. nicorae* PI508821	15	4	–	11
× *P. nicorae* CPI27707	1	–	–	1
× *P. rojasii* AK40732	7	5	1	1

S = sexual, A = apomictic; A/rS = apomictic with residual sexuality.
[a]Novo *et al.* 2017.

generally similar to bahiagrass, but with more than two seed racemes per stem (Hanson 1959). The species was introduced to cultivation by direct increase of particular accessions. In the Forages Fact Sheets of the Tropical Forages website, four cultivars are mentioned: two released in Georgia, USA, as cover plants in waterways or for seeding eroded areas, and two in Australia for turf. In addition, the accession CPI27707, used in the present research, has been considered a promising candidate for forage production (http://www.tropicalforages.info/key/Forages/Media/Html/Paspalum_nicorae.htm).

Oliveira & Valls (2008) placed the name *P. nicorae* Parodi as a synonym of *P. lepton* Schult., based on the leptomorphic rhizome observed in the photograph of the herbarium sheet containing the specimen that has been designed as the holotype of *P. lepton*, conserved in the PH Herbarium in Philadelphia. The photograph is available at http://plants.jstor.org/stable/10.5555/al.ap.specimen.ph00019182 in the JSTOR database of digitised plant specimens. The nomenclatural status of *P. lepton* is as follow: it was described with the name *Paspalum gracile* J. Le Conte, J. Phys. Chim. Hist. Nat. Arts 91:285. 1820, based on the collection: 'Muhlenberg. Gram. Sub Paspalo, *no.* 8. Habitat in Georgia'. Then J.A. Shultes, Mant. 2: 173. 1824, considering that '*gracile*' was an illegitimate name, because *P. gracile* was a name used previously by Rudge (Pl. Guian. 20, t. 26. 1805),

Fig. 2. Morphological characteristics of species of the Plicatula group of genus *Paspalum*. A: transverse wrinkles across sterile lemmas, a common feature of Plicatula group. B: Accession Hojs388 of *P. plicatulum* showing an occasional extravaginal cataphyllous rhizome emerging together with a dense clump of erect intravaginal shoots. C–E: Accession AK40811 of *P. compressifolium*; plant with bluish green leaves, and details of the base of a shoot with laterally compressed equitant leaf sheaths, and the typical inflorescence. F–G: *P. nicorae*, accessions PI508821 and CPI27707, respectively; root system together with profuse subterranean stems of long bowed rhizomes. H–J: *P. rojasii*, herbarium specimen *C.L.Quarin no. 4363* deposited at CTES, and details of the sheath–blade junction, and the dorsal view of a spikelet. Magnification bars: A = 1 mm; B, F and G = 2 cm; C = 10 cm; D and E = 1 cm; I and J = 1 mm.

