

Complete asynapsis resulting in 2n pollen formation in *Paspalum jesuiticum* Parodi (Poaceae)

R.A. Bernardo Filho¹, A.C.C. Santos¹, F.H.D. Souza², J.F.M. Valls³ and M.S. Pagliarini^{1†}

¹Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Maringá, PR, Brasil

²Embrapa Pecuária Sudeste, São Carlos, SP, Brasil

³Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brasil

Corresponding author: A.C.C. Santos

E-mail: anacasalvara@hotmail.com

Genet. Mol. Res. 13 (1): 255-261 (2014)

Received December 7, 2012

Accepted December 6, 2013

Published January 17, 2014

DOI <http://dx.doi.org/10.4238/2014.January.17.9>

ABSTRACT. Cytological investigation revealed complete asynapsis during microsporogenesis in 2 wild accessions of *Paspalum jesuiticum* collected in distinct Brazilian regions. Both accessions were hexaploid ($2n = 6x = 60$) and 60 univalents could be counted at diakinesis. In this phase, the majority of meiocytes exhibited univalents with both chromatids. After alignment at the metaphase plate, the chromatids segregated to the poles. Only 1 meiotic division (equational) occurred, and after cytokinesis, a dyad with 2n microspores was formed. The genetic constitution of the 2n gametes was equivalent to that of first division restitution (FDR). Since recombination did not occur, 100% transmission of parental heterozygosity could be expected from the FDR 2n gametes. The meiotic behavior of both accessions suggested that they resulted

from a recent natural hybridization event. The potential use of the 2n gametes in *Paspalum* breeding programs has been discussed.

Key words: Meiosis; Poaceae; Interspecific hybridization; Hexaploidy; Polyploidy

INTRODUCTION

Chromosome pairing during prophase plays a critical role in the ensuing sequence of meiotic events. Successful chromosome pairing during the early stages of the first meiotic division affects the viability of the resulting gametes (Visser et al., 1999). Synaptic mutants are nonetheless common in the plant kingdom, with several reported in various higher plant families (Koduru and Rao, 1981) and most observed in the family Poaceae (Singh, 2003). These mutants, mostly spontaneous, may result either from interspecific hybridization or from induced mutation (Pazy and Plitmann, 1996; Qu and Vorsa, 1999; Singh, 2003). The partial or complete loss of chromosome pairing during prophase and metaphase I can be attributed to only 1 or 2 processes, namely asynapsis or desynapsis. Asynapsis is the absence of chromosome pairing during the first meiotic division, whereas desynapsis is the failure to maintain association after the first synapsis in prophase (Golubovskaya, 1979). The action of asynaptic genes is recognizable when most or all of the chromosomes remain as univalents at diakinesis and metaphase (Visser et al., 1999).

Paspalum is a large genus that includes several important forage species native to natural tropical and subtropical grasslands in the New World (Chase, 1929). Polyploids and tetraploids predominate in the majority of *Paspalum* species, and most polyploid species have proven to be apomictic, usually with sexual diploid conspecific counterparts (Quarín, 1992). In this report, we have described the occurrence of complete asynapsis in 2 native hexaploid accessions of *Paspalum jesuiticum* Parodi and the cytological process that results in all of the 2n gametes.

First described by Parodi (1969), *P. jesuiticum* is a peculiar species, morphologically resembling members of the informal groups *Livida* and *Dilatata*, established by Chase (1929). *P. jesuiticum* shows dense, creeping, stoloniferous growth and relatively tender leaves compared to other native forage grasses and also has the potential for erosion control and as a lawn grass.

P. jesuiticum grows in 3 southern Brazilian states (Rio Grande do Sul, Santa Catarina, and Paraná), and its range extends to the adjacent province of Misiones in Argentina (Denham et al., 2010). It also grows in the southeastern states of Brazil, São Paulo and Minas Gerais (Valls and Oliveira, 2012).

