



Karyotype asymmetry in *Cynodon* Rich. (Poaceae) accessions

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Genet. Mol. Res. 15 (4): gmr15049152

Received September 2, 2016

Accepted October 19, 2016

Published December 2, 2016

DOI <http://dx.doi.org/10.4238/gmr15049152>

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ABSTRACT. *Cynodon* is a genus of plants with forage potential that has attracted the interest of breeders. These species have high morphological variability in a large number of varieties and cytotypes, hampering identification. This study aimed to determine the karyotype asymmetry index among accessions of *Cynodon* to discriminate between them. Karyotype symmetry was based on three estimates, which were compared. The basic number for the genus is $x = 9$. The results of the chromosome count and DNA quantification, respectively, were as follows: two diploid accessions ($2n = 2x = 18$ and 1.08 ± 0.094 to 1.17 ± 0.036 pg DNA and \pm standard deviation), one triploid accession ($2n = 3x = 27$ and 1.63 ± 0.017 pg DNA), four tetraploid accessions ($2n = 4x = 36$ and 1.88 ± 0.069 to 2.10 ± 0.07 pg DNA), and one pentaploid accession ($2n = 5x = 45$ and 2.55 ± 0.098 pg DNA). *C. incompletus* var. *hirsutus* had the longest total length of the haploid lot ($29.05 \mu\text{m}$), with

chromosomes that ranged from 1.7 to 6.2 μm in length. On the basis of the karyotype asymmetry indices, the accessions were divided into two groups: 1) *C. dactylon* var. *dactylon*, *C. transvaalensis*, *C. dactylon* var. *polevansii*, three accessions of *Cynodon* sp. and *C. nlemfuensis*; and 2) *C. incompletus* var. *hirsutus*. This is the first description of tetraploidy in *C. transvaalensis*. The karyotypic data facilitated a determination of the degree of proximity between the accessions.

Key words: Chromosome symmetry; *Cynodon*; DNA content; Polyploidy

INTRODUCTION

Cynodon Rich. (Poaceae) is a genus of perennial plants that is typical of tropical and subtropical regions and is widely adapted to various environments. It has high morphological variability and has multiple applications in various regions worldwide, such as its use in pasture and stored forage, soil cover and sward, on golf courses, and in gardens (Wu, 2011; Jewell et al., 2013).

Species of this group, particularly *Cynodon dactylon* (L.) Pers., have attracted the interest of breeders because of their ease of cultivation, high forage production, high nutritional value (Pedreira, 1996), high growth rate, grazing resistance, high carrying capacity, and rapid establishment (de Lima and Vilela, 2005). The main objective of forage breeding programs is to obtain genotypes with desirable agronomic traits, such as high forage production, persistence, tolerance to poorly drained soils and frosts, and forage quality for livestock systems, including, beef and milk cattle, horse feeding, and hay and silage production (Vilela, 2005).

Species of *Cynodon* have been separated into two groups based on their morphology (Nascimento et al., 2002). “Bermuda grasses”, represented by *C. dactylon*, have rhizomes and stolons, and “star grasses” [*C. plectostachyus* (K. Schum.) Pilg., *C. aethiopicus* Clayton & J.R. Harlan, and *C. nlemfuensis* Vanderryst] have only stolons. However, there are varieties and cytotypes with morphological similarities; consequently, many mistakes in classification occur (Caro and Sánchez, 1969; Harlan et al., 1970a; Clayton et al., 2006). In addition to the divergences in taxonomy, there is also controversy regarding the number of species and varieties of the genus, the records of which range from 9 to 14 species (Caro and Sánchez, 1969; Harlan et al., 1970a; Clayton et al., 2006). Thirteen species of *Cynodon* are recorded in the Plant List (<http://www.theplantlist.org/>), and for *C. dactylon*, one subspecies and 23 varieties are described. These ambiguities are a problem for conservation and the exchange of genetic resources, because many plants can be incorporated into botanical and germplasm collections or even incorrectly used in crosses.

