# Microsporogenesis in tetraploid accessions of *Brachiaria nigropedata* (Ficalho & Hiern) Stapf (Gramineae)

Karina Sayuri Utsunomiya*, Maria Suely Pagliarini* and Cacilda Borges do Valle**

* Department of Cell Biology and Genetics, State University of Maringá, Av. Colombo 5790, 87020-900, Maringá, Paraná, Brazil.
** Embrapa Beef Cattle, P. O. Box 154, 79002-970 Campo Grande, Mato Grosso do Sul, Brazil.

**ABSTRACT:** The genus *Brachiaria* (Trin.) Griseb. has achieved considerable importance to cattle production systems, as a result of the good production and adaptation of a few cultivars to poor and acid soils of the Brazilian savannas. Many of its species and accessions are polyploid and apomictic, which limits direct hybridization. To assist the breeding program, cytogenetic characterization has been undertaken on the accessions of *Brachiaria* collection at the Embrapa Beef Cattle Research Center. In this study, chromosome number and meiotic behavior are reported for the *Brachiaria nigropedata* (Ficalho & Hiern) Stapf collection. The 20 available accessions are tetraploid (2n = 4x = 36). Chromosomes paired preferentially as bivalents, but quadrivalents were found in high frequencies in some cells. Meiotic behavior was, in general, irregular, and varied among accessions. Most accessions presented more than 20% of abnormal tetrads. The most common meiotic abnormalities were those related to irregular chromosome segregation due to polyploidy, leading to micronuclei formation in the tetrad stage. A low frequency of other meiotic abnormalities such as the absence of cytokinesis, chromosome stickiness, cell fusion, anaphase bridges, and chromosome transfer among microsporocytes were also recorded in some accessions. Limitations of these accessions for use in hybridization programs are discussed.

## Introduction

*Brachiaria* (Trin.) Griseb. is a large genus comprising of about 100 species distributed throughout the tropics, especially in Africa (Renvoize *et al*., 1996). The forage potential of these grasses was first recognized about 50 years ago, particularly in tropical Australia. The major impact of the genus, however, was realized only in the past three decades, when a handful of *Brachiaria* cultivars, derived directly from natural occurring germplasm, were widely sown in tropical America (Miles *et al*., 1996). Current estimates of the acreage of the *Brachiaria* pastures in Brazil range from 50 to 70 million hectares. *Brachiaria decumbens* Stapf cv. Basilisk and *Brachiaria brizantha* (A. Rich.) Stapf cv. Marandu are the most widely grown varieties in the acid soils of Brazil.

The rapid expansion of acreage did not occur without problems, and current available cultivars are now recognized as having serious limitations: cv. Basilisk lacks resistance to a serious insect pest - spittlebugs - and cv. Marandu requires higher soil fertility and good drainage (Valle *et al*., 1993).

Two decades ago, an extensive germplasm collection trip was undertaken by the International Center for Tropical Agriculture (CIAT Colombia), in East Africa

with the support of International Board for Plant Genetic Resources (IBPGR) (Keller-Grein *et al*., 1996). This germplasm was later distributed to several latin American countries. Studies on the cytology and genetics of several *Brachiaria* species have opened up new opportunities and challenges in the improvement of this important forage (Miles *et al*., 1996). To date, new varieties of *Brachiaria* are being developed, either by selecting superior genotypes from natural diversity or by intra- and interspecific hybridization to obtain novel genetic combinations. In each case an adequate germplasm base was essential. An important part of the CIAT germplasm collection was introduced into Brazil during 1986 and 1987, and is presently maintained in plots at the Embrapa Beef Cattle Research Center. Whereas some species are represented in the collection by a great number of accessions, other species, such as *B. nigropedata*, are represented by a few accessions only.