MATERIAL AND METHODS

Two native accessions of *P. jesuiticum* collected in distinct Brazilian regions were analyzed. The accession BRA-020648 (collection No. JValls 14185) was collected in Juiz de Fora, MG (21°46'S; 43°22'W; 885 m a.s.l.), and the accession BRA-025003 (collection No. FDS-010) was collected in São Bento do Sul, SC (26°23'S; 49°32'W; 750 m a.s.l.). Both accessions are maintained as live plants in the *Paspalum* germplasm collection at the Embrapa Pecuária Sudeste, located in São Carlos, SP.

Young inflorescences were collected and fixed in 3:1 ethyl alcohol:acetic acid for 24 h at room temperature, transferred to 70% (v/v) aqueous alcohol solution, and stored in a freezer until use. Meiocytes were obtained by squashing anthers in a drop of 1% propionic-carmin. Chromosome number was counted in meiocytes at diakinesis. All meiotic phases were evaluated under light microscopy and meiotic abnormalities were scored. A total of 774 meiocytes were evaluated in the accession BRA-020648 and 1242 in the accession BRA-025003. Photomicrographs were taken using an Olympus microscope (CX30) and the AnalySIS getIT software.

RESULTS AND DISCUSSION

Occurrence of asynapsis

Both accessions of *P. jesuiticum* revealed complete asynapsis at diakinesis (Table 1), at a point in which a total of 60 univalents (Figure 1A) were counted, revealing that the accessions were hexaploids ($2n = 6x = 60$). The sister chromatids could be visualized in each univalent (Figure 1B). One (Figure 1A) or 2 nucleoli (Figure 1B) were found in the meiocytes. Asynapsis has previously been shown to occur in a hexaploid accession of *P. jesuiticum* collected in the State of Rio Grande do Sul, Brazil (Fernandes et al., 1974), but no details of its meiotic behavior were provided. Asynapsis was also reported in an apomictic tetraploid accession of *P. secans* Hitchc. & Chase (Snyder, 1961). Interestingly, in the highlands and in the quaternary littoral southern region, the natural distribution area of *P. jesuiticum* overlaps with that of the Torres biotype, *P. dilatatum* Poir, another taxon with $2n = 60$ chromosomes and almost total asynapsis (Fernandes et al., 1968; Burson et al., 1991).

Table 1. Meiotic behavior in the asynaptic accessions of *Paspalum jesuiticum*.

Phase	No. of meiocytes	
	BRA-020648	BRA-025003
Diakinesis with 60 univalents	168	90
Metaphase with a non-congregated genome	118	221
Metaphase with 60 congregated univalents	122	322
Normal anaphase	45	7
Normal telophase	36	158
Normal cytokinesis after telophase	178	248
Dyad	107	196
Total	774	1242

To date, several studies have considered the relevance of asynapsis and desynapsis to plant breeding and to $2n$ gamete formation (Baker et al., 1976; Zickler and Kleckner, 1999).

Meiotic behavior and evidence of recent natural hybridization

Both accessions presented the same meiotic behavior (Table 1). Univalents varied between accessions in terms of their ability to reach the metaphase plate. While 40 univalents converged to the plate, 20 (10 + 10) were found either grouped or scattered between the poles (Figure 1C-G). Such meiotic behavior is typical of recently formed natural hybrids resulting from parental genomes with dissimilar meiotic rhythms. This phenomenon has previously

been found in 1 accession of *P. subciliatum* Chase (Adamowski et al., 1998), has been reported several times in species of *Brachiaria*, and is presently accepted (Shirasuna, 2012) within the circumscription of *Urochloa* (Risso-Pascotto et al., 2004; Mendes et al., 2006; Mendes-Bonato et al., 2006; Boldrini et al., 2009, 2010; Ricci et al., 2010). Although several polyploids were formed as a result of genome duplication events occurring several millions of years ago, Wendel and Doyle (2005) consider it an active and ongoing process, termed paleopolyploidization; the meiotic behavior of the *P. jesuiticum* accessions reported here reinforces this view.