assigned the new name *Paspalum leptos* Schult. (correct name: *P. lepton*) to the plant material described by J. Le Conte. The American botanist H.E. Muhlenberg (1753–1815), native of Pennsylvania, left his herbarium and types to the PH Herbarium. The question is whether the plant specimen whose photograph was used to synonymise *P. nicorae* Parodi with *P. lepton* Schult. is really the true specimen described by J. Le Conte. The herbarium sheet with this specimen is conserved at PH Herbarium, number 01076650, barcode symbol with number 00019182, and a stamped coloured script of 'TYPE COLLECTION'. Curiously enough, there is a complete absence of information on the herbarium sheet regarding the collector name, the collection locality and date. A label in Le Conte's handwriting: '*Paspalum gracile mihi*' is sticking below the specimen mounted on the right side of the sheet. However, the whole sheet should have been mounted more recently than Le Conte' s annotation and therefore it is not possible to determine to which specimen it refers. Moreover, there is just a small label with the printed name of the botanist Agnes Chase, an authority in grass systematics, with a handwritten annotation 'accepted as type of *P. gracile* LC = *P. plicatulum* Mix.' Despite the fact that the annotation on this label certainly belongs to the Chase's script, it remains doubtful whether this label, which has no date, was destined for the specimen mounted on that herbarium sheet as observed nowadays. The doubt arises from Chase's comments in her taxonomic revision of the North American species of *Paspalum* (Chase 1929): 'A Le Conte specimen, with the name in his script, in the Academy of Natural Sciences, Philadelphia, is the upper part of a culm with 4 racemes. This is accepted as the type since it agrees with the description. . .'. Then, Chase indicated that the name was presumably changed to *P. leptos* because the existence of the name *P. gracile* Rudge, and pointed out that 'Schultes quotes Le Conte's description'. It seems evident that her comments were not referring to the present herbarium sheet, whose photograph was used to select the holotype by Oliveira & Valls (2008). This specimen could not be described by Chase as 'the upper part of a culm. . .' because there are two pieces mounted on the sheet: on the left side, an entire culm bearing a cataphyllous rhizome of over 10-cm long in the base and an inflorescence of four racemes in the tip, and on the right side an upper part of a culm with four racemes. Chase′s comments seem to refer to the specimen on the right side of the sheet only. The plant mounted on the left side probably corresponds to a different collection. She would not affirm that the specimen that she accepted as the type specimen was in agreement with the description, since the Le Conte's description had not mentioned any kind of rhizome, while in the photograph of the specimen, now selected as holotype, such a large rhizome is clearly evident. In our opinion, only the specimen mounted on the right side should be considered the holotype of *P. gracile* J. Le Conte. Therefore, we prefer to maintain *P. nicorae* Parodi as a valid name until doubts about the type specimen described by Le Conte are resolved. When *P. nicorae* was synonymised with *P. lepton* (Oliveira & Valls 2008), even the authors expressed some doubts when they indicated that the collector name and the collection locality of the specimen, whose photograph they saw, was probably Muhlenberg, since the name of the collector is not written on the herbarium sheet. In addition, it is worth noting that the specific epithet '*nicorae*' was used by Parodi (1943) as a new name for *P. plicatulum* var. *arenarium* Arechav., rising the variety of Arechavaleta to the species category, and considering that the name *P. arenarium* was illegitimate since it had been previously used. The type specimen of *P. plicatulum* var. *arenarium* Arechav. is conserved in the Herbarium of the National History Museum in Vienna, a photograph of which is available at the database http://plants.jstor.org/stable/10.5555/al.ap.specimen.w19160035184. The accessions PI508821 and CPI27707 have long, arched, cataphyllous rhizomes (Fig. 2F, G) and general morphological characteristics as observed in the type specimen, although accession PI508821 is a biotype with narrower leaves than what is typical for the species.

The botanical name of *P. rojasii* Hack. has been erroneously applied to plant material related to different strains of *P. guenoarum* Arech. This nomenclatural confusion was partially clarified by Oliveira & Valls (2008). These authors focused the controversy on the different morphological characteristics between the two species and in the justification for including *P. kempfffii* Killeen and *P. macedoi* Swallen into the synonymy of *P. rojasii* Hack. However, there are still incongruities between some morphological characteristics of *P. rojasii* described by Oliveira & Valls (2008) and the original Hackel's botanical description. For example, number of nodes per culm: 3–4 *versus* 5–6; spikelet size variation of 2.8–4.0 mm width × 2.1–7.0 mm length *versus* 2.5 × 3.5 mm, respectively. Moreover, it should be noted that Hackel originally described a hairy plant with dense adpressed white-greyish pubescence covering the leaf sheaths, both sides of leaf blades and the spikelets. According to the original description the inflorescences have two racemes, erect for the uppermost one, ascending and somewhat separated from the main axis for the second one, as may be observed in the photograph of the type specimen (collector *T.Rojas no. 10122*) conserved in BM Herbarium of the British Museum of Natural History, London. There is a fragment of the specimen *T.Rojas no. 10122* in the Gaspar Xuárez Herbarium (BAA), University of Buenos Aires, Argentina, together with a drawing made by the botanist L.R. Parodi in 1935 from a duplicated specimen of *T.Rojas no. 10122* conserved at the Genève Herbarium (G), Conservatoire et Jardin Botaniques de la Ville de Genève, Switzerland. The observation of the fragment and the drawing confirmed the dense pubescence covering the leaves, the sterile lemma and the glume of spikelets, as well as a few erect or semi-erect racemes per inflorescence. The accession AK40732 was obtained through seed gathered from the herbarium specimen *A.Krapovickas no. 40732* collected in 1987, and maintained since then under cultivation at FCA-UNNE, Corrientes, Argentina. A herbarium sample collected from the cultivated plant, bearing the collection number *C.L.Quarin no. 4363*, was deposited at the CTES Herbarium, Corrientes, Argentina. It has two or three (rare four) racemes per inflorescence and densely pubescent leaves and spikelets. Both specimens, *A.Krapovickas no. 40732* and *C.L.Quarin no. 4363* (Fig. 2H–J), match the original Hackel's botanical description and the morphological characteristics observed in the photograph of the type specimen conserved at BM Herbarium.