Breeding difficulties in the genus *Brachiaria* are associated with polyploidy and asexual reproduction. Natural tetraploid (2n = 4x = 36) populations are widespread, whereas sexuality is rare in most species and usually at lower ploidy levels (Valle and Savidan, 1996). Polyploids have generally been classified as highly to obligate aposporous apomicts, which propagate clonally by seeds. Apomictic accessions cannot be improved by using traditional breeding schemes, but sexual diploid, artificially tetraploidized genotypes of a few species have been used to facilitate the introgression of valuable agronomic characteristics from apomictic species (Valle and Miles, 1994; Miles and Valle, 1996). Taking into consideration the correlation between polyploidy and apomixis in the genus, the knowledge of basic characteristics, such as ploidy level and chromosome behavior in meiosis, is essential for a successful breeding program. *Brachiaria nigropedata* is a species still untested. About 67% of the collection present at the Roodeplaat Grassland Institute of the African Research Council (RGI/ARC) belongs to this species (Keller-Grein *et al*., 1996). In this case, most accessions were obtained through direct collection conducted mainly in Zimbabwe and the diversity gathered does not represent all the variation present in nature, according to its collectors (Keller-Grein *et al*., 1996). *Brachiaria nigropedata* grows on soils of granitic origin and is considered a valuable component of natural grasslands in Southern Africa. It is reported to have high forage value and tends to disappear under heavy grazing (Oudtshoorn, 1992). Its agronomical value is under evaluation at Embrapa Beef Cattle Cen-

ter. Cytological characteristics such as chromosome number and meiotic behavior are reported here for the accessions available in Campo Grande, Mato Grosso do Sul, Brazil.

## Materials and Methods

Cytogenetic studies were carried out on 20 accessions of *B. nigropedata* of the Embrapa Beef Cattle Center *Brachiaria* collection grown in Campo Grande (state of Mato Grosso do Sul, Brazil), which comprises of approximately 475 accessions of 15 species maintained in plots. Site characteristics are: (climate type Aw: tropical humid savanna (average annual precipitation = 1526 mm; average temperature = 22°C); altitude 520 m; latitude = 20° 28' S; longitude = 55° 40' W); poor Dark Red Latossol (59% sand; 8% silt; 33% clay; pH = 4.2).

Inflorescences were collected for meiotic studies, fixed in ethanol: acetic acid (3:1) for 24h and stored under refrigeration until use. Microsporocytes were prepared by squashing and then staining with 0.5% propionic carmine. All meiotic phases were evaluated in five plants per accession. More than 1400 microsporocytes were analyzed per accession. Chromosome associations were evaluated in 20 microsporocytes at diakinesis per accession. Photomicrographs were made using a Kodak Imagelink – HQ, ISO 25 black and white film.