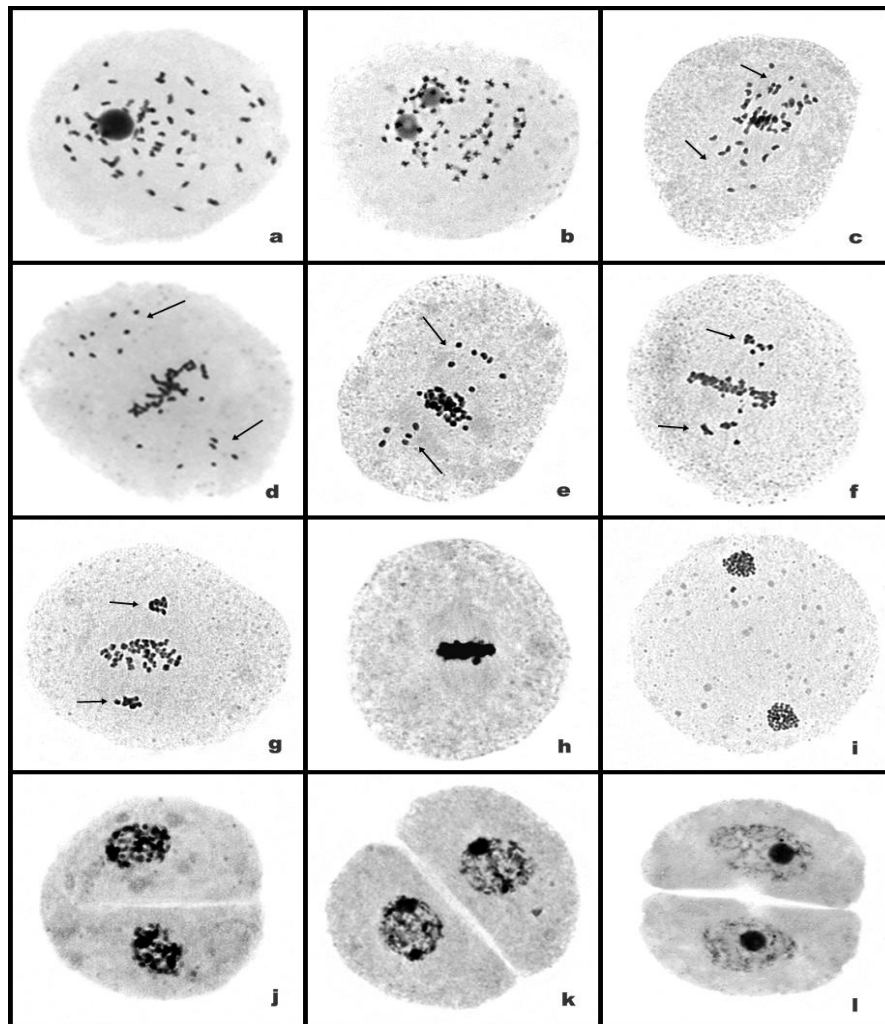


Figure 1. Aspects of microsporogenesis in *Paspalum jesuiticum* (accession BRA-025003). **A.** Meiocyte in diakinesis with 60 univalents and one nucleolus. **B.** Meiocyte in diakinesis with 60 univalents showing the sister chromatids and two nucleoli. **C-G.** Meiocytes in metaphase showing one genome congregated at the metaphase plate (40 univalents) and the other with 20 scattered univalents (arrows). **H.** Metaphase with both genomes congregated at the plate. **I.** Telophase showing the segregated chromatids. **J.** Occurrence of cytokinesis after telophase. **K-L.** Dyads with $2n$ microspores.

