

Meiotic behavior and chromosome number of *Urochloa adspersa* (Trin.) R. D. Webster from the Brazilian Chaco

M.F. Felismino¹, R.L.S. Maior², G.A. Damasceno Jr.¹, A. Pott¹ and M.S. Pagliarini^{2†}

¹Departamento de Botânica, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brasil

²Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, PR, Brasil

[†]*In memoriam*

Corresponding author: M.F. Felismino

E-mail: mariferrari82@gmail.com

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ABSTRACT. This is the first report of meiotic division in *Urochloa adspersa* (Trin.) collected from the Brazilian Chaco. Meiotic analyses were performed on three specimens of *U. adspersa* named G10, G15, and G16. Inflorescences were collected and fixed in a mixture of ethanol and acetic acid (3:1, v/v) for 24 h and then stored in 70% alcohol. Diakinesis revealed different chromosome numbers and ploidy levels. All three plants were polyploids: G10 and G15

exhibited $2n = 6x = 54$ chromosomes (arranged in 27 bivalents), while G16 exhibited $2n = 4x = 36$ chromosomes (18 bivalents). Meiotic behavior was mainly normal in the hexaploid G15 and the tetraploid G16 (5.3 and 6.2% of the cells were abnormal, respectively), revealing only a few meiotic abnormalities that are common to polyploids, i.e., those related to irregular chromosome segregation. G10 exhibited other meiotic abnormalities during meiosis II, such as chromosome stickiness, irregular spindle orientation, and irregular cytokinesis, which led to the formation of a few triads, resulting in 16.9% of the cells being abnormal. The origin of these abnormalities is discussed, and we suggest that the genes that control meiotic steps may be present in the *Urochloa* gene pool.

Key words: Microsporogenesis; Polyploidy; Native grass; *Brachiaria*; Chaco grass

INTRODUCTION

Chaco vegetation covers approximately 800,000 km² of Argentina, Paraguay, and Bolivia, including the western edge of the Mato Grosso do Sul State in Brazil (Hueck, 1972). The Brazilian Chaco is in the municipality of Porto Murtinho, and is restricted to a narrow strip located between the Paraguay and Apa Rivers that is not easily identified and defined on regional-scale maps (Prado et al., 1992). Prado et al. (1992) stated that the vegetation of Porto Murtinho was the only one that could be considered true Chaco. However, during the last few decades half of the original vegetation of the Brazilian Chaco has been cleared (Silva et al., 2011), and most of this heterogeneous landscape has been converted to agricultural fields and pastures.

Poaceae is one of the largest and most important families of angiosperms because of its economic and ecological importance, and Brazil contains approximately 197 genera and 1368 species of this family (Longhi-Wagner et al., 2005). We consider the Poaceae to be the most representative family of herbaceous vegetation in the Chaco of Porto Murtinho (A. Pott and J.F.M. Valls, personal communication). Knowledge of the diversity and biology of these species, including chromosome number, represents a starting point for the characterization of the herbaceous vegetation of this environment and a significant contribution to our knowledge of the Chaco as a whole.

Urochloa adspersa (Trin.) R. D. Webster (synonym, *Brachiaria adspersa* (Trin.) Parodi) is native to South America, the West Indies, and the southeast of the USA (Seiffert, 1984; Morrone and Zuloaga, 1992), although in some regions it is considered naturalized (Shirasuna, 2013). There have only been a few cytogenetic studies conducted on this genus, most of which were on species of the genus *Brachiaria* (Miles et al., 1996; Karia et al., 2006; Valle and Pagliarini, 2009). Although many researchers use the name *Brachiaria*, we are following the taxonomic revision of Morrone and Zuloaga (1992).

This study investigated the meiotic behavior, chromosome numbers, and ploidy levels of *U. adspersa* specimens collected from different sites in the Brazilian Chaco.

MATERIAL AND METHODS

Plants were collected from various sites in the Porto Murtinho municipality in March 2012: 1) Fazenda Santa Vergínia (22°01'12.5"S, 57°52'10.2"W); 2) Fazenda Retiro Conceição (21°40'58.2"S, 57°46'44.2"W); and 3) Fazenda Bala (21°41'42.4"S, 57°42'55.8"W), and the three plants collected at these sites were named G10, G15, and G16, respectively. We conducted systematic walks over the collection sites and collected inflorescences for both taxonomic and cytogenetic studies. Specimens of the collected material were deposited in the CGMS Herbarium of Universidade Federal de Mato Grosso do Sul (UFMS) after identification. The inflorescences were fixed in a mixture of ethanol and acetic acid (3:1, v/v) for 24 h and then stored in 70% alcohol. Cells undergoing microsporogenesis were analyzed by the squashing technique using 1% propionic carmine, and images were taken with a Leica DM5500B microscope (São Paulo, Brazil) and edited using the PhotoScape software (<http://www.photoscape.org/>).