## Results and Discussion

The 20 accessions of *B. nigropedata* analyzed were tetraploid (2n = 4x = 36). Previous chromosome countings for this species revealed the occurrence of diploid (2n = 2x = 18) (De Wet and Anderson, 1956; Spies and Du Plessis, 1986) and tetraploid (2n = 4x = 36) (Moffet and Hurcombe, 1949; Hoshino and Davides, 1988) accessions. Two basic chromosome numbers, x = 7 and x = 9, have been generally accepted for the genus *Brachiaria*, with most species presenting chromosome numbers multiples of 9 (Basappa *et al*., 1987; Valle and Savidan, 1996), as also found for *B. nigropedata*. The available literature about the genus *Brachiaria* indicates a prevalence of tetraploidy (Sotomayor-Ríos *et al*., 1968; Bernini and Marin-Morales, 2001; Mendes-Bonato *et al*., 2002). Polyploidy is common among grasses. Stebbins (1956) estimated that 70% of the Gramineae family are natural polyploids.
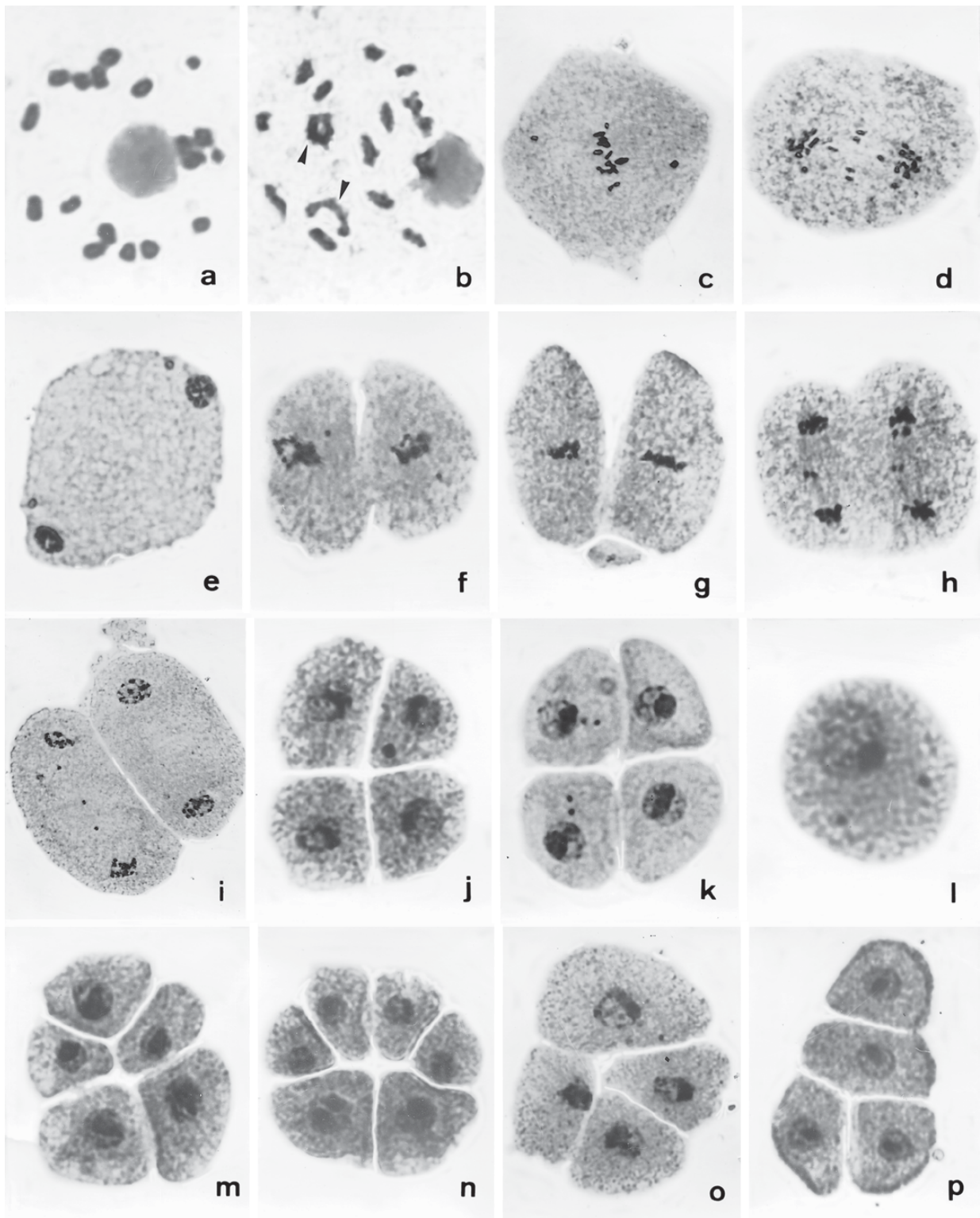
**FIGURE 1.** Aspects of the irregular microsporogenesis in the tetraploid acessions (2n = 4x = 36) of *B. nigropedata*. (a) Diakinesis with 18 II, (b) Diakinesis with 2 IV and 14 II (a, b: 1000X), (c) Metaphase I with precocious chromosome migration to the poles, (d) Anaphase I with laggards, (e) Telophase I with micronucleus in the both poles, (f) Metaphase II with micronucleus in the both cells, (g) Metaphase II with the micronuclei eliminated as a microcyte, (h) Anaphase II with laggards, (i) Telophase II with micronuclei in one cell, (j) tetrad with one micronuclei in one microspore, (k) Tetrad with two micronuclei in two microspores, (l) Released microspore with two micronuclei, (m) Pentad, (n) Hexad, and (o, p) Irregular positioning of microspores in the tetrad (c to p: X 400).

The polyploid condition of *B. nigropedata* accessions under analysis was also revealed by the pattern of chromosome association at diakinesis. Uni-, bi-, tri- and, quadrivalents were detected at this stage (Fig. 1a, b), with prevalence of bivalents (Table 1), as also observed in tetraploid accessions of *B. brizantha* (Mendes-Bonato *et al*., 2002). However, in some cells, chromosomes associated mainly as quadrivalents. According to Stebbins (1947), low frequency of multivalents is an argument used in advocating segmental allopolyploidy. In segmental allopolyploids, the genomes are not identical; they result from hybridization of closely related diploid species (AA´A'A') followed by the doubling of the chromosome numbers. As a result of preferential pairing between identical chromosomes, mainly bivalents are formed and few multivalents are thus present. Although this hypothesis has been accepted for many decades, Sybenga (1994, 1996a) pointed out that this character is not necessarily a reliable indication of pairing affinity, and thus of homology, because even true autopolyploids may form quadrivalents with frequencies substantially lower than the theoretically possible or expected. Advanced studies of genome constitution in the genus *Brachiaria* involving genomic "in situ" hybridization could help elucidate the origin of polyploid accessions.

