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Cytogenetic studies in three diploid species of *Andropogon* (*Andropogoneae*), section *Leptopogon*

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Abstract

Karyotypes can provide a relevant information about relationships and evolutionary origin among species of the *Andropogon* genus. This paper presents the karyotype, C^+ and DAPI/CMA_3 banding and DNA content of three diploid (2n=20) species belonging to section *Leptopogon*: *A. selloanus, A. macrothrix* and *A. gyrans*. Karyotypes of the three diploid species are symmetrical. We propose a karyotype formulae (18m + 2sm) for each of them. The three species show a pair of metacentric chromosomes with a terminal secondary constriction on short arms. Fluorochrome banding revealed different constitutive heterochromatin patterns and CMA_3^+/$DAPI^-$ terminal bands related to the nucleolar organizer region in each species. Nuclear DNA content was estimated by flow cytometry ranged from 2.22 to 2.61 pg. FISH technique revealed that these three species have two 45S rDNA loci at the distal ends of the short arms of two metacentric chromosomes. We compare the genomes of the diploids *A. selloanus, A. macrothrix* and *A. gyrans*, and the triploid *A. ternatus* using GISH. These technique allowed us to confirm the hypotheses that the *A. selloanus, A. macrothrix* and *A. gyrans* constitute a homogeneous group that share a common S genome that comprises just one of the genomes in the triploid *A. ternatus.*

Key words: *Andropogon*, C/DAPI/CMA_3 banding, Genomic *In Situ* Hybridization, karyotype, *Leptopogon* sect.

Resumen

Los cariotipos pueden proporcionar una información relevante sobre las relaciones y el origen evolutivo entre las especies del género *Andropogon*. Este trabajo presenta el cariotipo, bandeo C^+ y DAPI / CMA_3 y contenido de ADN de tres especies diploides (2n = 20) pertenecientes a la sección *Leptopogon*: *A. selloanus*, *A. macrothrix* y *A. gyrans*. Los cariotipos de las tres especies diploides son simétricos. Proponemos la fórmula cariotípica (18m + 2sm) para cada una de ellas. Las tres especies muestran un par de cromosomas metacéntricos con una constricción secundaria terminal en los brazos cortos. El bandeo fluorocrómico reveló diferentes patrones de heterocromatina constitutiva y bandas CMA_3^+/ $DAPI^-$ terminales relacionadas con la región organizadora nucleolar en cada especie. El contenido de ADN nuclear se estimó mediante citometría de flujo variando de 2,22 a 2,61 pg. La técnica FISH reveló que estas tres especies poseen dos loci 45S rDNA en los extremos distales de los brazos cortos de dos cromosomas metacéntricos. Comparamos los genomas de los diploides *A. selloanus, A. macrothrix* y *A. gyrans*, y del triploide *A. ternatus* utilizando GISH. Esta técnica nos permitió confirmar la hipótesis de que *A. selloanus, A. macrothrix* y *A. gyrans* constituyen un grupo homogéneo que comparte un genoma S común le cual comprende solo uno de los genomas en el triploide *A. ternatus*.

Palabras clave: *Andropogon*, bandeo C/DAPI/ CMA_3, Hibridación *In Situ* Genómica, cariotipo, sección *Leptopogon*.

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Introduction

Andropogon L. is a pantropical genus of grasses composed by 100 (Clayton & Renvoize 1986) to 120 (Campbell & Windisch 1986) species, distributed mainly in the grasslands of Africa and the Americas. It is considered that the basic chromosome number is x = 10 (Gould 1967; Stebbins 1975; Campbell 1983; Norrmann 1985; Davidse *et al.* 1986) with only a few exceptions in the Old Word: x = 9 in *A. distachyus* L.; x = 8 in *A. abbysinicus* Fresen.; and x = 7 in *A. manii* Hook. f. (Davidse *et al.* 1986). While most African species are diploids or tetraploids (2n = 2x = 20; 4x = 40) (Campbell 1983; Campbell & Windisch 1986; Norrmann 1999), american *Andropogon* species are usually diploid or hexaploid (2n = 6x = 60) (Gould 1967; Norrmann 1985; Campbell & Windisch 1986).

Stapf (1917, 1919) proposed four sections for African species: *Andropogon, Leptopogon, Notosolen* and *Piestium*. Gould (1967) suggested the incorporation of American species into the first three taxonomic sections mentioned above; these were currently recognized by Clayton & Renvoize (1986).

Leptopogon is considered the most advanced section within the genus (Clayton & Renvoize 1986; Campbell & Windisch 1986). In America, the section is mainly represented by two complexes: *Andropogon virginicus* complex, a monophyletic group with a center of diversity on the Coastal Plain of the southeastern United States (Campbell 1983) and the *Andropogon lateralis* complex geographically distributed in Central and South America, which is constituted entirely by hexaploid species (Campbell 1983; Norrmann 2009).

American diploids of this section are represented by twelve species. Nine of them belong to the *A. virginicus* complex, distributed over much of the northern of America (Campbell 1983), and the other three are distributed in Central and South America: *A. selloanus* (Hack.) Hack., *A. macrothrix* Trin. and *A. leucostachyus* Kunth. Another South American species, *A. ternatus* (Spreng.) Nees. maintains permanent triploidy (2n = 3x = 30) by transmitting one genome through the egg cell and two genomes through the sperm nucleus (Norrmann & Quarín 1987). This species may be best regarded as a diploid with an additional accessory chromosomes set (Norrmann 1985; Norrmann & Quarín 1987). Other species without formal determination of chromosome number but probably diploids due to their exomorphological characteristics (Gould 1967; Norrmann 1985) are the Central American *A. cubensis* Hack., *A. reedii* Hitchc. et Ekman, *A. reinoldii* León and *A. nashianus* Hitchc. and the South American species *A. sanlorenzanus* Killeen, *A. carinatus* Nees, *A. diuturnus* Sohns, *A. sincoranus* Renvoize, *A. palustris* Pilg. and *A. ingratus* Hack. most of them belonging to the Section *Leptopogon*.

With the aim to increase the cytogenetic knowledge of this genus, we report for the first time the karyotype formulae, the total DNA content, the distribution and variability of constitutive heterochromatin and the number and location of ribosomal organizer regions in three American diploid species of Sect. *Leptopogon* (*A. selloanus*, *A. macrothrix* and *A. gyrans*). By GISH technique we compared the genomes of the diploids *A. selloanus, A. macrothrix* and *A. gyrans*, and the triploid *A. ternatus* confirming the hypotheses that the South American (*A. selloanus, A. macrothrix*) and the North American (*A. gyrans*) species share a common S genome that comprises just one of the genomes in the South American triploid *A. ternatus.*

Material and Methods

Plant material

Living plants from naturally occurring populations were transplanted to clay pots at the experimental garden of the Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste (FCA-UNNE), Corrientes, Argentina. Vouchers of the studied specimens (N) were deposited at the herbarium of the Instituto de Botánica del Nordeste (CTES) and at the United States National Herbarium (US). The origin of the studied material is cited in Table 1.