DISCUSSION

The female parent is a synthetic plant obtained by doubling the chromosome number of a diploid plant. Consequently, it has

four ten-homologous chromosomes sets, which theoretically might associate to form ten quadrivalents at meiosis. However, the complete association of all homologous chromosomes to form exclusively quadrivalents is a very rare event in autotetraploid plants. Bivalents and quadrivalents are usually observed at meiosis in autotetraploids. Our synthetic autotetraploid forms an average of 2.8 quadrivalents at meiosis, with a maximum of 7. All natural tetraploids used as male parents formed some quadrivalents per pollen mother cell (PMC), varying from 0.3 to 4.8 (average per PMC), with maximum quadrivalents per PMC from 2 to 7. Comparing these results with the meiotic chromosome paring of the synthetic autotetraploid, it could be assumed that, in general, these natural apomictic tetraploids originated by autoploidy or by segmental alloploidy.

The mean and the maximum quadrivalent chromosome associations of both natural accessions of *P. plicatulum* (Hojs388 and ML5) were low in comparison with the equivalent values observed in the colchicine-induced autotetraploid genotype 4PT (mean 2.8 and maximum of 7 per PMC). This behaviour suggests that accessions Hojs388 and ML5 of *P. plicatulum* are not typical autotetraploids. This may be especially true for accession Hojs388, which had a high number of bivalents per PMC (mean = 18.9) and a low number of quadrivalents (mean 0.3, maximum 2 per PMC). However, the average number of male parent chromosomes associated by autosyndesis (MPCau) observed in 4PT × Hojs388 hybrids was the lowest one (mean 14.6) among all recovered inter- or intraspecific hybrids, and varied from 8 to 20 s per PMC. Therefore, the maximum value of 20 MPCau in the hybrids and the wide range of variation (8–20 per PMC) could indicate that Hojs388 made a contribution of two partially homologous chromosome sets to its intraspecific hybrids. Each one of the ten chromosomes of one set should be homologous or may have some degree of homology with each one of the second set, because in at least some PMC we observed up to 20 MPCau. A similar behaviour of chromosome pairing was observed in accession ML5 of *P. plicatulum* and its intraspecific hybrids with 4PT genotype. However, the number of MPCau (mean 17.7) was higher and less variable (range 14–20 per PMC) in 4PT × ML5 than in 4PT × Hojs388 hybrids. Therefore, both indigenous accessions of *P. plicatulum*, Hojs388 and ML5, may be considered segmental allotetraploids, which contributed to their hybrids with two homeologous chromosome sets, although there are more affinities between the two chromosome sets of ML5 than between the two sets of Hojs388. It should be noted that the intraspecific hybrids between 4PT × Hojs388 indicated that there is a higher possibility of gene exchange between Hojs388 and 4PT than between 4PT and ML5 because the average and the total number of chromosomes involved in allosyndetic associations (MPCal) in 4PT × Hojs388 (mean 3.6; maximum 8 per PMC) was higher than in 4PT × ML5 (mean 2; maximum 6). The hybrid families obtained from 4PT × ML5 and 4PT × Hojs388 crosses are fertile (Novo *et al.* 2017) and share some homologous chromosomes which pair during meiosis. Thus, gene transfer is a potential target for a genetic plant improvement programme. Considering that accessions Hojs388 and ML5 of *P. plicatulum* and their hybrids with the synthetic autotetraploid genotype fit the cytological parameters of segmental allotetraploids, the question remains concerning their type of polyploidisation process. The question is whether they originated from independent hybridisation events between two closely related diploid species, which then naturally duplicated their chromosomes and simultaneously acquired the ability for apomictic reproduction, or whether accessions Hojs388 and ML5 originated as new apomictic autotetraploids, derived from two different diploid biotypes of *P. plicatulum* which then evolved toward segmental alloploidy through mutations or eventual hybridisations with a close related species.

Cytological analyses of two male parents used for interspecific crosses, *P. compressifolium* and *P. rojasii,* showed meiotic chromosome associations typical of autotetraploid plants. The plant material of *P. compressifolium* was the same individual plant of the accession AK40811, whose meiotic chromosome behaviour was previously reported by Quarin *et al.* (1996), with most chromosomes synapsed as bivalents or quadrivalents. Based exclusively on this meiotic chromosome behaviour, it was proposed that the tetraploid cytotype of *P. compressifolium* originated by autoploidy. Now the meiotic chromosome configurations observed in a 4PT *P. plicatulum* × *P. compressifolium* hybrid is in agreement with that proposition. The vast majority of the male parent chromosomes joined primarily by autosyndesis (MPCau) in the hybrid, indicating that the two chromosome sets received from *P. compressifolium* were homologous genomes. On the other hand, the meiotic chromosome behaviour suggests autoploidy for *P. compressifolium*, but the number of quadrivalents (mean 1.4, average 0–4 per PMC) and the MPCal observed in the *P. plicatulum* × *P. compressifolium* hybrid suggest partial homology or homeology between the chromosome sets afforded by each species. Consequently, these species share important variants of the same genomic formula.