Despite asynchrony in early metaphase, metaphases were nonetheless formed as the 20 scattered univalents reached the metaphase plate before anaphase (Figure 1H). Equational chromatid division occurred in anaphase, leading to normal telophase (Figure 1I). Equational chromatid division was previously reported in synaptic mutants of tomato (Ramanna, 1983; Jongedijk et al., 1991), blueberry (Vorsa and Ortiz, 1992), and *Vaccinium darrowi* Camp (Qu and Vorsa, 1999). Only 1 meiotic division (equational) occurred, and after cytokinesis (Figure 1J), a dyad with two 2n microspores (Figure 1K and L) was formed. Dyads that generated two 2n fertile pollen grains were also observed in the above-mentioned synaptic mutants, as well as in an asynaptic mutant of *P. secans* (Snyder, 1961).

The fact that 2 nucleoli were found in diakinesis in some meiocytes provided further evidence that recent natural hybridization resulted in the accessions analyzed (Figure 1B). Two nucleoli were also recently reported in hybrids of *Brachiaria* (Mendes et al., 2006; Boldrini et al., 2009, 2010). The genetic and functional consequences of uniting 2 divergent genetic systems into a common nucleus in only 1 parental cytoplasm can only be speculated at present (Wendel and Doyle, 2005). According to Liu et al. (2001), the critical periods of this process are during and immediately after allopolyploidy formation, when the 2 distinct genomes are first brought into contact, thereby requiring a diverse array of accommodations.

2n gametes and *Paspalum* breeding

Gametes with the somatic chromosome number (2n gametes) are the result of a modified meiosis that affects the micro- and megasporogenesis stages. 2n gametes appear frequently in the plant kingdom, with their occurrence having been reported in species of many families, including Poaceae (Veilleux, 1985). The absence of cytokinesis has been demonstrated to result in 2n gamete formation in accessions of several species of *Paspalum* (Pagliarini et al., 1999). Such spontaneous polyploidy is an important phenomenon both for evolution and for the development of new cultivars.

More than 70% of all flowering plants are estimated to be polyploids. The two main recognized modes of polyploidization are asexual, which occurs through somatic doubling of the chromosome complement, and sexual, which occurs through the formation of functioning 2n gametes (Harlan and De Wet, 1975). However, Veilleux (1985) and Bretagnolle and Thompson (1995) proposed that polyploidization via 2n gametes may be a particularly common event among higher plants.

In the accessions considered in the present study, 100% of the gametes presented somatic chromosome numbers (2n gametes). However, their frequency of occurrence varies across plant species, even within species or genotypes with 2n gamete formation. The frequency of 2n pollen production varies from 0.05 to 99% across species (Veilleux, 1985). In *V. darrowi*, only 5% of 2n gametes formed after desynapsis (Qu and Vorsa, 1999). Fertile 2n gametes have been demonstrated in synaptic mutants of tomato (Ramanna, 1983; Jongedijk et al., 1991), blueberry (Vorsa and Ortiz, 1992), and *V. darrowi* (Qu and Vorsa, 1999). The importance of meiotic mutants to breeding has been highly emphasized previously. In particular, Peloquim (1982) discussed the potential applications of 2n gametes formed by synaptic mutants for transmitting maximal amounts of heterozygosity in the potato.

More than 10 abnormal mechanisms leading to 2n gamete formation have been reported to date (Bretagnolle and Thompson, 1995). The genetic constitution of 2n gametes fall

into 2 general categories: the first or second division restitution (FDR or SDR, respectively). If the 2n gamete contains 1 sister chromatid of each replicated chromosome, it is considered to be an FDR 2n gamete. If the 2n gamete includes both sister chromatids of 1 of the homologous chromosomes, it is considered to be an SDR 2n gamete. In the *P. jesuiticum* accessions analyzed in the present study, asynapsis caused separation of the univalent sister centromeres at anaphase, followed by cytokinesis after telophase, thus resulting in an FDR 2n gamete.