Cytogenetic information, such as karyotype characterization and nuclear DNA quantification, is important in the discrimination of species, varieties, and cytotypes, and for germplasm handling, and has been successfully used in grasses including *Pennisetum* (Techio et al., 2002, 2010), *Paspalum* (Vaio et al., 2005), *Setaria* (Nani et al., 2015), and *Brachiaria* (Nani et al., 2016). However, cytogenetic studies have not been conducted on *Cynodon*. The existing information is limited to chromosome counts and the determination of ploidy level, without considering taxonomic differences. The genus has the basic chromosome number $x = 9$ (Dhaliwal and Gupta, 2011; Zhi-Yun et al., 2013), but plants with different ploidy levels

have been described, including diploid ($2n = 2x = 18$), triploid ($2n = 3x = 27$), tetraploid ($2n = 4x = 36$), and, in smaller numbers, pentaploid ($2n = 5x = 45$) and hexaploid ($2n = 6x = 54$) (Harlan et al., 1970a; Wu and Taliaferro, 2009). The goal of this study was to determine karyotype asymmetry among *Cynodon* accessions, in order to discriminate between them and establish their genetic relationships.

MATERIAL AND METHODS

Plant material

We evaluated seven *Cynodon* accessions (Table 1) that were donated by Embrapa Gado de Leite, Juiz de Fora, Minas Gerais State, Brazil. The accessions were obtained from the Active Germplasm Bank, United States Department of Agriculture, Tifton, GA, USA.

Table 1. Identification of accessions/species of the genus *Cynodon*.

Accession	Identification*
PI 291151-20	<i>Cynodon dactylon</i> var. <i>dactylon</i> (L.) Pers.
PI289923-09	<i>Cynodon transvaalensis</i> Burt-Davy
PI 289750-10	<i>Cynodon dactylon</i> var. <i>polevansii</i> (Stent) J.R. Harlan & de Wet
PI 29175301 PL 15	<i>Cynodon incompletus</i> var. <i>hirsutus</i> (Stent) de Wet & J.R. Harlan
PI 291755-24	<i>Cynodon</i> sp
PI 224148-28	<i>Cynodon</i> sp
PI 224147-13	<i>Cynodon</i> sp
ERX 7**	<i>Cynodon nlemfuensis</i> Vanderryst

*Standardization of synonyms followed that of the United States Department of Agriculture (<https://npgsweb.ars-grin.gov/gringlobal/site.aspx?id=22>). ***Cynodon nlemfuensis* (purple star grass) accession developed by Embrapa Gado de Leite breeding program.

Cytogenetic analysis

For cytogenetic evaluations, root tips were pre-treated with cycloheximide (0.025%) at room temperature for 2 h. The roots were then fixed in Carnoy's solution (three parts of absolute ethyl alcohol to one part acetic acid) and stored at -20°C until slide preparation. The roots were subjected to enzymatic digestion with an enzyme mix containing Onozuka R10 cellulase (0.7%), cellulase (0.7%), pectolyase (1%), and cytohellicase (1%) (Sigma-Aldrich) at 37°C for 60 min. Slides were prepared by the flame-drying technique (Dong et al., 2000), and stained with 5% Giemsa. Photomicrographs of the metaphases were taken with a microscope (Axio Lab. A1, Zeiss) with an image-capture camera (AxioCam ICc1), and analyzed using the AxioVision 4.8.2 software.

Chromosome measurements and karyotype analysis

Morphometric characteristics of the chromosomes [mean lengths of the short arm (c) and the long arm (l)] were obtained using the software MicroMeasure 3.3 (Reeves and Tear, 2000). We then calculated the total length of the chromosome, the total length of the haploid

lot (TLHL), and the relative length of each chromosome. The morphological classification of the chromosomes was based on the ratio of arms proposed by Levan et al. (1964). This information was used for assembling karyograms of the *Cynodon* accessions with Adobe Photoshop. Ten metaphases were measured per accession.

TLHL values were tested by analysis of variance (ANOVA) and the Tukey test at the 5% probability level using the software Sisvar (Ferreira, 2011), in order to compare the accessions.

To determine karyotype symmetry, we employed the criteria of Stebbins (1971), Zarco (1986), and Peruzzi and Eroğlu (2013). Stebbins (1971) considers the ratio of the arms and the lengths of the chromosomes to assess karyotype symmetry. The criteria of Zarco (1986) are based on the indices A1, intrachromosomal asymmetry (relative to the centromere position), and A2, interchromosomal asymmetry (relative to size). Peruzzi and Eroğlu (2013) propose the use of M_{CA} parameters (mean centromeric asymmetry) for intrachromosomal asymmetry and CV_{CL} (coefficient of variation of chromosome length) for interchromosomal asymmetry.