Bivalent and quadrivalent chromosome associations also predominated in *P. rojasii*, suggesting autoploidy as the most reasonable assumption for the origin of tetraploid *P. rojasii*. The very occasional VI or VIII chromosome associations observed in *P. rojasii* might suggest the existence of some chromosome rearrangement among different chromosomes of the same chromosome set. Therefore, the unusual hexavalent observed in the hybrid 4PT *P. plicatulum* × *P. rojasii* should be composed by four chromosomes from *P. rojasii* and two from 4PT. The number of IVs (mean 3.4, average 1–6) observed in the *P. plicatulum* × *P. rojasii* hybrid indicated an important level of homology between chromosome sets provided by both species. We observed a high proportion of the male parent chromosomes synapsed by allosyndesis with chromosomes of the two homologous sets coming from autotetraploid female parent (mean 7.0, average 12 per PMC), which is a confirmation that *P. rojasii* is an autotetraploid, and that it shares basically the same genome formula with autotetraploid 4PT *P. plicatulum*. A new taxonomic issue emerges from these results and from some morphological similarities observed between the accession AK40732 of *P. rojasii* and the plant from which the 4PT plant was obtained (diploid *P. plicatulum*, accession AH14). They share a high genome homology, a general hairiness covering the whole ground plant organs, and a few racemes per inflorescence (usually two or three), butthe accession AH14 has smaller spikelet (average 2.69-mm long × 1.59-mm wide) than *P. rojasii* (average 3.5-mm long × 2.5-mm wide). The accession AH14 might be the diploid cytotype of *P. rojasii* rather than a strain of the diploid

cytotype of *P. plicatulum* as it was considered when chromosome duplication was done (Sartor *et al.* 2009) and in several subsequent reports. The taxonomic status of *P. rojasii*, the limits of its morphological variation, and its relationship with other species of the Plicatula group need further analyses.

The other male parents used for interspecific crosses were *P. lenticulare* V11893 and two accessions of *P. nicorae* (PI508821 and CPI27707). Their meiotic chromosome behaviours were not in agreement with typical allotetraploid origins. Otherwise, the low to moderate presence of quadrivalent associations per PMC might be an indication that these species do not have four completely homologous genomes (chromosome sets) of typical autotetraploids. However, a high average of MPCau in their interspecific hybrids was observed. Moreover, in all hybrids there were some PMCs having up to 20 MPCau, which is an indication that there is an important level of homology between chromosomes of the two genomes afforded by male parents. Therefore, tetraploid *P. lenticulare* and tetraploid *P. nicorae* could have originated through autoploidy from diploid cytotypes of two different species, both closely related to diploid *P. plicatulum* cytotype, probably followed by still incipient diploidisation events such as genome reorganisation and some structural chromosome changes. Autoploidy had been proposed for the tetraploid cytotype of *P. lenticulare* by Espinoza *et al.* (2001), based on cytological research involving five accessions collected in Paraguay, Bolivia and Brazil. Our cytological results in tetraploid accession V11893 agree basically with this previous report. Moreover, the high proportion of MPCau in *P. plicatulum* × *P. lenticulare* hybrid indicated that the two chromosome sets afforded by *P. lenticulare* were largely constituted by homologous rather than homeologous chromosomes. We obtained similar results in two accessions of *P. nicorae*. Many accessions of this species from very different geographic origins, embracing a wide range of its natural distribution were cytologically investigated by several authors (Bashaw *et al.* 1970; Burson & Bennett 1970; Fernandes *et al.* 1974; Pagliarini *et al.* 2001; Reis *et al.* 2008). All investigated accessions were tetraploid and bivalents and quadrivalents were the main chromosome associations observed during meiosis. In general, the authors suggested that *P. nicorae* could be considered either a segmental allotetraploid or an autotetraploid. Because there was a low average of quadrivalents per cell, the authors suggested that the species has two closely related but not identical genomes. The speculation that autoploidy was in the origin of *P. lenticulare* and *P. nicorae* is better endorsed in the case of *P. lenticulare* than in *P. nicorae*. Following the information of Espinoza *et al.* (2001), who reported diploidy and sexual reproduction for *P. limbatum* Henrard, and the nomenclatural criterion of Oliveira & Valls (2008), who synonymised *P. limbatum* with *P. lenticulare*, we recognise that a diploid cytotype of *P. lenticulare* exists, and it might be the basis for autoploidy of the tetraploid cytotype. The morphological and physiological characteristics of these materials consistently differed from typical *P. plicatulum*, with branched flowering culms, compressed blade sheaths, usually higher number of racemes per inflorescence, and specific time of flowering support the criteria of Killeen (1990) and Oliveira & Valls (2008) who considered *P. lenticulare* as a good species. We agree that these materials should deserve a specific name, although it is necessary to clearly identify the herbarium specimen mentioned in the Kunth's botanical description of *P. lenticulare*. Chase (1929) synonymised *P. lenticulare* Kunth to *P. plicatulum* Michx. She mentioned different specimens deposited in European herbaria that might be the plant described by Kunth. However, her comments indicated the difficulty in establishing which specimen has a better concordance with Kunth's description. Until such time as this is determined, we agree with Oliveira & Valls (2008) to use *P. lenticulare* as a valid botanical name for these materials.