Sexual polyploidization represents an interesting alternative method for the growing interest in exotic germplasm exploitation, both for increasing allelic diversity and for transferring useful traits from wild related species into cultivated varieties. Potato breeding offers a good example of this potential (Carputo et al., 2000). The high efficiency of 2n gametes (100%) found in the accessions studied here could be of special interest to *Paspalum* breeding programs aiming at interspecific hybridization. However, since the meiotic behavior of both accessions suggests a recent history of natural hybridization, and since the genomes involved in the phenomenon are yet to be identified, further studies are necessary before these 2n gametes could be used in breeding programs. Moreover, their use in breeding programs is also hindered by the fact that polyploid accessions are generally apomictic (Quarín, 1992), resulting in a reproductive barrier to crossing (Miles, 2007).

ACKNOWLEDGMENTS

The authors are grateful to UNIPASTO, CNPq, and Fundação Araucária (PR) for financial support.

REFERENCES

- Adamowski EV, Pagliarini MS and Batista LAR (1998). Chromosome elimination in *Paspalum subciliatum* (Notata Group). *Sex. Plant Reprod.* 11: 272-276.
- Baker BS, Carpenter AT, Esposito MS, Esposito RE, et al. (1976). The genetic control of meiosis. *Annu. Rev. Genet.* 10: 53-134.
- Boldrini KR, Micheletti PL, Gallo PH, Mendes-Bonato AB, et al. (2009). Origin of a polyploid accession of *Brachiaria humidicola* (Poaceae: Panicoideae: Paniceae). *Genet. Mol. Res.* 8: 888-895.
- Boldrini KR, Pagliarini MS and Valle CB (2010). Evidence of natural hybridization in *Brachiaria humidicola* (Rendle) Schweick. (Poaceae: Panicoideae: Paniceae). *J. Genet.* 89: 91-94.
- Bretagnolle F and Thompson JD (1995). Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytol.* 129: 1-22.
- Burson BL, Voigt PW and Evers GW (1991). Cytology, reproductive behavior and forage potential of hexaploid dallisgrass biotypes. *Crop Sci.* 31: 636-641.
- Carputo D, Barone A and Frusciant L (2000). 2n gametes in the potato: essential ingredients for breeding and germplasm transfer. *Theor. Appl. Genet.* 101: 805-813.
- Chase A. (1929). The North American Species of *Paspalum*. Contributions from the United States National Herbarium, Washington.
- Denham SS, Morrone O and Zuloaga FO (2010). Estudios en el género *Paspalum* (Poaceae, Panicoideae, Paniceae): *Paspalum denticulatum* y especies afines. *Ann. Miss. Bot. Gard.* 97: 11-33.
- Fernandes MIBM, Barreto IL and Salzano FM (1968). Cytogenetics, ecologic and morphologic studies in Brazilian forms of *Paspalum dilatatum*. *Can. J. Genet. Cytol.* 10: 131-138.
- Fernandes MIBM, Barreto IL, Salzano FM and Sacchet AMOF (1974). Cytological and relationships in Brazilian forms of *Paspalum* (Gramineae). *Caryologia* 27: 455-465.
- Golubovskaya IN (1979). Genetic control of meiosis. *Int. Rev. Cytol.* 58: 247-290.
- Harlan JR and de Wet JMJ (1975). On Ö Winge and a prayer: the origins of polyploidy. *Bot. Rev.* 41: 361-390.
- Jongedijk E, Ramanna MS, Sawor Z and Hermesen JGT (1991). Formation of first division restitution (FDR) 2n megaspores