Methods

Chromosome preparations

At least three plants per species and ten metaphase plates per individual were used for chromosome measurements in three species of *Andropogo*n. For mitotic chromosome preparations root tips were collected from potted plant and pre-treated with 0.002 M 8-hydroxyquinoleine solution at room temperature for 5 h, fixed in absolute ethanol/glacial acetic acid 3:1 (v/v) for 24 hours and stored in ethanol 70% at -20 °C, according to Greizerstein (1987), with minor modifications.

Cytological analysis

For karyotype analysis the root tips were stained following Feulgen's technique and squashed in a drop of 2% acetic orcein (Jong 1997).

Table 1 – Locality and voucher number of the studies material used in this work.

Species	Collector and locality
Andropogon gyrans (Michx.)	G.A. Norrmann 332. Estados Unidos, Georgia, Thomasoville
Andropogon selloanus Hack. (Hack.)	G.A. Norrmann 229. Argentina, Prov. Corrientes, Corrientes
Andropogon macrothrix Trin.	G.A. Norrmann 81. Argentina, Prov. Corrientes, 17 km al sur de San Roque
Andropogon ternatus (Spr.) Nees	G.A. Norrmann 74. Argentina, Prov. Corrientes, 30 km S de Curuzú Cuatiá

Chromosome measurements were performed using the computer application MicroMeasure 3.3 program (<http://www.colostate.edu/Depts/Biology/MicroMeasure/>). The karyotype was built using imaging processing. The nomenclature followed for karyotype description was proposed by Levan *et al.* (1964). Chromosome morphology was determined using the centromeric index (CI) (short arm x 100/total length). Accordingly, chromosomes were classified as metacentric (*m*) = 50–37.5 and submetacentric (*sm*) = 37.5–25. Mean karyotype values for each species were represented as haploid complements in the idiograms. Chromosomes were ordered primarily by morphology and then by decreasing size. Karyotype asymmetry was estimated using two numerical parameters according to Romero Zarco (1986): A_1 and A_2. The first index measure intrachromosomal asymmetry index, ranging from zero to one. It has been estimated using the following equation where b_i is the average length for short arms in every homologous chromosomes pairs, B_i is the average length for long arms in every homologous chromosomes pairs, *n* is the number of homologues chromosomes pairs:

$$A_1 = 1\text{-}(1)$$

and the interchromosomal index

$$A_2 = S/ \quad (2)$$

where S is the standard deviation, and the mean of chromosome length. Both indexes are independent of chromosome number and chromosome size. Parameters and estimated index means were compared by analysis of variance (ANOVA); Tuckey's test was used to examine karyotype similarity between species.

Fluorochrome banding DAPI-CMA_3

For the description of CMA_3^+/$DAPI^+$ bands distribution pattern we followed to Guerra (2000). Fluorchrome banding was performed in all species according to Schweizer (1976). The root tips were digested using a solution containing 2% cellulose (w/v) and 20% pectinase (v/v) for 3 h at 37 °C and dissected in 45% (v/v) aqueous acetic acid, the root tips were squashed under a coverslip. After removal of the coverslip with carbon dioxide, slides were air dried for 2 h at room temperature and then kept at -20 °C until use. Slides were double stained with DAPI (4', 6-diamidino-2-phenylindole) and CMA_3 (Chromomycin A_3) and mounted in 1:1 (v/v) McIlvaine´s Ph7 buffer-glycerol.

C-banding

The C-banding technique was performed according to Schweizer (1976) with minor modifications, using DAPI instead of Giemsa stain.

In situ hybridization

Was performed on mitotic cells according to Cuadrado & Jouve (1994) with minor modifications. The probe containing the 45S ribosomal DNA (45S rDNA) sequences amplified from *Triticum aestivum*, was labelled by Bionick Labelling System (Invitrogen) following manufacturer's instructions and detected with Streptavidin Cy3 (red). Total genomic DNA from dry collected leaves was isolated using the Wizard Genomic DNA purification kit (Promega) and labeled using DIG High Prime (Roche) or a Biotin Nick Translation kit (BioNick Labelling System-Invitrogen) according to manufacturer's instructions. Slides were counterstained with 4', 6-diamidino-2-phenylindole (DAPI) (1 µg in bi-distillate water) for 10 min at room temperature, and subsequently mounted in antifade solution. GISH was performed with a hybridization stringency of 85%. Slides were treated with sheep anti-digoxigenin–FITC and streptavidine-Cy3 conjugate, to detect digoxigenin- (green) and biotin- (red) labeled probes, respectively. Slides were counterstained with DAPI (4', 6-diamidino-2-phenylindole).

Fluorescence microscopy and image acquisition

Chromosomes were viewed and photographed with a Leica DMLB equipped with a computer-assisted Leica DFC350 FX CCD digital camera system and Leica IM50 software, version 4.0 (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK); with appropriate filters for TRITC, FITC, and DAPI excitation.

Determination of DNA content

Flow cytometry analyses were performed using young leaves and following the recommendations of the Partec kit CySatin PI Absolute P, which was used to prepare the samples. Briefly, 0.5 cm^2 of leaves material was placed in a Petri dish with a comparable amount of leaves from an internal standard (*Paspalum intermedium*, Sch28857 (diploid, 2C =1.51 pg) (Vaio *et al.* 2007; Galdeano *et al.* 2016). After adding 0.5 ml of extraction buffer (Nuclei Extraction Buffer-CyStain PI Absolute P) from Partec, the tissue was chopped with a razor blade. Following a 2 min incubation, the chopped material was filtered through a 50 µm nylon mesh into the sample tube with 1.5 ml of staining solution buffer with Propidium Iodide (Doležel & Bartoš 2005). The mixture was incubated for 2 min at room temperature and analyzed by flow cytometry. At least three replicates and 5.000 nuclei were measured in each sample using a Partec PA II flow cytometer (Partec GmbH, Münster, Germany) with the detector operating at 480 nm. The absolute 2C-value of the DNA content of each sample was calculated according to Doležel & Bartoš (2005) employing the following formula:

Sample 2C DNA content = x standard 2C DNA content DNA (pg). (3)

The monoploid genome size (1Cx) was calculated dividing the 2C-value by the ploidy level (Greilhuber *et al.* 2005). The factor for conversion of pg to Mbp is 978 (Doležel *et al.* 2003). Data was analyzed using PA II's Partec FloMax software. Statistical analyses to estimate the genome size of each species were performed using InfoStat software, version 2015 (Di Rienzo *et al.* 2015). The differences in DNA content were tested by analysis of variance (ANOVA), and comparisons between means were performs using Tukey's method. Significance was evaluated at $p > 0.05$.