Cytoembryological studies on ten accessions of *P. nicorae* introduced to the USA from South America led Burson & Bennett (1970) to the conclusion that the species reproduces by obligate apomixis through processes of apospory, parthenogenesis and pseudogamy. More recently, Sartor *et al.* (2011) analysed the ploidy level in three natural populations from northeast Argentina, combining classical chromosome counts in root tips of a few individuals and the DNA content of a total of 262 plants measured by flow cytometry, and all were tetraploid. The relative DNA content of embryo and endosperm tissues of seeds harvested from 30 plants per population indicated that the majority of the seed were formed through apomictic pathways (apospory + parthenogenesis + pseudogamy), showing a DNA embryo:endosperm ratio of 2:5, while 2.5–5.0% of the seeds were produced by means of sexuality (embryo:endosperm DNA ratio = 2:3), suggesting facultative apomixis with some chances for sexual reproduction. However, the potentiality for sexual reproduction of a facultative apomictic *Paspalum* species may change substantially depending upon the stage of the reproductive process when it was determined. The relative frequency of a sexual pathway, in relation to the apomictic pathway, declined drastically through consecutive stages of the plant life cycle, from gametogenesis to seed formation and adult offspring (Hojsgaard *et al.* 2013). Therefore, the low chances for sexual reproduction observed in *P. nicorae* at seed stage (2.5–5.0%) may result in a very occasional sexually originated individual among a large progeny. Notwithstanding, the potentiality exists and hence a new apomictic 4x genotype may be formed every so often by recombination. On the other hand, it is known that the degree of sexual reproduction may increase when the adequate environmental conditions for flowering decrease at the end of the season, as for example in *P. notatum* (Rebozzio *et al.* 2011). Similarly, it was demonstrated in *Eragrostis curvula* (Schrad.) Nees that different stress conditions can alter the expression of sexual reproduction in facultative tetraploid apomictic cultivars, and when the stress stops the reproductive mode shifts back to the apomixis original level (Rodrigo *et al.* 2017). If tetraploid *P. nicorae* originated by autoploidy, the diploid ancestor should be extremely rare in the wild or might have become extinct, because there are many references in the literature concerning the ploidy level of the species, but a diploid plant has never been found. If a diploid cytotype of *P. nicorae* exists, it should be restricted to a very special habitat. On the other hand, the hypothesis of an allotetraploid origin for this species through interspecific crosses is hard to endorse. All tetraploid species of the Plicatula group analysed so far are apomictic (Novo *et al.* 2017), hence hybridisation between two closely related tetraploid species should be an unlikely event, due to the low occurrence of effective sexual reproduction, and temporal and geographic reproductive barriers.

Our results confirm that in most species of the genus *Paspalum* there exists a strong association between apomixis and autoploidy or between apomixis and segmental alloploidy derived from original autoploidy; cytological analyses proved cytogenetic relationships between *P. plicatulum* and four different species of the Plicatula group: *P. rojasii, P. compressifolium*, *P. nicorae* and *P. lenticulare*, allosyndetic chromosome pairing and seed fertility of their hybrids suggest the feasibility of gene transfer among species.

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