As the 20 accessions were tetraploid, meiotic behavior was found to be irregular (Table 2). The most common meiotic abnormalities were those related to irregular chromosome segregation, such as precocious chromosome migration to the poles in metaphase I (Fig. 1c), laggards in anaphase I (Fig. 1d) and anaphase II (Fig. 1h), leading to micronucleus formation at telophase I (Fig. 1e) and telophase II (Fig. 1i). The behavior of micronuclei in both telophases was varied. In some cells, after the first division, micronuclei remained as such during the second division (Fig. 1f), whereas in others, these were eliminated in microcytes (Fig. 1g). After telophase II, the micronuclei also showed dissimilar behavior; when containing a single chromosome, they re-

**TABLE 1.**

**Accession code, range of chromosome association, and average association/cell.**

| Accession code | Range of chromosome association | | | | Average association/cell | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | I | II | III | IV |
| N 190 | (0-2) | (7-18) | (0-0) | (0-5) | 0.30 | 14.95 | 0.00 | 1.45 |
| N 191 | (0-2) | (8-18) | (0-0) | (0-5) | 0.10 | 13.45 | 0.00 | 2.25 |
| N 192 | (0-2) | (9-18) | (0-0) | (0-4) | 0.20 | 16.80 | 0.00 | 0.55 |
| N 193 | (0-2) | (6-14) | (0-0) | (2-6) | 0.40 | 10.30 | 0.00 | 3.75 |
| N 194 | (0-3) | (10-18) | (0-1) | (0-4) | 0.25 | 13.30 | 0.05 | 2.25 |
| N 195 | (0-3) | (5-18) | (0-1) | (0-5) | 0.15 | 11.95 | 0.05 | 2.95 |
| N 196 | (0-4) | (8-16) | (0-0) | (1-5) | 0.50 | 12.35 | 0.00 | 2.70 |
| N 197 | (0-4) | (8-18) | (0-1) | (0-5) | 0.50 | 12.75 | 0.05 | 2.45 |
| N 198 | (0-2) | (4-14) | (0-1) | (1-7) | 0.20 | 9.60 | 0.10 | 4.05 |
| N 199 | (0-2) | (6-16) | (0-1) | (1-6) | 0.20 | 10.55 | 0.10 | 3.60 |
| N 200 | (0-3) | (8-14) | (0-1) | (2-5) | 0.40 | 11.55 | 0.10 | 3.05 |
| N 201 | (0-2) | (8-16) | (0-0) | (1-5) | 0.70 | 13.25 | 0.00 | 2.20 |
| N 202 | (0-3) | (4-16) | (0-1) | (1-7) | 0.45 | 10.85 | 0.15 | 3.35 |
| N 203 | (0-4) | (6-16) | (0-0) | (1-6) | 0.40 | 11.50 | 0.00 | 3.15 |
| N 204 | (0-2) | (4-18) | (0-0) | (0-7) | 0.20 | 12.60 | 0.00 | 2.65 |
| N 207 | (0-4) | (8-18) | (0-1) | (0-4) | 0.75 | 12.25 | 0.05 | 1.75 |
| N 208 | (0-2) | (0-18) | (0-0) | (0-9) | 0.30 | 11.45 | 0.00 | 3.20 |
| N 209 | (0-4) | (9-18) | (0-0) | (0-4) | 0.50 | 14.45 | 0.00 | 1.65 |
| N 210 | (0-2) | (10-18) | (0-0) | (0-4) | 0.45 | 14.10 | 0.05 | 1.80 |
| N 211 | (0-4) | (8-16) | (0-0) | (1-5) | 0.50 | 11.75 | 0.00 | 3.00 |

mained as micronuclei inside the microspore (Fig. 1j, k, l) although when constituted by a group of chromosomes, they were isolated as microspores of different sizes, giving rise to polyads (Fig. 1m, n). Tetrads with abnormal shapes were also observed (Fig. 1o, p). Similar meiotic behavior was reported in polyploid accessions of other *Brachiaria* species (Pritchard, 1967; Sotomayor-Ríos *et al*., 1968; Valle, 1986; Valle *et al*., 1987, 1989; Basappa *et al*., 1987; Mendes-Bonato *et al*., 2001a; Risso-Pascotto *et al*., 2003). The percentage of abnormal tetrads among the 20 accessions of *B. nigropedata* ranged from 14.10 to 65.80%, but in 15

accessions, that is, 75% of the total, the percentage ranged from 20 to 40%. Such frequency of abnormal meiotic products was higher than that found in *B. brizantha* (Mendes-Bonato *et al*., 2002), where a considerable number of tetraploid accessions presented less than 15% abnormal tetrads.