DNA quantification

To estimate the DNA content, three samples of each accession evaluated (Table 1) were quantified. Young *Cynodon* leaf tissue (20-30 mg) was macerated with the same amount of material from *Pisum sativum* (internal reference standard, 2C DNA content = 9.09 pg) on a Petri dish containing 1 mL cold buffer (Marie and Brown, 1993) to obtain a nuclear suspension (Dolezel, 1997). To this nuclear suspension, we added 25 μ L propidium iodide, and for each sample we quantitated at least 10,000 nuclei.

The analysis was performed on a FACSCalibur™ cytometer (BD Biosciences), and histograms were obtained using the CellQuest™ software and analyzed in WinMDI 2.9. The nuclear DNA content of the plants was estimated by comparison with the G1 peak position of the internal reference standard. DNA content values were compared by ANOVA and the Tukey test using Sisvar.

RESULTS

Data on the chromosome number and ploidy level of all eight *Cynodon* accessions evaluated (Figures 1 and 2 and Table 2) were corroborated by the estimated nuclear DNA content. Whereas the basic number of chromosomes proposed for the genus is $x = 9$, the species *C. nlemfuensis* (ERX 7) and *C. incompletus* var. *hirsutus* (PI 29175301 PL 15) were diploid ($2n = 2x = 18$), with the smallest amount of nuclear DNA (1.08 ± 0.094 and 1.17 ± 0.036 pg, respectively) (Figure 1A and B). *Cynodon* sp (PI 291755-24) was triploid with $2n = 3x = 27$ and 1.63 ± 0.017 pg of DNA (Figure 1C). The accessions *C. dactylon* var. *dactylon* (PI 291151-20), *C. transvaalensis* (PI 289923-09), and *Cynodon* sp (accessions PI 224148-28 and PI 224147-13) were tetraploid ($2n = 4x = 36$, Figure 1D-G), with the nuclear DNA content varying between 1.88 ± 0.069 and 2.10 ± 0.07 pg. In terms of DNA content, there were no significant differences between these four accessions/species, but they were statistically different to the other species. *C. dactylon* var. *polevansii* (PI 289750-10) had the highest nuclear DNA content (2.55 ± 0.098 pg), which was compatible with its higher ploidy level ($2n = 5x = 45$ chromosomes) (Figure 1H).

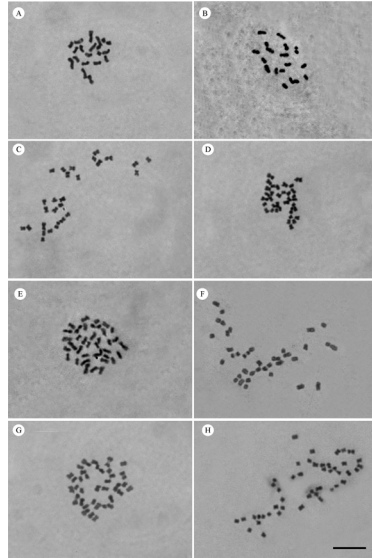


Figure 1. Mitotic metaphases of *Cynodon* accessions. **A.** *C. incompletus* var. *hirsutus* (PI 29175301 PL 15; $2n = 2x = 18$); **B.** *C. nlemfuensis* (ERX 7; $2n = 2x = 18$); **C.** *Cynodon* sp (PI 291755-24; $2n = 3x = 27$); **D.** *C. dactylon* var. *dactylon* (EGL1; $2n = 4x = 36$); **E.** *C. transvaalensis* (PI 289923-09; $2n = 4x = 36$); **F.** *Cynodon* sp (PI 224148-28; $2n = 4x = 36$); **G.** *Cynodon* sp (PI 224147-13; $2n = 4x = 36$); **H.** *C. dactylon* var. *polevansii* (PI 289750-10; $2n = 5x = 45$). Bar = 10 μ m.