Results

General karyotype features

Mitotic chromosomes and karyotypes are shown in Figure 1. Chromosome number, karyotype formulae and the parameters analyzed for the species are summarized in Table 2.

The karyotypes of *A. selloanus*, *A. macrothrix* and *A. gyrans* are reported for the first time in this paper. Morphological analysis showed that all the species have their complement mainly consisting of metacentric (*m*) and submetacentric (*sm*) chromosomes (Fig. 1d; Tab. 2), like those reported by Okoli & Olorode (1983) for *Andropogon tectorum* Schum. & Thonn. (2n = 20), from the south of Nigeria (Africa).

The mean chromosome length varied between 3.20 µm (*A. selloanus*), 3.75 µm (*A. gyrans*) and 5.06 µm (*A. macrothrix*), and the total length of the karyotype range from 64.25 µm (*A. selloanus*), 75.24 µm (*A. gyrans*) and 101.40 µm (*A. macrothrix*) (Tab. 2).

All karyotypes showed chromosomes with secondary constrictions. Although they were borne by different chromosome pairs, they always were located on the *m* chromosomes at the distal end of the short arms (Fig. 1d). In *A. selloanus* and *A. macrothrix* one secondary constriction was observed in the pair 9 and pair 5 respectively. However, in *A. gyrans* a pair of secondary constrictions was observed in both homologous of the pair 7. In all the studied cells of *A. selloanus* and in a few of *A. macrothrix*, it was observed that one chromosome of the pairs of homologous has a clearly visible satellite and the other chromosome present a proximal segment which became evident with a bright C^+ and CMA_3^+/$DAPI^-$ bands.

The intrachromosomal asymmetry index (A_1) ranged from 0.14 to 0.21, while the interchromosomal index (A_2) ranged from 0.13 to 0.16. These values indicate small variation among the chromosome arms in the different species; however, data for the A_2 indexes showed differences among the size of the different chromosomes in each species. Dates are plotted in a scatter diagram (Fig. 2a).

Flow cytometry

The measurements of 2C-value for the species ranged from 2.23 pg to 2.61 pg (Tab. 2; Fig. 2b-d). Significant differences in DNA content between the three species were found by ANOVA and Tuckey's test at $p > 0.05$.

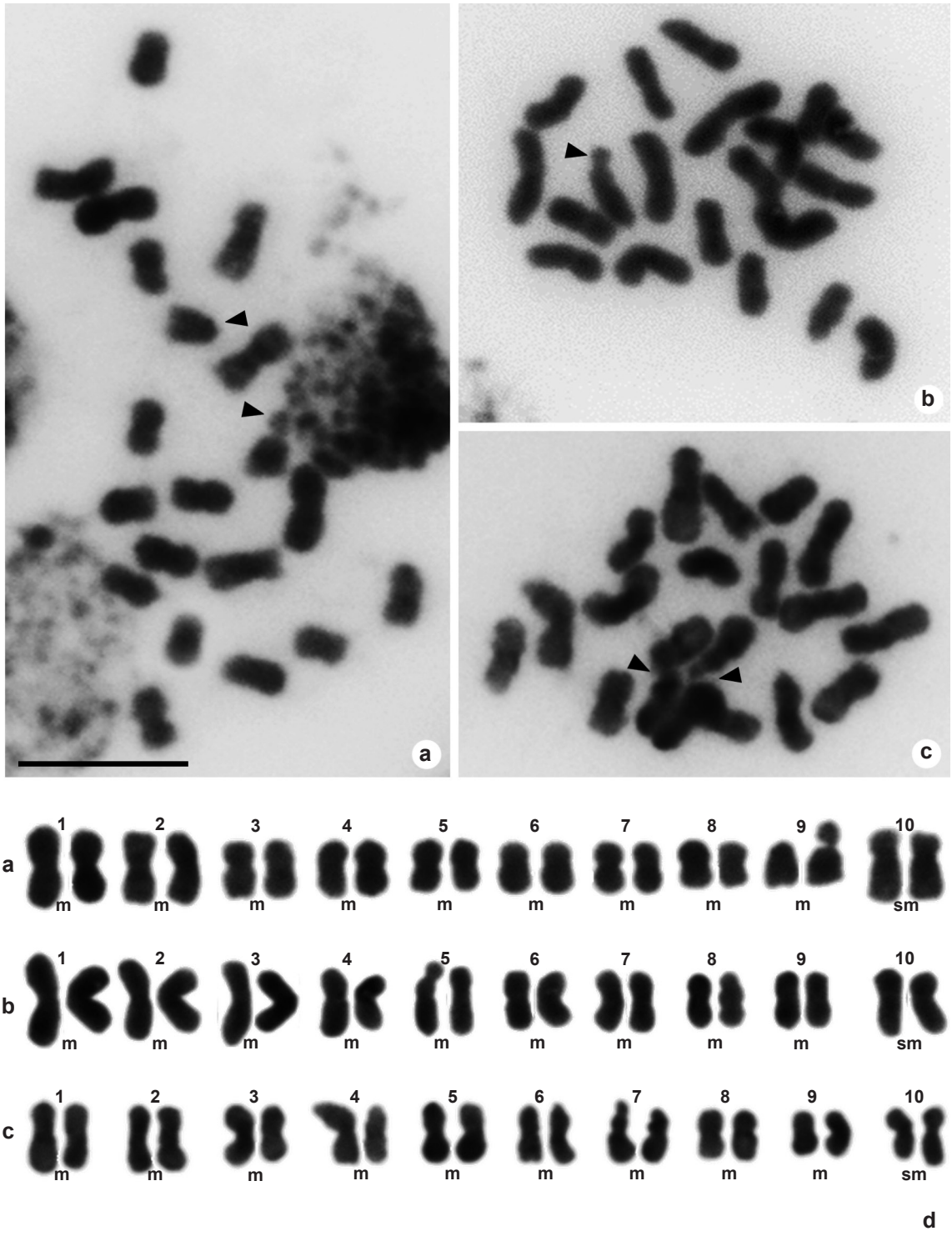

Figure 1 – Mitotic metaphase and karyograms of a. *Andropogon selloanus*; b. *A. macrothrix*; c. *A. gyrans*. d. Karyograms. The arrowhead indicates the sat chromosomes. Scale bar: 5 µm.