Other meiotic abnormalities were detected in low frequencies in some accessions of *B. nigropedata*. Absence of cytokinesis after first and/or second meiosis, leading to monad, dyad, and triad formation was found in ten accessions (Fig. 2a to c). In eight of them, the percentage of affected cells was lower than 0.5%, but in
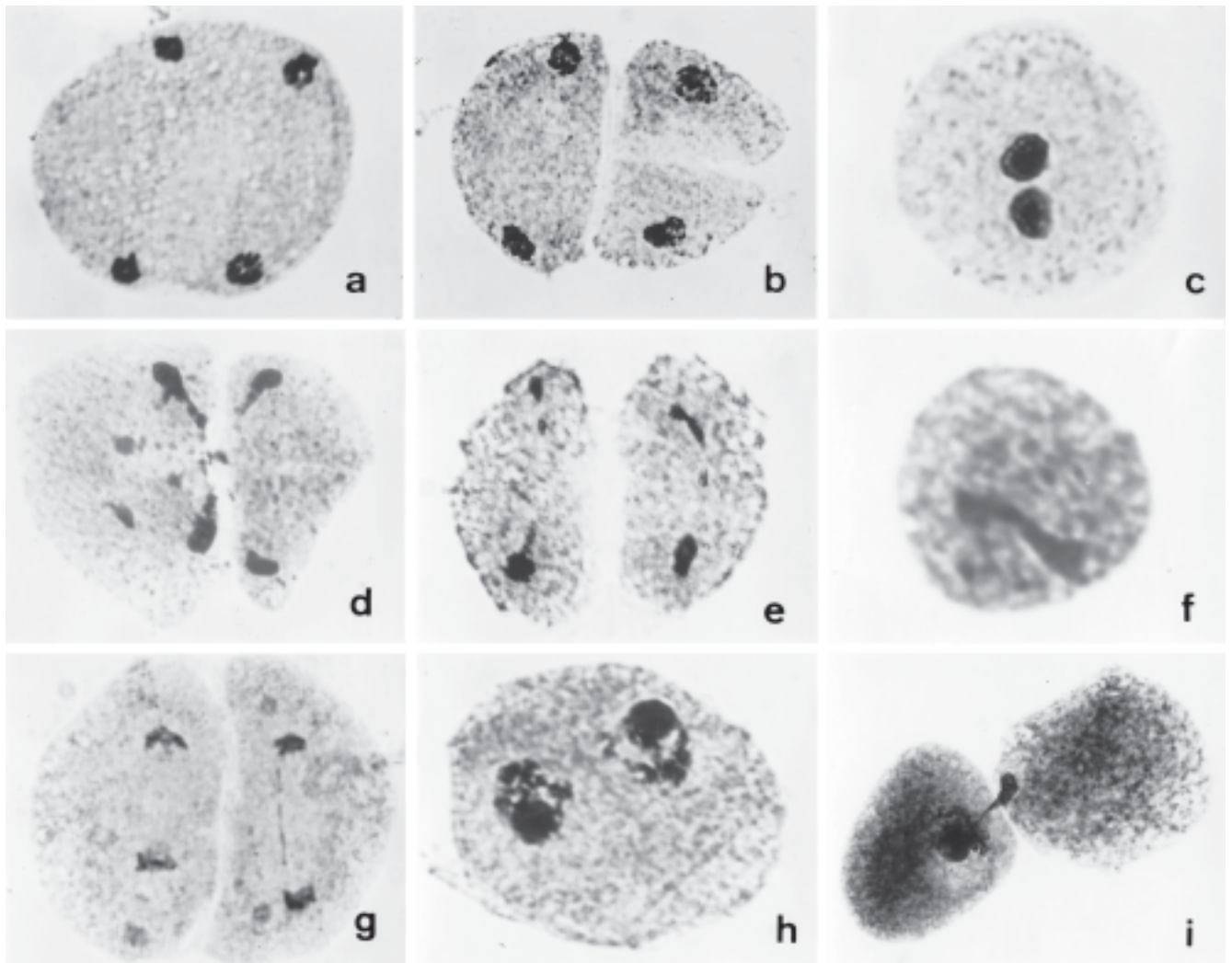