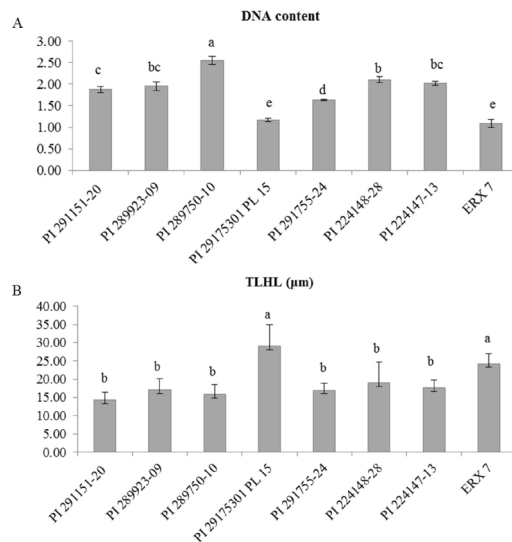


Figure 2. Nuclear DNA content (pg) and \pm standard deviation (**A**) and total length of the haploid lot (TLHL) (mm) (**B**) of *Cynodon*. *C. incompletus* var. *hirsutus* (PI 29175301 PL 15; $2n = 2x = 18$); *C. nlemfuensis* (ERX 7; $2n = 2x = 18$); *Cynodon* sp (PI 291755-24; $2n = 3x = 27$); *C. dactylon* var. *dactylon* (PI 291151-20; $2n = 4x = 36$); *C. transvaalensis* (PI 289923-09; $2n = 4x = 36$); *Cynodon* sp (PI 224148-28; $2n = 4x = 36$); *Cynodon* sp (PI 224147-13; $2n = 4x = 36$); *C. dactylon* var. *polevansii* (PI 289750-10; $2n = 5x = 45$). Means followed by different letters were significantly different by the Tukey test at the 5% probability level.

Table 2. *Cynodon* accession chromosome numbers.

Accession	Species	Chromosome number
PI 291151-20	<i>Cynodon dactylon</i> var. <i>dactylon</i>	2n = 4x = 36
PI 289923-09	<i>Cynodon transvaalensis</i>	2n = 4x = 36
PI 289750-10	<i>Cynodon dactylon</i> var. <i>polevansii</i>	2n = 5x = 45
PI 29175301 PL 15	<i>Cynodon incompletus</i> var. <i>hirsutus</i>	2n = 2x = 18
PI 291755-24	<i>Cynodon</i> sp	2n = 3x = 27
PI 224148-28	<i>Cynodon</i> sp	2n = 4x = 36
PI 224147-13	<i>Cynodon</i> sp	2n = 4x = 36
ERX 7	<i>Cynodon nlemfuensis</i>	2n = 2x = 18

With the exception of the diploid accession *C. incompletus* var. *hirsutus* (PI 29175301 PL 15) that had only submetacentric chromosomes (sm) with a 9sm karyotype formula, the karyotypes of the other accessions were composed of only metacentric chromosomes (m) (Figure 3 and Table 3). *C. nlemfuensis* (ERX 7) and *C. incompletus* var. *hirsutus* (PI 29175301 PL 15), despite having a lower amount of DNA, had the greatest total lengths of the haploid lot (24.21 and 29.05 mm, respectively), and significantly differed from the other accessions (Figure 2). In *C. nlemfuensis* (ERX 7) and *C. incompletus* var. *hirsutus* (PI 29175301 PL 15) accession, the lengths of the largest and smallest chromosome pairs corresponded to 15.88 and 7.92%, respectively, of the TLHL. Among the polyploid accessions, PI 224147-13 (*Cynodon* sp) had the largest and smallest chromosome pairs, which corresponded to 17.18 and 7.44%, respectively, of the TLHL (Table 4).