Table 2 – Chromosome number, karyotype formulae, total chromosome length (TCL), mean centromeric index (CI), asymmetry indexes (A_1-A_2), DNA content, heterochromatin refers to C^+-DAPI band and number of 45S rDNA sites of *A. gyrans*, *A. selloanus* and *A. macrothrix*. Abbreviations: NB: number of heterochromatin bands; Pe: pericentromeric; Di: distal; Ter: terminal. Note: Values in column DNA content data followed by different letter are significantly different.

								Heterochromatin C-DAPI$^+$				N° rDNA sites
Species	**2n**	**Karyotype Formulae**	**TCL (µm)**	**CI ẋ (µm)**	**A1**	**A2**	**DNA content ẋ (pg) ± SE**	**NB Pe**	**NB Di**	**NB Ter**	**%**	**45 S**
A. gyrans	20	9 m + 1 sm	75.24	45.98	0.14	0.13	2.23 ± 0.02a	20	2	2	16.19	2
A. selloanus	20	9 m + 1 sm	64.25	44.93	0.17	0.16	2.49 ± 0.02b	20	4	6	26.25	2
A. macrothrix	20	9 m + 1 sm	101.40	43.56	0.21	0.16	2.61 ± 0.03c	20	4	8	24.23	2

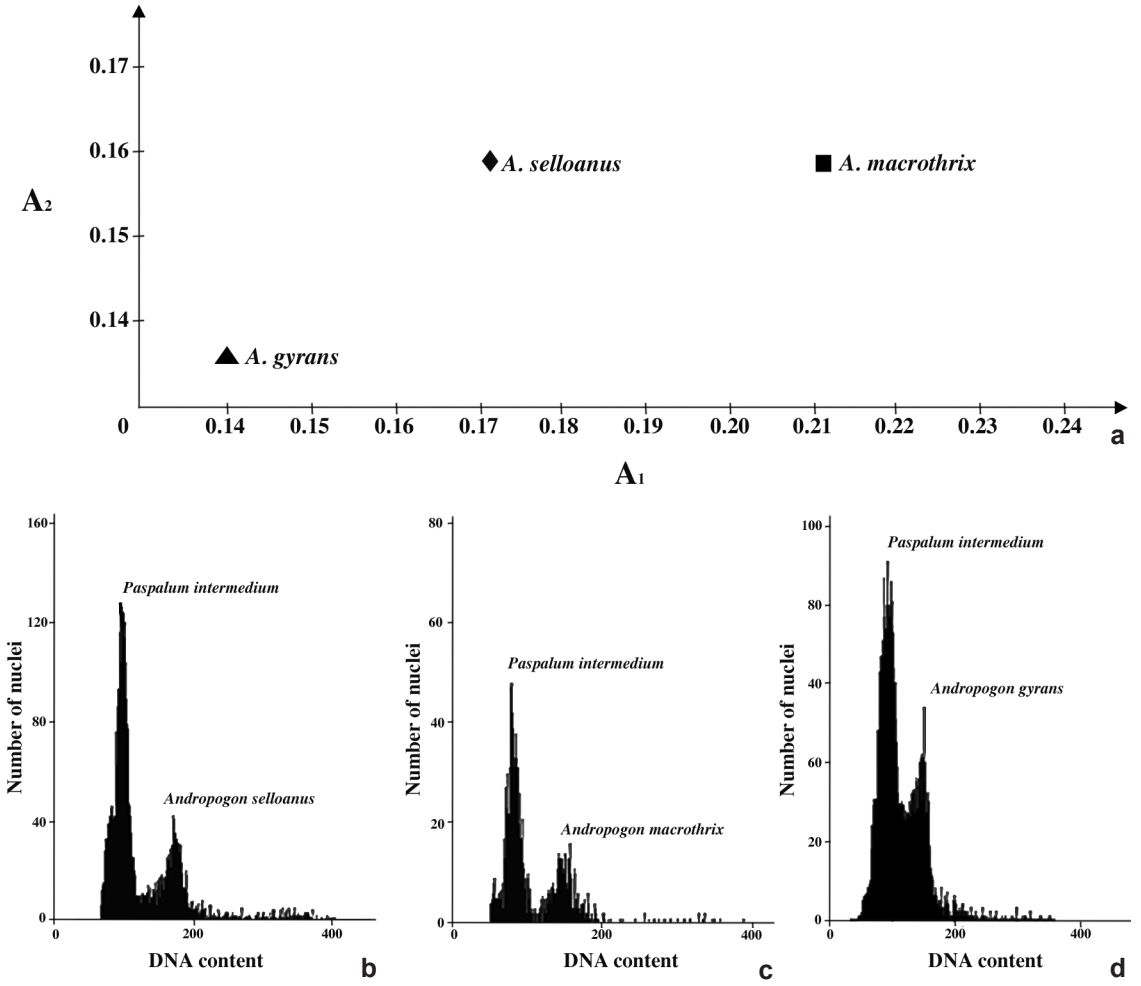

Figure 2 – a. Scatter diagram showing the relation between intrachromosomal (A_1) and interchromosomal (A_2) asymmetry indexes. b-d. Histogram illustrating the absolute DNA content of *Paspalum intermedium* (standard) and b. *A. selloanus*, c. *A. macrothrix*, d. *A. gyrans* obtained by flow cytometric analysis of propidium iodide stained nuclei isolated from young leaves. Values of A_1, A_2 and DNA content are summarized in Table 2.

Heterochromatin distribution

The pattern of C^+-DAPI heterochromatin distribution showed that the karyotypes of *A. selloanus, A. macrothrix* and *A. gyrans* had conspicuous pericentromeric fluorescent bands in all chromosomes pairs (Fig. 3). Furthermore, *A. selloanus* showed two pairs of *m* chromosomes with terminal and distal C^+ bands (ranged from 0.24–0.39 µm), occupying both