RESULTS

Diakinesis revealed different chromosome numbers and ploidy levels. G10 and G15 exhibited 27 bivalents in all of the cells undergoing diakinesis (Figure 1a and b), while G16 exhibited a multivalent configuration (16 bivalents and one quadrivalent) in only one of the 38 cells analyzed, resulting in 97.4% of cells having 18 bivalents (Figure 1c and d). Therefore, the chromosome numbers were $2n = 4x = 36$ for G16 and $2n = 6x = 54$ for G10 and G15, with $x = 9$ as the basic chromosome number.

Meiosis was mainly normal in all of the specimens analyzed (Table 1; Figure 1e, g, i, and j). G10 exhibited the most abnormal meiosis, with 16.9% of cells being abnormal (Table 1). Abnormalities were observed in meiosis I and II, and consisted of precocious chromosome migration to the poles in metaphases I and II and laggard chromosomes in anaphases I (Figure 1h) and II, which led to the formation of micronuclei in telophases I and II. Some of these micronuclei persisted to tetrads and microspores, but at a very low frequency (Table 1). These abnormalities are common in polyploids, and confirm the polyploid status of the species. However, G15 and G16 exhibited nearly normal meiosis, with only 5.3 and 6.2%, respectively, of cells being abnormal (Table 1).

One type of meiotic abnormality observed in the three plants was non-oriented bivalents at the equatorial plate during metaphase I (4.3, 4.2, and 25.3% for G10, G15, and G16, respectively; Figure 1f). Other meiotic abnormalities were only observed in G10, such as chromosome stickiness during prophase II (12.8%), an irregular spindle during metaphase II (33.3%), a lack of cytokinesis (observed in one cell) during anaphase II (14.3%), and possible restitution nuclei (also observed in one cell) during telophase II (4.1%; Figure 1k), which probably originated from the spindle irregularity during metaphase II and could have been related to the observed triads (17.9%; Figure 1l) as a result of nucleus restitution.

Table 1. Number and percentage (in parentheses) of normal cells per meiotic phase, types of abnormality observed per meiotic phase, and number and percentage (in parentheses) of abnormal cells in *Urochloa adspersa* G10, *U. adspersa* G15, and *U. adspersa* G16 from the Brazilian Chaco.

Phase	<i>U. adspersa</i> (G10) (2n = 6x = 54)	<i>U. adspersa</i> (G15) (2n = 6x = 54)	<i>U. adspersa</i> (G16) (2n = 4x = 36)
Metaphase I	38 (82.6)	63 (87.5)	61 (73.5)
- precocious migration	6 (13.1)	6 (8.3)	1 (1.2)
- non-oriented bivalent	2 (4.3)	3 (4.2)	21 (25.3)
Anaphase I	23 (85.2)	40 (90.9)	80 (98.8)
- laggards	4 (14.8)	4 (9.1)	1 (1.2)
Telophase I	45 (97.8)	45 (97.8)	60 (100)
- micronuclei	1 (2.2)	1 (2.2)	-
Prophase II	37 (78.7)	55 (100)	30 (100)
- micronuclei	4 (8.5)	-	-
- chromosome stickiness	6 (12.8)	-	-
Metaphase II	8 (53.4)	6 (100)	-
- precocious migration	2 (13.3)	-	-
- irregular spindle	5 (33.3)	-	-
Anaphase II	5 (71.4)	-	1 (100)
- laggards	1 (14.3)	-	-
- lack of cytokinesis	1 (14.3)	-	-
Telophase II	22 (91.7)	-	4 (100)
- micronuclei	1 (4.2)	-	-
- restitutional nuclei	1 (4.1)	-	-
Tetrads	21 (75)	10 (100)	83 (94.3)
- micronuclei	2 (7.1)	-	5 (5.7)
- triads	5 (17.9)	-	-
Microspores	24 (92.3)	30 (100)	152 (98.1)
- micronuclei	2 (7.7)	-	3 (1.9)
Total number of cells	266	263	502
Total number of abnormal cells	45 (16.9)	14 (5.3)	31 (6.2)