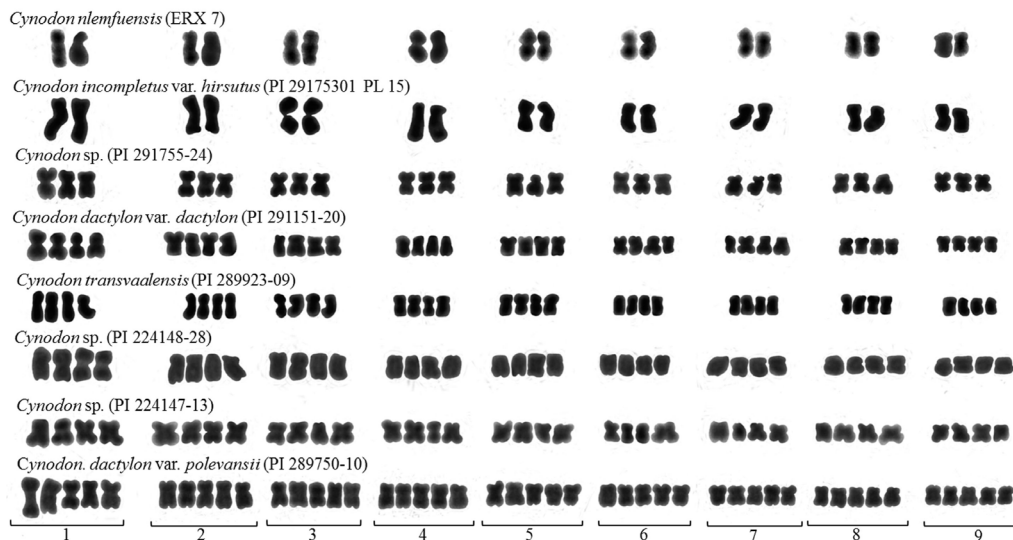


Figure 3. Karyotypes of *Cynodon* accessions. **A.** *C. nlemfuensis* (2n = 2x = 18); **B.** *C. incompletus* var. *hirsutus* (PI 29175301 PL 15; 2n = 2x = 18); **C.** *Cynodon* sp (PI 291755-24; 2n = 3x = 27); **D.** *C. dactylon* var. *dactylon* (PI 291151-20; 2n = 4x = 36); **E.** *C. transvaalensis* (PI 289923-09; 2n = 4x = 36); **F.** *Cynodon* sp (PI 224148-28; 2n = 4x = 36); **G.** *Cynodon* sp (PI 224147-13; 2n = 4x = 36); **H.** *C. dactylon* var. *polevansii* (PI 289750-10; 2n = 5x = 45). Bar = 10 μ m.

Table 3. Arm ratio and karyotypic formulae of *Cynodon* accessions.

Chromosome	Accessions and arm ratio							ERX 7
	PI 291151-20	PI 289923-09	PI 289750-10	PI 29175301 PL 15	PI 291755-24	PI 224148-28	PI 224147-13	
1	1.489	1.57	1.393	2.069	1.638	1.643	1.39	1.57
2	1.524	1.56	1.551	2.618	1.565	1.619	1.388	1.5
3	1.552	1.474	1.436	2.238	1.593	1.597	1.391	1.34
4	1.498	1.52	1.35	2.097	1.542	1.518	1.387	1.26
5	1.421	1.54	1.451	2.365	1.496	1.557	1.35	1.27
6	1.412	1.511	1.368	1.998	1.533	1.574	1.445	1.24
7	1.412	1.388	1.372	1.864	1.355	1.448	1.427	1.22
8	1.363	1.55	1.4	2.211	1.383	1.548	1.367	1.2
9	1.38	1.395	1.343	1.798	1.509	1.395	1.349	1.2
Karyotype formula	9m	9m	9m	9sm	9m	9m	9m	9m

PI 291151-20, *C. dactylon* var. *dactylon*; PI 289923-09, *C. transvaalensis*; PI 289750-10, *C. dactylon* var. *polevansii*; PI 29175301 PL 15, *C. incompletus* var. *hirsutus*; PI 291755-24, PI 224148-28, and PI 224147-13, *Cynodon* sp; ERX 7, *C. nlemfuensis*; m, metacentric; sm, submetacentric.

Table 4. Mean relative length (%) and karyotype symmetry estimates of chromosomes of *Cynodon* accessions according to the criteria of Stebbins (1971), Zarco (1986), and Peruzzi and Eroğlu (2013).