- through pseudohomotypic division in ds-1 (desynapsis) mutants of diploid potato: routine production of tetraploid progeny from 2xFDR x 2xFDR crosses. *Theor. Appl. Genet.* 82: 645-656.
- Koduru PRK and Rao MK (1981). Cytogenetics of synaptic mutants in higher plants. *Theor. Appl. Genet.* 59: 197-214.
- Liu B, Brubaker CL, Mergeal G, Croon RC, et al. (2001). Polyploid formation in cotton is not accompanied by rapid genomic changes. *Genome* 44: 321-330.
- Mendes-Bonato AB, Pagliarini MS, Risso-Pascotto C and Valle CB (2006). Cytogenetic evidence for genome elimination during microsporogenesis in interspecific hybrid between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae). *Genet. Mol. Biol.* 29: 711-714.
- Mendes DV, Boldrini KR, Mendes-Bonato AB, Pagliarini MS, et al. (2006). Cytological evidence of natural hybridization in *Brachiaria brizantha* Stapf (Gramineae). *Bot. J. Linn. Soc.* 150: 441-446.
- Miles JW (2007). Apomixis for cultivar development in tropical forage grasses. *Crop Sci.* 47: S238-S249.
- Pagliarini MS, Takayama SY, Freitas PM, Carraro LR, et al. (1999). Failure of cytokinesis and 2n gamete formation in Brazilian accessions of *Paspalum*. *Euphytica* 108: 129-135.
- Parodi LR (1969). Estudios sistemáticos sobre las Gramineae-Panicaceae argentinas y uruguayas. *Darwiniana* 15: 65-111.
- Pazy B and Plitmann U (1996). Asynapsis in *Cistanche tubulosa* (Orobanchaceae). *Plant Syst. Evol.* 201: 271-273.
- Peloquim SJ (1982). Meiotic mutants in potato breeding. *Stadler Genet. Symp.* 14: 99-109.
- Qu L and Vorsa N (1999). Desynapsis and spindle abnormalities leading to 2n pollen formation in *Vaccinium darrowii*. *Genome* 42: 35-40.
- Quarín CL (1992). The nature of apomixis and its origin in panicoid grasses. *Apomixis Newsletter* 5: 8-15.
- Ramanna MS (1983). First division restitution gametes through fertile desynaptic mutants of potato. *Euphytica* 32: 337-35.
- Ricci GC, Pagliarini MS and Valle CB (2010). Genome elimination during microsporogenesis in two pentaploid accessions of *Brachiaria decumbens* (Poaceae). *Genet. Mol. Res.* 9: 2364-2371.
- Risso-Pascotto C, Pagliarini MS, do Valle CB and Jank L (2004). Asynchronous meiosis in an interspecific hybrid of *Brachiaria ruziziensis* and *B. brizantha*. *Plant Cell Rep.* 23: 304-310.
- Shirasuna RT (2012). Urochloa. In: Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Available at [http://floradobrasil.jbrj.gov.br/2012/FB020516]. Accessed October 21, 2012.
- Singh RJ (2003). Plant Cytogenetics, 2nd edn. CRC Press, Boca Raton.
- Snyder LA (1961). Asyndesis and meiotic non-reduction in microsporogenesis of apomictic *Paspalum secans*. *Cytologia* 26: 50-61.
- Valls JFM and Oliveira RC (2012). *Paspalum*. In: Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Available at [http://floradobrasil.jbrj.gov.br/2012/FB013476]. Accessed October 21, 2012.
- Veilleux R (1985). Diploid and polyploid gametes in crop plants: mechanisms of formation and utilization in plant breeding. *Plant Breed. Rev.* 3: 253-287.
- Visser NC, Spies JJ and Venter HJT (1999). The presence of synaptic and chromosome disjunction mutants in *Cenchrus ciliaris* (Poaceae: Paniceae). *Bothalia* 29: 327-334.
- Vorsa N and Ortiz R (1992). Cytology of 2n pollen formation in a blueberry aneuploid ($2n = 4x + 9 = 57$). *J. Hered.* 83: 346-349.
- Wendel JF and Doyle JJ (2005). Polyploidy and evolution in plants. In: Diversity and Evolution in Plants. (Henry R, ed.). CABI Publishing, Oxon. 97-117.
- Zickler D and Kleckner N (1999). Meiotic chromosomes: integrating structure and function. *Annu. Rev. Genet.* 33: 603-754.