**FIGURE 2.** Aspects of some rare abnormalities. (a to c) Abnormalities related to absence of cytokinesis: telophase II without the first cytokinesis (a), triad originated by absence of the second cytokinesis in one cell (b), and a binucleate microspore (c); (d to f) Meiotic phases showing chromosome stickiness: prophase II (d), telophase II (e), and microspore (f); (g) Bridge in anaphase II; (h) Cell fusion; and (i) Chromosome transfer among microsporocytes (X 400).

two, N 194 and N 195, affected cells reached 2.4 and 2.9%, respectively. This abnormality was also reported in a pentaploid accession of *B. brizantha* (Risso-Pascotto *et al.*, 2003). Absence of cytokinesis, when accompanied by restitutional nuclei, lead to 2n gamete formation. Restitutional nuclei were described in *B. brizantha* (Risso-Pascotto *et al.*, 2003), but not found in *B. nigropedata*. Here all cells affected by absence of cytokinesis maintained isolated nuclei.

Chromosome stickiness (Fig. 2d to f) was detected in nine accessions, ranging from 0.25 to 5.0%. A severe case of stickiness was reported affecting meiosis in *B. brizantha* (Mendes-Bonato *et al.*, 2001b), *B. decumbens* (Mendes-Bonato *et al.*, 2001a), and several other accessions of different *Brachiaria* species under analysis. Chromosome bridges (Fig. 2g) affecting less than 0.5% of cells was found in 12 accessions; they may result from chromosome stickiness such as found in *B. brizantha* (Mendes-Bonato *et al.*, 2001b), and *B. decumbens* (Mendes-Bonato *et al.*, 2001a). No more

than 0.5% of cells, in four accessions, was affected by cell fusion (Fig. 2h). This abnormality has been reported to occur in *B. brizantha* (Mendes-Bonato *et al.*, 2001c; Risso-Pascotto *et al.*, 2003), and also in several accessions of other *Brachiaria* species under analysis. An abnormality, which has never been reported in the genus *Brachiaria* until now, was observed quite frequently in six accessions analyzed: chromosome transfer among microsporocytes (Fig. 2i). In accession N 193 up to 9% of cells displayed it. In general, the phenomenon occurred between two cells, involving total or partial transference of the genetic material.

The genus *Brachiaria* is extensively used and became the most important tropical pasture in cattle production systems in the poor and acid soils of the Brazilian savannas. The two *Brachiaria* cultivars under wide use in the country are native to East Africa and natural apomictic tetraploids. The lack of diversity represents an obvious risk to the ecosystem as well as to cattle production. Therefore rational exploitation of the diver-