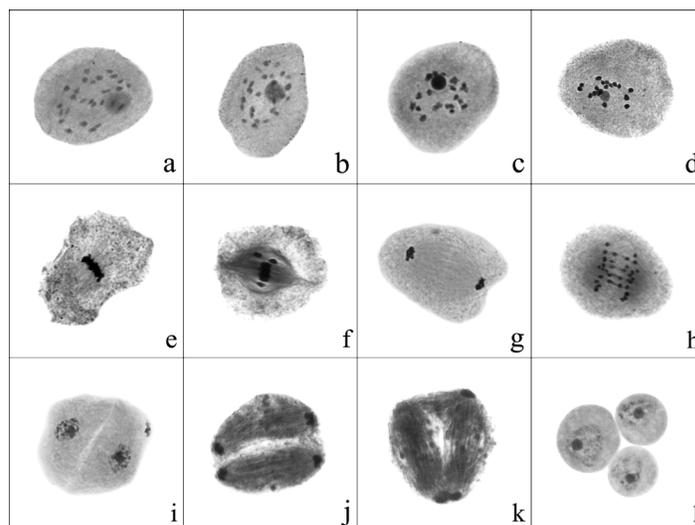


Figure 1. Meiosis in polyploid *Urochloa adspersa* from the Brazilian Chaco (40X magnification). **a.** *U. adspersa* G10 (2n = 6x = 54) with 27 bivalents; **b.** *U. adspersa* G15 (2n = 6x = 54) with 27 bivalents; **c.** *U. adspersa* G16 (2n = 4x = 36) with 18 bivalents; **d.** *U. adspersa* G16 with 18 bivalents; **e.** Normal metaphase I in *U. adspersa* G16; **f.** Non-oriented bivalents in hexaploid *U. adspersa*; **g.** Normal anaphase in *U. adspersa* G16; **h.** Anaphase I with laggard chromosomes in *U. adspersa* G16; **i.** Prophase II in *U. adspersa* G16; **j.** Normal anaphase II in hexaploid *U. adspersa*; **k.** Telophase II with possible restitution nuclei in *U. adspersa* G10; **l.** Triad in *U. adspersa* G10.

DISCUSSION

Morrone and Zuloaga (1992) revised the taxonomy of this group and classified several species into the genus *Urochloa*. We have decided to follow their taxonomic revision, based on taxonomic evidence from the collected specimens. There is much debate regarding the taxonomy of *Brachiaria* and *Urochloa*. Tórres-González and Morton (2005) conducted a phylogenetic study based on nucleotide-base sequence polymorphisms in the internal transcribed spacer region of nuclear ribosomal DNA, and were unable to separate *Brachiaria* from *Urochloa*. The classification of these species is still a matter of great discussion, but was not the focus of the present study.

Little is known about the cytology of the *Urochloa* grasses, despite their great number of species and agronomic and economic importance, and their broad distribution and use in the tropics. This is the first cytological report that has investigated meiotic behavior in *U. adspersa* in Brazil, specifically from the Brazilian Chaco. Morrone and Zuloaga (1992) reported that Argentine plants are polyploid, with $2n = 54$. The chromosome numbers of no more than 44 *Brachiaria* and/or *Urochloa* species have been reported; however, there have been few detailed studies of their karyotypic morphologies (Valle et al., 1987; Bernini and Marin-Morales, 2001). Many of the genus' species that have no agronomic interest, or are not included in active germplasm collections have not been cytologically studied.

Most angiosperms are polyploids, and polyploidy plays an important role in their evolutionary history (Leitch and Bennett, 1997; Meyers and Levin, 2006; Soltis et al., 2009). Polyploidy is very common in grasses, and according to Stebbins (1956) approximately 70% of grasses exhibit polyploidy. Polyploidy also predominates in the genus *Brachiaria*, particularly tetraploidy (Letteriello et al., 1999; Mendes-Bonato et al., 2002a,b). Penteado et al. (2000) studied ploidy levels using flow cytometry in all *Brachiaria* accessions of economic importance that were maintained by Embrapa Beef Cattle (Campo Grande, MS, Brazil), and found a wide variation in ploidy levels between and within species, ranging from diploids to heptaploids. However, none of these studies included *U./B. adspersa* in their analyses.

Darlington and Wylie (1955) determined that $x = 7$ and $x = 9$ are the basic chromosome numbers in *Brachiaria*. The majority of researchers agree with these numbers. Recently, Riso-Pascotto et al. (2006) described $x = 6$ for *B. dictyoneura*. Our analyses reveal that *U. adspersa* is also a polyploid, with $2n = 4x = 36$ and $2n = 6x = 54$ chromosomes, derived from $x = 9$. No multivalent formation was observed in the hexaploids G10 and G15, and a single quadrivalent configuration was observed during diakinesis in the tetraploid G16; the absence or a low frequency of multivalent formation suggests the occurrence of allopolyploidy. The low frequency of multivalent formation in tetraploidal *U. adspersa* suggests it could be a segmental allotetraploid, i.e., the parental genomes are only partially homologous (Stebbins, 1956).