Chromosome	Accession and mean relative length (%)							ERX 7
	PI 291151-20	PI 289923-09	PI 289750-10	PI 29175301 PL 15	PI 291755-24	PI 224148-28	PI 224147-13	
1	16.03	16.396	15.535	15.887	15.669	16.592	17.181	15.567
2	13.708	13.814	13.071	13.425	13.347	14.076	13.827	13.757
3	12.262	12.483	12.146	12.18	12.144	12.767	12.195	12.759
4	11.385	11.497	11.386	11.718	11.492	11.531	11.197	11.332
5	10.696	10.587	10.723	10.868	10.672	10.711	10.549	10.468
6	10.033	9.907	10.213	10.025	10.079	9.902	9.995	10.055
7	9.424	9.23	9.682	9.248	9.505	9.077	9.158	9.371
8	8.716	8.565	9.091	8.725	8.955	8.237	8.448	8.754
9	7.742	7.517	8.148	7.92	8.134	7.104	7.444	7.934
Stebbins	4b	4b	4a	2b	4a	4b	4b	4a
A1	0.309	0.332	0.288	0.528	0.336	0.351	0.279	0.527
A2	0.232	0.2504	0.202	0.225	0.211	0.269	0.268	0.225
M _{CA}	18.336	19.988	16.879	36.046	20.319	21.342	16.258	35.953
CV _{CL}	23.297	25.040	20.229	22.557	21.187	26.968	26.871	22.576

PI 291151-20, *C. dactylon* var. *dactylon*; PI 289923-09, *C. transvaalensis*; PI 289750-10, *C. dactylon* var. *polevansii*; PI 29175301 PL 15, *C. incompletus* var. *hirsutus*; PI 291755-24, PI 224148-28, and PI 224147-13, *Cynodon* sp; ERX 7, *C. nlemfuensis*; A1, intrachromosomal asymmetry; A2, interchromosomal asymmetry; M_{CA}, mean centromeric asymmetry; CV_{CL}, coefficient of variation of chromosome length.

Based on the symmetry criteria proposed by Stebbins (1971), the karyotypes were divided into three categories: 4a for *C. dactylon* var. *polevansii* (PI 289750-10), *Cynodon* sp (PI 291755-24), and *C. nlemfuensis* (ERX 7); 4b for *C. dactylon* var. *dactylon* (PI 291151-20), *C. transvaalensis* (PI 289923-09), and *Cynodon* sp (PI 224148-28 and PI 224147-13); and 2b for *C. incompletus* var. *hirsutus* (PI 29175301 PL 15) (Table 4). The estimated values of asymmetry obtained according to Zarco (1986) and Peruzzi and Eroğlu (2013) were identical and formed two groups. *C. incompletus* var. *hirsutus* (PI 29175301 PL 15) had the most asymmetric karyotype (A1 = 0.528, A2 = 0.225, M_{CA} = 36.046, and CV_{CL} = 22.557), while *C. nlemfuensis* (ERX 7) had the most symmetric (A1 = 0.237, A2 = 0.224, M_{CA} = 22.355, and CV_{CL} = 22.462) (Table 4 and Figure 4A and 4B).

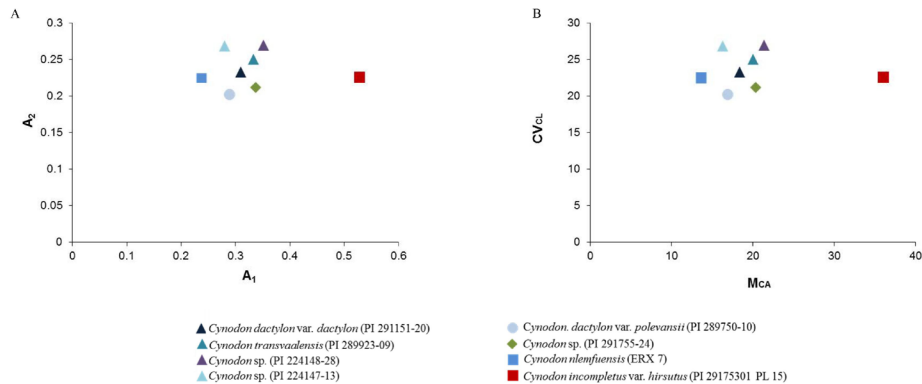


Figure 4. Karyotype asymmetry in *Cynodon* accessions. **A.** Indices of intrachromosomal asymmetry (A_1) and interchromosomal asymmetry (A_2) as proposed by Zarco (1986). **B.** Mean centromeric asymmetry (intrachromosomal asymmetry) (MCA) and coefficient of variation of the chromosomal length (interchromosomal asymmetry) (CV_{CL}) according to Peruzzi and Eroğlu (2013).