**TABLE 2.**

**Accession code, number of cells analyzed, and percentage of abnormal cells in each meiotic phase.**

| Accession code | N° of cells | Percentage of abnormal cells | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PRO I | MET I | ANA I | TEL I | PRO II | MET II | ANA II | TEL II | TETR |
| N 190 | 1456 | 8.16 | 9.13 | 15.50 | 26.90 | 21.26 | 40.24 | 24.13 | 38.00 | 32.20 |
| N 191 | 1645 | 0.00 | 11.30 | 32.10 | 33.52 | 49.30 | 62.50 | 46.94 | 23.30 | 19.23 |
| N 192 | 1528 | 0.00 | 16.10 | 28.50 | 14.30 | 8.26 | 18.70 | 36.20 | 22.72 | 27.06 |
| N 193 | 1658 | 10.22 | 1.57 | 32.60 | 29.40 | 25.17 | 30.14 | 1.57 | 26.47 | 33.00 |
| N 194 | 1518 | 0.00 | 3.70 | 14.40 | 11.76 | 7.00 | 13.70 | 14.30 | 16.15 | 16.70 |
| N 195 | 1649 | 1.35 | 10.85 | 60.40 | 59.00 | 55.56 | 66.20 | 36.00 | 68.60 | 65.80 |
| N 196 | 1543 | 0.00 | 25.90 | 47.00 | 57.05 | 56.00 | 62.32 | 31.17 | 50.00 | 34.50 |
| N 197 | 1665 | 1.10 | 4.00 | 34.03 | 32.90 | 32.04 | 25.40 | 16.15 | 27.03 | 25.13 |
| N 198 | 1532 | 9.32 | 14.73 | 44.57 | 29.93 | 37.50 | 82.80 | 24.57 | 28.60 | 31.90 |
| N 199 | 1717 | 0.00 | 15.10 | 56.73 | 58.30 | 48.90 | 69.52 | 47.36 | 58.80 | 62.50 |
| N 200 | 1708 | 0.00 | 4.70 | 35.47 | 22.60 | 49.03 | 38.20 | 43.13 | 36.25 | 29.90 |
| N 201 | 1711 | 2.70 | 6.00 | 35.60 | 34.96 | 32.50 | 35.64 | 24.65 | 21.50 | 29.00 |
| N 202 | 1692 | 0.00 | 18.52 | 58.10 | 50.00 | 40.14 | 55.00 | 49.67 | 44.74 | 36.74 |
| N 203 | 1725 | 0.00 | 9.94 | 34.30 | 32.64 | 16.13 | 35.33 | 25.52 | 35.14 | 32.64 |
| N 204 | 1501 | 0.00 | 6.00 | 30.92 | 27.53 | 29.10 | 32.92 | 27.80 | 21.90 | 22.76 |
| N 207 | 1714 | 0.00 | 7.20 | 25.60 | 24.43 | 21.35 | 30.70 | 28.00 | 25.93 | 28.10 |
| N 208 | 1607 | 0.00 | 2.43 | 2690 | 27.60 | 29.65 | 41.00 | 32.15 | 21.90 | 24.50 |
| N 209 | 1667 | 0.00 | 4.60 | 27.80 | 12.65 | 12.16 | 29.73 | 18.20 | 11.20 | 14.10 |
| N 210 | 1819 | 0.00 | 4.32 | 32.00 | 13.70 | 13.80 | 35.80 | 21.90 | 31.50 | 26.13 |
| N 211 | 1782 | 0.00 | 8.16 | 21.40 | 39.02 | 36.22 | 60.80 | 25.74 | 47.90 | 39.80 |

sity present in the germplasm, especially of species with good forage value such as *B. nigropedata*, is a fundamental alternative. Despite identifying promising accessions, the breeding program in effect at Embrapa Beef Cattle Center depends on compatible sexual accessions to act as female genitor in hybridizations. Tetraploid apomictic accessions with nearly regular meiosis are used as male genitor, thus wide cytological screening is a pre-requisite. Work underway with the *Brachiaria* germplasm collection at Embrapa has revealed the occurrence of a single sexual diploid accessions and a few apomictic tetraploid accessions with considerable low frequency of meiotic abnormalities in *B. brizantha* (Mendes-Bonato *et al*., 2002), a species that provides a wealth of genetic variation of desired attributes for gene introgression. Previous studies performed on determination of mode of reproduction of *B. nigropedata* (unpublished data) have revealed that all accessions are apomictic. Taking into account that the present cytological studies revealed that all accessions are tetraploid and display high levels of meiotic abnormalities, their use in hybridization programs is indeed limited. Only three accessions (N 209, N 194, and N 191) presented less than 20% of abnormal tetrads and can be considered proper genitors in crosses.

## References

Basappa GP, Muniyamma M, Chinnappa CC (1987). An investigation of chromosome numbers in the genus *Brachiaria* (Poaceae:Paniceae) in relation to morphology and taxonomy. Can J Bot. 65: 2297-2309.

Bernini C, Marin-Morales MA (2001). Karyotype analysis in *Brachiaria* (Poaceae) species. Cytobios 104: 157-171.

De Wet JMJ, Anderson L (1956). Chromosome numbers in Transvaal grasses. Cytologia 21: 1-10.

Hoshino T, Davides G (1988). Chromosome numbers in grasses (Poaceae) from Southern Africa, I. Ann Miss Bot Gard. 75: 866-871.

Keller-Grein G, Maass BL, Hanson J (1996). Natural variation in *Brachiaria* and existing germoplasma collections, in *Brachiaria:* Biology, Agronomy, and Improvement. In: Miles JW, Maass BL, Valle CB (eds.), CIAT, Colombia, pp. 16-42.

Mendes-Bonato AB, Pagliarini MS, Silva N, Valle CB (2001a). Meiotic instability in invader plants of signal grass *Brachiaria decumbens* Stapf (Gramineae). Acta Scient. 23: 619-625.

Mendes-Bonato AB, Pagliarini MS, Valle CB, Penteado MIO (2001b). A severe case of chromosome stickiness in pollen mother cells of *Brachiaria brizantha* (Hochst) Stapf (Gramineae). Cytologia 66: 287-291.