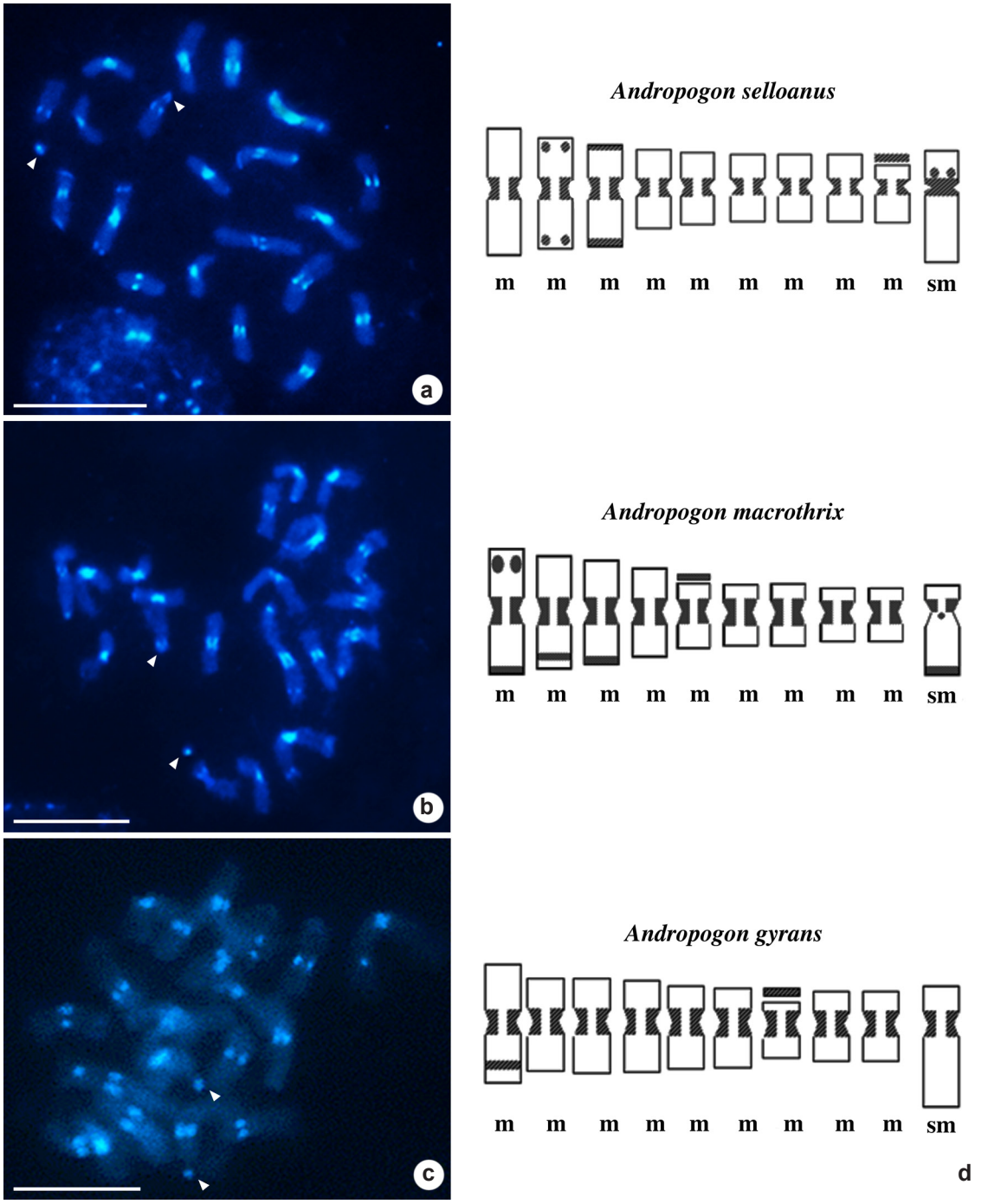

Figure 3 – Metaphase chromosomes with C/DAPI banding and bands idiograms: a. *Andropogon selloanus*; b. *A. macrothrix*; c. *A. gyrans.* The arrowhead indicates satellites C^+/DAPI. d. Idiograms showing the distribution of C^+/DAPI banding patterns. Scale bar: 5 µm.

chromosomic arms (Fig. 3a,d). The total amount of heterochromatin per complement was 26.25% of the total karyotype length. In *A. macrothrix,* the pattern of C^+-DAPI heterochromatin distribution was similar than *A. selloanus* showing three pairs of *m* chromosomes with terminal and distal C^+ bands (ranged from 0.21–0.2 µm), which occupy one or both chromosomic arms. The s*m* chromosomes pairs from *A. macrothrix* showed terminal C^+ bands and a particular pattern of pericentromeric bands (Fig. 3b,d). The total amount of heterochromatin per complement was 24.23% of the total karyotype length. In *A. gyrans*, only one pair of *m* chromosomes showed distal C^+ bands (0.38 µm) in one chromosomic arm. The rest of the *m* chromosomes and the *sm* pair showed pericentromeric C^+ bands (Fig. 3c-d). The total amount of heterochromatin per complement was 16.19% of the total karyotype length. In the three species studied, the heterochromatin distribution among the karyotypes was also conserved. The *m* chromosomes pairs had medium-sized bands (0.55 µm in *A. selloanus,* 0.49 µm in *A. macrothrix* and 0.45 µm in *A. gyrans*). The s*m* chromosomes pairs bore the largest pericentromeric bands (0.81 µm in *A. selloanus*, 0.82 µm in *A. macrothrix* and 0.63 µm in *A. gyrans*).

In all species sat regions of the sat chromosomes shows bright C^+ heterochromatic bands (Fig. 3d).

CMA_3/DAPI bands distribution pattern

Most chromosomes of *A. selloanus* present fully and partially heterochromatic arms CMA_3^+/$DAPI^+$ (Fig. 4a-b,g). In this case, the heterochromatin occupies the complete arm and extending towards to the pericentromeric region involved partially the other arm. It is interesting to point out the presence of a pair of *m* chromosomes completely $DAPI^+$/CMA_3^+. In addition, other chromosomes, showed AT-rich and GC-rich heterochromatic bands in pericentromeric and telomeric position (range from 0.42–0.45 µm).

In *A. macrothrix*, the double staining with CMA_3/DAPI revealed a bands distribution pattern preferentially distributed in some pericentromeric and telomeric regions. Most of these bands are AT-rich or GC-rich DNA. Two pairs of metacentric chromosomes and the submetacentric pair had small pericentromeric $DAPI^+/CMA_3^-$ (*i.e.*, brighter with CMA and duller with DAPI) (range from 0.35–0.38 µm) bands and one pair presented a heterochromatic arm $CMA_3^+/DAPI^+$ (Fig. 4c-d,g).

Andropogon gyrans exhibit eighteen bright bands which are similar in size (0.45 µm). These bands were distributed in pericentromeric position, being most of them AT-rich DNA. Eight AT-rich and GC-rich heterochromatic sites were clearly observed in telomeric region. These bands are similar in size (0.3 µm) and distributed in one arm from four metacentric pairs of chromosomes. The sat-chromosome pairs showed a heterochromatic $CMA_3^+/DAPI^+$ short arm. Only one pair of chromosomes did not showed bands. In the three species, bright GC-rich heterochromatin band was observed in the secondary constriction occupying its total area (Fig. 4e-g).

Chromosome mapping of the 45S rDNA genes by FISH

The FISH pattern of the 45S rDNA gene concerning number, position and size of loci not differ between the three species studied here. This technique showed that each species had one pair of 45S rDNA loci localized at the distal ends of the short arms of two metacentric chromosomes (Fig. 4h-j). The size of the 45S rDNA loci is similar for the three species range 0.4–0.45 µm. These brightest loci coincides with the site where the secondary constriction is located.