Abnormal meiotic cells were observed in tetraploidal and hexaploidal *U. adspersa* at low frequencies (Table 1). Valle and Savidan (1996) reported that the natural diploids *Brachiaria brizantha*, *Brachiaria decumbens*, and *Brachiaria ruziziensis* exhibit regular meiosis, with chromosomes pairing in nine bivalents. Tetraploid forms, however, exhibit irregular meiosis, with univalents and quadrivalents being formed. The most common abnormalities found in polyploids are related to irregular chromosome segregation in both divisions: precocious chromosomes in metaphase plates and laggards in anaphases lead to the formation of micronuclei in telophases, which can lead to unbalanced pollen grains, affecting pollen fertility (Valle, 1986; Mendes-Bonato et al., 2001a,b; Junqueira-Filho et al., 2003;

Utsunomiya et al., 2005). Few meiotic abnormalities were observed in the tetraploid G16 and hexaploid G10 and G15, and were related to irregular chromosome segregation (Table 1). One tetraploid access of *Brachiaria humidicola* exhibits regular meiosis, with 18 bivalents during diakinesis and sexual reproduction (Valle and Glienke, 1991), but other tetraploid accessions exhibit irregular meiosis (Valle, 1986). *Brachiaria mutica* and *Brachiaria arrecta* are tetraploid species; the first exhibits irregular chromosome pairing and reproduction by apomixis, and the latter exhibits fairly regular bivalent pairing and sexual reproduction (Valle, 1986). Risso-Pascotto et al. (2009a) observed meiotic abnormalities at a low frequency in two hexaploid accessions of *Brachiaria dura*, as well as a few multivalent chromosome associations at diakinesis, and suggested that hexaploidy probably resulted from chromosome doubling. The accumulated evidence from several studies on *Brachiaria* point to an association between polyploidy and apomixis, with irregular meiosis, on the one hand, and sexual reproduction and regular chromosome pairing on the other (Valle and Savidan, 1996; Valle and Pagliarini, 2009). Valle and Pagliarini (2009) reported the modes of reproduction of many *Brachiaria* accessions, including one of *B. adspersa*, which reproduces sexually.

The presence of non-oriented bivalents at the equatorial plate was observed in G10, G15, and G16 (4.3, 4.2, and 25.3%, respectively). Non-oriented bivalents can occur due to chromosomes being unable to attach to the equatorial plate. This may be related to the kinetochore. Factors that could prevent the attachment of the spindle fibers to the kinetochore were discussed by Nicklas and Ward (1994). These bivalents can be incorporated (or not) into the telophase nucleus. If incorporated, one of the telophase I nuclei would have one extra chromosome, and the other would have a missing chromosome, generating two microspores: $n + 1$ and $n - 1$, respectively. If they are not incorporated and originate microspores, both telophase I nuclei would have one missing chromosome, generating four aneuploid microspores ($n - 1$). Regardless of the non-oriented bivalent behavior, aneuploid gametes would be formed (Pagliarini, 2001).

Other abnormalities observed in the hexaploid G10 were related to chromosome stickiness during prophase II (12.8%), a defective spindle during metaphase II (33.3%), and a lack of cytokinesis during anaphase II (14.3%), which could have resulted in the few triads observed (17.9%). Chromosome stickiness is related to intense chromatin clustering, which can begin during pachytene and continue to metaphase I. At anaphase I, chromosomes form bridges that can be broken by mechanical forces, producing several chromatin fragments. Gaulden (1987) postulated that chromosome stickiness results from the defective behavior of one or two types of specific non-histone proteins involved in chromosome organization. Chromosome stickiness has been observed in several *Brachiaria* accessions and hybrids (Utsunomiya et al., 2005; Mendes-Bonato et al., 2001b, 2007; Risso-Pascotto et al., 2009b; Felismino et al., 2010; Ricci et al., 2011).

Risso-Pascotto et al. (2003) and Gallo et al. (2007) analyzed penta-, tetra-, and hexaploid species of *Brachiaria*, and observed that a lack of, or abnormal, cytokinesis favored the rejoining of nuclei (restitutional nucleus) and the formation of monads, dyads, and triads, and consequently $2n$ gametes. Although polyploidy is common in *Brachiaria* its origin is still unknown, but $2n$ gametes could be a source of polyploidization.

The observation that these types of abnormalities occur in several *Brachiaria* species indicate that the genes controlling these meiotic steps are also present in the *Urochloa* gene pool, and if so, could also be present in *U. adspersa*. Cytological studies are important for providing information on the basic biology of species, from their chromosome numbers and ploidy levels to their evolutionary history, and even their population genetics and distribution

patterns. We intend to study other Chaco species, since few studies have been conducted on the native vegetation of this biome. The Brazilian Chaco is very important and almost unknown, but human activity is becoming more intense and there is great risk of biome degradation, and consequently a loss of biodiversity.

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