Mendes-Bonato AB, Pagliarini MS, Valle CB, Penteado MIO (2001c). Archesporial syncytes restricted to male flowers in a hexaploid accession of *Brachiaria brizantha* (Hochst) Stapf (Gramineae). Nucleus 44: 137-140.

Mendes-Bonato AB, Forli F, Pagliarini MS, Valle CB, Penteado MIO (2002). Chromosome numbers and microsporogenesis in *Brachiaria brizantha* (Gramineae). Euphytica 125: 419-425.

Miles JW, Valle CB (1996). Manipulation of apomixis in *Brachiaria* breeding, in *Brachiaria:* Biology, Agronomy, and Improvement. In: Miles JW, Maass BL, Valle CB (eds.), CIAT, Colombia, pp. 164-177.

Miles JW, Maass BL, Valle CB (1996). *Brachiaria:* Biology, Agronomy, and Improvement. CIAT/Embrapa, CIAT Publication No. 259, Cali, Colombia, 288 pp.

Moffett H, Hurcombe R (1949). Chromosome numbers of South African grasses. Heredity 3: 369-373.

Oudtshoorn FPVan (1992). Guide to Grasses of South Africa. Briza Publikasies Cc, 166 pp.

Pritchard AJ (1967). Apomixis in *Brachiaria decumbens* Stapf. J. Aust Agric Sci. 33: 264-265.

Renvoize SA, Clayton WD, Kabuye CHS (1996). Morphology, taxonomy, and natural distribution of *Brachiaria* (Trin.) Griseb., in *Brachiaria:* Biology, Agronomy, and Improvement. In: Miles JW, Maass BL, Valle CB (eds.), CIAT, Colombia, pp. 1-15.

Risso-Pascotto C, Pagliarini MS, Valle CB, Mendes-Bonato AB (2003). Chromosome number and microsporogenesis in a pentaploid accession of *Brachiaria brizantha* (Gramineae). Plant Breed. 122: 136-140.

Sotomayor-Ríos A, Velez-Fortuno J, Woodbury R, Schertz KF, Sierra-Bracero A (1968). Description and cytology of a form of Signalgrass (*Brachiaria brizantha* Stapf.) and its agronomic behavior compared to Guineagrass (*Panicum maximum* Jack.). J Agric Univ PR. 44: 208-220.

Spies JJ, Du Plessis H (1986). Chromosome studies on African plants, I. Bothalia 16: 87-88.

Stebbins GL (1947). Types of polyploids: their classification and significance. Adv Genet. 1: 403-429.

Stebbins GL (1956). Cytogenetics and evolution of the grass family. Amer J Bot. 43: 891905.

Sybenga J (1994). Preferential pairing estimates from multivalent frequencies in tetraploids. Genome 37: 1045-1055.

Sybenga J (1996a). Chromosome pairing affinity and quadrivalent formation in polyploids: do segmental allopolyploids exist? Genome 39: 1176-1184.

Valle CB (1986). Cytology, mode of reproduction, and forage quality of selected species of *Brachiaria* Griseb. Ph.D. thesis, Illinois University of Illinois, Illinois.

Valle CB, Singh RJ, Miller DA (1987). Pachytene chromosomes of *Brachiaria ruziziensis* Germain et Evrard. Plant Breed. 78: 75-78.

Valle CB, Savidan YH, Jank L (1989). Apomixis and sexuality in *Brachiaria decumbens* Stapf, in Proc. XVI Int. Grassl. Congr**.** France, INRA, pp. 407-408.

Valle CB, Glienke C, Leguizamon GC (1993). Breeding of apomictic *Brachiaria* through interspecific hybridization. Proc. XVII Int. Grassl. Congr., New Zealand: NZIAS, 1: 427-428.

Valle CB, Miles JM (1994). Melhoramento de gramíneas do gênero *Brachiaria*. In: Peixoto A.M., Moura J.C.de and Faria V.P.de. XI Simpósio sobre Manejo da Pastagem, Piracicaba. FEALQ, pp. 1-23.

Valle CB, Savidan YH (1996). Genetics, cytogenetics, and reproductive biology of *Brachiaria*, in *Brachiaria:* Biology, Agronomy, and Improvement. In: Miles JW, Maass BL, Valle CB (eds.), CIAT, Colombia, pp. 147-163.