Genomic relationship by GISH technique

Andropogon selloanus (2x), *A. macrothrix* (2x), *A. gyrans* (2x) and *A. ternatus* (3x) genomes were studied by GISH technique. The total genomic DNA from one species was labeled and used as a probe on metaphase chromosomes from the other species

When the probe from *A. macrothrix* was hybridized on mitotic chromosomes preparation of *A. selloanus*, the fluorescent signal was bright and uniform along their entire length, labeling all 20 chromosomes (Fig. 5a-b).

The hybridization of *A. gyrans* mitotic chromosomes with total genomic DNA probe from *A. selloanus* showed that, at least ten chromosomes, are completely hybridized with strong and uniform signal and the remaining chromosomes show hybridization fluorescent signals with different intensities on one chromosome arm (Fig. 5c-d).

To analyze the relationship among diploid and polyploid genomes, total genomic DNA probe from the diploid species *A. selloanus* were hybridized on mitotic chromosomes of the triploid species *A. ternatus*. Hybridization signals were distributed over only 20 out of the 30 chromosomes showing green fluorescence (Fig. 5e-f), albeit stronger signal on certain chromosomal region and weak and disperse

signal in other regions. It is important to note the presence of some pairs of chromosomes with a single hybridized chromosome arm, suggesting the presence of intergenomic rearrangements.

Discussion

Chromosome analysis consider that species with homologous genomes may show similar karyotypes and display similar patterns

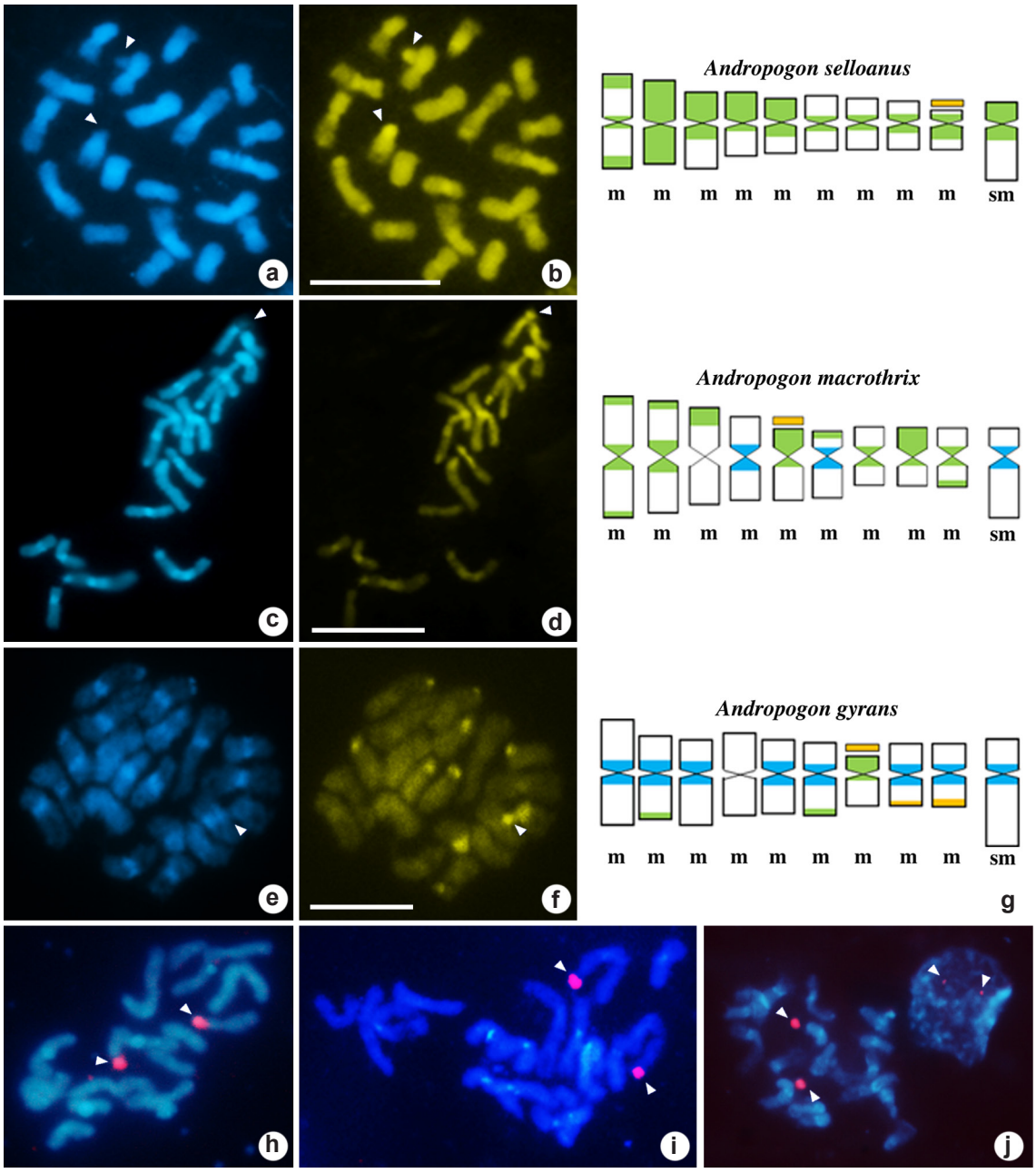

Figure 4 – Metaphase chromosomes. a-f. CMA_3/DAPI banding. a. DAPI. b. CMA_3 of A. selloanus; c. DAPI. d. CMA_3 of A. macrothrix; e. DAPI. f. CMA_3 of A. gyrans. The arrowhead indicates the satellites CMA_3+/DAPI- band. g. Idiograms showing the distribution of $DAPI^+$ (light blue), CMA^+ (yellow) and CMA^+/ $DAPI^+$ (green) banding patterns. h-j. FISH with 45S rDNA: h. *A. selloanus*, i. *A. macrotrix*, j. *A. gyrans* showig two signals. The arrowhead indicates the 45S rDNA sites. Scale bar=5 µm.

of chromosome markers, therefore we used cytogenetic techniques to explore the chromosomes of this three diploids species to obtain information about the presence of the S genome within section *Leptopogon*.

The karyotype formula of 18 m + 2 sm was constant within the three species. The centromeric index indicates that all karyotypes are moderately symmetric. The *A. macrothrix* karyotype is the least symmetrical with little variation in length between its chromosomes compared to those of the other two species, and it has the longest chromosomes.

Our C^+-DAPI banding analysis evidenced a very characteristic pattern of heterochromatin distribution. Although the terminal bands had the same shape, the interstitial bands showed different aspects in both South American species, some as small blocks and others as small points. The presence of big heterochromatic bands at pericentromeric position with shaped like ribs on the whole chromosomes is a repeating pattern in all the three species. However, although in the three species the distribution pattern of heterochromatin is similar, the low amount of heterochromatin by karyotype present in *A. gyrans* is due to the absence of more terminal and interstitial bands, which are present in the other two species. Fluorochrome banding has been a great tool in the identification of homologous chromosomes pairs and its characterization (Moscone *et al.* 1996). According to Guerra (2000), the preferential localization of heterochromatin in the pericentromeric region of all, or almost all, the chromosomes of the karyotypes is a common pattern for species with small chromosomes.

Concerning the nature of the heterochromatin bands, CMA/DAPI staining demonstrated that the three species had a different pattern of heterochromatin distribution bands. In *A. selloanus* most of the heterochromatin bands were presented

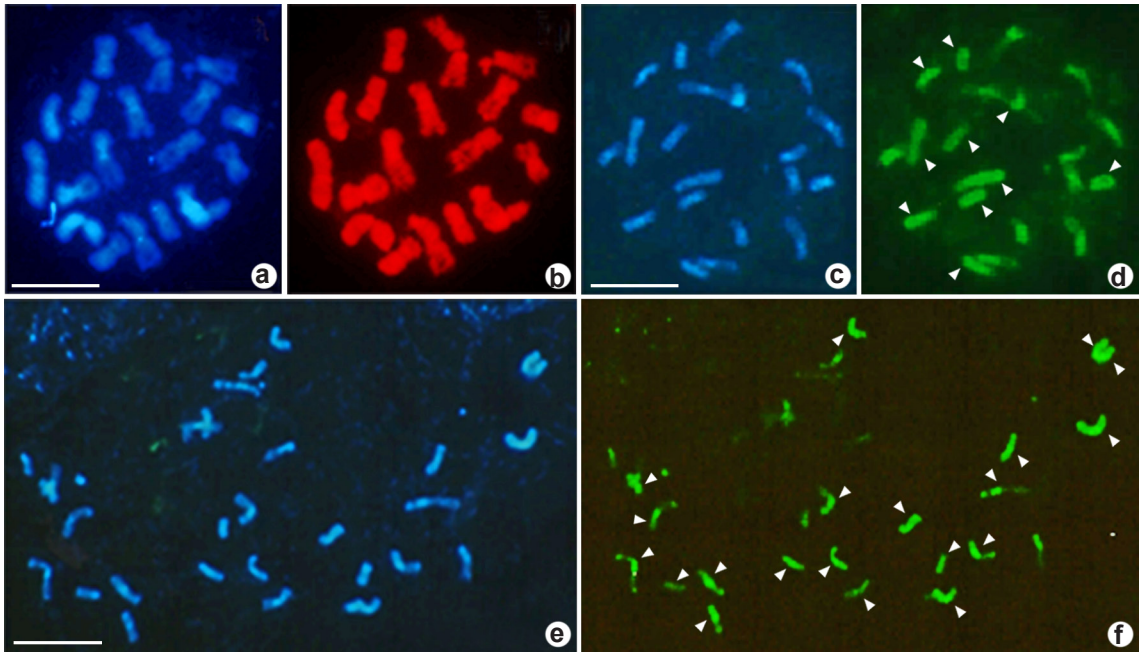

Figure 5 – GISH on somatic metaphase cells from root tips of diploid and triploid taxa of *Andropogon*. Sites of probe hybridization fluoresce red with Cy3-conjugated avidin and green with antidigoxigenin-FITC. Counterstaining with DAPI. (a-b) *A. selloanus* (2n=2x=20) probed with genomic DNA from *A. macrothrix* (2n=2x=20) and detected with red fluorescence. (a) DAPI-staned chromosomes; (b) GISH showing all 20 chromosomes fluorescing red. (c-d) *A. gyrans* (2n=2x=20) probed with genomic DNA from *A. selloanus* and detected with green fluorescence. (c) DAPI-stained chromosomes; (d) GISH showing at least ten chromosomes completely hybridized (arrowhead) and the remaining with signal on one chromosome arm. (e-f) *A. ternatus* (2n=3x=30) probed with genomic DNA from *A. selloanus* and detected with green fluorescence. (e) DAPI-stained chromosomes; (f) GISH showing 20 chromosomes fluorescein green (arrowhead), albeit stronger signal on certain chromosomal region and weak and disperse signal in other regions. Scale bar = 10 μm

as large blocks AT-rich and GC-rich sequences forming heterochromatic arms. Here, it is interesting to point out the presence of a pair of *m* chromosomes completely $DAPI^+/CMA_3^+$, suggesting the presence of the same type of heterochromatin all along the chromosome. This pattern of heterochromatin distribution is not usual but it seems that it is not uncommon in the genus *Andropogon*, so that in the hexaploid species *A. gerardii, A. exaratus* and in the interspecific hybrids where *A. exaratus* is one of the progenitors a similar phenomenon is observed (Hidalgo *et al.* in press).

On the other hand, the double staining with CMA_3/DAPI in *A. macrothrix,* revealed a different pattern of heterochromatin distribution bands than *A. selloanus*. Such banding pattern is predominantly centromeric and telomeric and it composed of small AT-rich or GC-rich sequences in most of its chromosomes. Instead, *A. gyrans* exhibits heterochromatic bands in all chromosome pairs except one. Most of the bands presented were AT-rich distributed in pericentromeric position and very few terminal band were GC-rich. In these three diploid species, we found that the NORs and NOR-associated heterochromatin was GC-rich, being this a rule in plants (Schweizer 1976).

The analysis of band patterns showed us that heterochromatin is not randomly distributed but rather that it is preferentially located. The NOR-associated heterochromatin is an example, displaying a very marked distribution at telomeric sites from sat-chromosomes. This heterochromatin type is very frequently in these diploids species being positively stained by C-banding techniques and by fluorochromes with high affinity for GC-rich chromatin.

The analysis of the distribution of heterochromatin, located preferentially in the chromosomal regions, is one of the important aspects of its investigation (Guerra 2000), suggesting that heterochromatin has a functional and evolutionary meaning that might not be the same for all species, there are only trends or preferential patterns for different genomes and karyotypes (Guerra 2000). This C^+- and DAPI/ CMA_3 banding technique is a very useful tool for comparative studies of genomes and for the analysis of evolutionary and phylogenetic relationships between species, becoming more important in the process of cytogenetic discrimination between species.

The secondary constrictions were observed in all species and were located in the short arm, close to the telomere region on the *m* chromosomes. They displayed a conspicuous heterochromatin distribution patterns showing bright bands revealed by C^+/DAPI and double staining technique CMA_3^+/DAPI¬. In both South American species, C^+ heterochromatin bands were found in one satellite of the sat chromosome and in the proximal segment of its homologous chromosome. These results suggest that these pairs are heterozygous for the length of the satellite. On the other hand, in *A. gyrans* the secondary constrictions were observed in both homologous chromosomes with bright C^+ heterochromatin bands. These differences in the morphology of the sat-chromosomes between the South American diploid species and the North American one could be due to small structural rearrangements that would have been suffered *A. gyrans* chromosomes. In addition, it was noted that in the three species the double staining with CMA_3/DAPI demonstrated that bright GC-rich heterochromatin band was observed in the secondary constriction occupying its total area.

The most characteristic heterochromatin detected with fluorochromes was the heterochromatin associated to the NOR region, usually CMA^+ and $DAPI^-$ (Guerra 2000). This clearly differentiated from other heterochromatic types due it contains rDNA genes, although it may also contain repeated sequences not related to the NOR (Cuadrado & Jouve 1994). Our analysis of 45S rDNA genes demonstrated that the three species had one pair of 45S rDNA loci localized at the distal ends of the short arms of two metacentric chromosomes displaying a high homomorphy in the FISH pattern of homologous chromosomes. The number, location and size of the 45S rDNA loci were highly conserved in these species with large heterochromatic block. Both the number and position of 45S rDNA sites detected by FISH always coincided with the C^+/DAPI bands and $CMA^+/DAPI^-$ bands presented in the distal region of the secondary constrictions observed in the karyotypes of these species.

Heterochromatin could be an additional component of the genome, as is usual in many plants (Greilhuber 1982, 1984), and there may be a correlation between karyotype length, number and length of C-bands, amount of C-heterochromatin and nuclear DNA content. Nevertheless, comparing the three species studied here we observe an opposite correlation: *A. macrothrix* with the largest karyotype had the greatest nuclear DNA content but only 2% less

in the heterochromatin content than *A. selloanus*. However, *A. selloanus* with the highest content of heterochromatin and only 0.12 pg less of nuclear DNA content than *A. macrothrix* had the smallest karyotype length of the three species. On the other hand, *A. gyrans* with less nuclear DNA content had the lowest heterochromatin content of the three, perhaps because of its lower content in terminal and interstitial bands, but its karyotype length is larger than *A. selloanus*. These results could be due to the great variation in the repetitive sequences which are dispersed in the genome.

Genome analysis in section Leptopogon

In the present work, the information we obtained was used to suggest the existence of interspecific relationships between *Andropogon* diploid species belonging to the section *Leptopogon*. These diploid species could be grouped according to their karyotype features. In this sense, the entities with this karyotype patterns also have heterochromatic bands in all chromosomes; thus, they are homogeneous in their gross karyotypic structure defining the S genome. Therefore, this would confirm that *A. selloanus, A. macrothrix* and *A. gyrans* shared a basic genome called "S" after *A. selloanus* (Norrmann & Quarín 1987). Their affinities in rDNA loci and heterochromatic banding patterns are in accordance with data on crossability. The two South American diploids species with the S genome, *A. selloanus* and *A. macrothrix*, are the most similar, self-pollinating (Norrmann 1985) and fully fertile (Norrmann & Quarín 1991). In southern South America, while *A. selloanus* grows in very varied environments, in natural or altered fields, in isolated form or in small and spaced populations, *A. macrothrix* lives in places with high humidity in the soil. They are sympatric in large areas of Argentina, Paraguay,

Brazil and Uruguay where natural hybridization is possible although in low degree (Galdeano & Norrmann 2000). These authors found that *A. selloanus* dominated a dense population on both sides of the roads, while *A. macrothrix* grew exclusively in small low areas of the same places. There they found an intermediate individual growing in the middle of de population.

On the other hand, *A. gyrans*, a member of a closely interrelated North American group, the *Virginicus* complex, whose monophyly has been demonstrated by classical taxonomy (Campbell 1983), is self-pollinating and cleistogamous. Its center of diversity is on the Coastal Plain of the southeastern United States (Campbell 1983). Also shares karyotype formulae, high homomorphy in the rDNA gene FISH patterns than the South American diploids and not only the presence of heterochromatin in the pericentromeric region of all their chromosome pairs are similar, but also the relative amount of heterochromatin present in each chromosome.

The donor of the S genome

Our results confirm that *A. selloanus, A. macrothrix* and *A. gyrans* could constitute a homogeneous group that share a basic genome. The use of GISH with genomic DNA of *A. macrothrix* and *A. gyrans* on mitotic chromosomes of *A. selloanus,* reinforces the hypothesis of the existence of a basic genome, originally defined by *A. selloanus* and also shared by the North American diploid *A. gyrans* (Norrmann *et al.* 2004).

When Norrmann *et al.* (2004) performed GISH studies with DNA from the South American diploids species with S genome on two South American hexaploid species *A. lateralis* and *A. bicornis* (members of the *A. lateralis* complex and belonging to the section *Leptopogon*), they observed that the S genome only found homologous sequences in less than half of the hexaploid chromosomes. However, these homologous sequences were not uniformly labeled along their entire length, but mainly concentrated in the pericentromeric regions, the remaining chromosomes showed only a very weak hybridization signal, as the results they observed when the hybridization with DNA from *A. gyrans* was performed on meiotic chromosomes from the hexaploids species.

A similar homology pattern was observed in the hybridization performed on mitotic chromosomes from the rare South American triploid *A. ternatus* with genomic DNA from *A. selloanus*, suggesting that a form of the S genome would be one of the genomes involved in its formation. The low sequence homology between them may possibly be a result of a common ancestry. The lack of sequence homology, suggest that there has been some divergence of the repetitive sequences in the distal regions of the S genome chromosomes since the polyploid was formed, as was observed in hexaploids. So that, they no longer hybridize to the S genome probe. Therefore, the triploid species seems to have a closer relationship with *A. selloanus* indicating

that the diploid species might have participated in the origin of the South American triploid. In fact, an interespecific cross between these two species was successful (Norrmann & Quarín 1987). It is interesting to point out the presence of partially hybridized chromosomes in the studied polyploid species that would indicate the occurrence of chromosome rearrangements. This is in agreement with Soltis & Soltis (1999) who postulated that the intra- and intergenomic reorganization is a frequent event in polyploid species.

The results obtained in the present paper, together with those from classical cytogenetic, morphological studies, interspecific crosses and molecular approaches; support the hypothesis that *A. selloanus* may be the most probable donor of one of the genomes involved in the origin of the poliploids from the section *Leptopogon* in South America.

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