DISCUSSION

The predominant level of ploidy for the accessions was tetraploid, although diploid, triploid, and pentaploid accessions were also observed and validated by DNA quantification. Silva and Snaydon (1995) evaluated 480 plants from 32 populations of *C. dactylon* collected from Sri Lanka; most were $2n = 4x = 36$, and about 20% were $2n = 2x = 18$. The variation in ploidy levels (from diploid to hexaploid) in *Cynodon* has been described by several authors (Forbes and Burton, 1963; Harlan et al., 1970a; Dhaliwal and Gupta, 2011). This indicates that there is wide genetic variability in the group that is expressed as morphological, phenotypic, and biochemical variability, which are important characteristics for the origin and evolution of species (Schifino-Wittmann, 2004) and the development of improved cultivars (Wu and Taliaferro, 2009).

The chromosome counts of *C. dactylon* var. *dactylon* (PI 291151-20), *C. incompletus* var. *hirsutus* (PI 29175301 PL 15), and *C. nlemfuensis* (ERX 7) agreed with the observations made by Harlan et al. (1970b) in genotypes from Africa and Asia. The chromosome numbers of *C. transvaalensis* (PI 289923-09) and *C. dactylon* var. *polevansii* (PI 289750-10) ($2n = 4x = 36$ and $2n = 5x = 45$, respectively) differed from those reported by Harlan et al. (1970a), who cited $2n = 2x = 18$ and $2n = 4x = 36$, respectively, for these species.

This is the first record of tetraploidy in *C. transvaalensis* (accession PI 289923-09). This species is known as diploid ($2n = 2x = 18$), is endemic to South Africa, and is recognized as a species by its distinctive morphology (de Wet and Harlan, 1970), which includes a small size, erect posture, thin, linear, and green leaves, and yellow inflorescences with two to four racemes in a single spiral (de Wet and Harlan, 1971). The occurrence of tetraploid plants, such as the *C. transvaalensis* accession PI 289923-09, suggests the occurrence of chromosomal duplication or the production of unreduced gametes in diploid species, which may be elucidated through meiotic analysis. This is important information for interspecific hybridization purposes, because *C. transvaalensis* (diploid) is used in crosses with *C. dactylon* var. *dactylon* (tetraploid) to produce hybrid cultivars that are triploid and infertile (Wu and Taliaferro, 2009). The existence of a tetraploid *C. transvaalensis* opens up new possibilities

for combinations with the tetraploid *C. dactylon* for the possible generation of fertile hybrids, depending on the genomic/chromosomal affinity between them.

The triploidy ($2n = 3x = 27$) that was observed in the accession PI 291755-24 (*Cynodon* sp) indicates that they are hybrid plants. Previous studies have reported triploid intra- and interspecific hybrids, natural and induced, in *Cynodon* (Forbes and Burton, 1963; de Wet and Harlan, 1970; Gupta and Srivastava, 1970). According to Gupta and Srivastava (1970), there are two theories for the origin of triploid cytotypes: 1) natural crosses between diploid and tetraploid individuals; and 2) natural crosses between diploid individuals with an unreduced gamete and a reduced gamete. de Wet and Harlan (1970) described *C. x magennisii* Hurcombe as a natural triploid that originated from a cross between *C. dactylon* (tetraploid) and *C. transvaalensis* (diploid), and proposed that *C. x magennisii* Hurcombe would be an allopolyploid. An induced triploid was first described by Forbes and Burton (1963), who conducted a cross between diploid and tetraploid *C. dactylon* to originate a triploid hybrid. They detected a high frequency of trivalents in the hybrids, indicating homology between the genomes of tetraploid and diploid species. Harlan et al. (1970b) observed that artificial triploid hybrids from a cross between *C. incompletus* (diploid) and *C. dactylon* (tetraploid) formed nine bivalents and nine univalents. They concluded that there was no homology between the genomes of these species, and hybrids from this cross were infertile.

The origin of *C. dactylon* var. *dactylon* (tetraploid), such as accession PI 291151-20, has been associated with the union of the unreduced gametes of the ancestor *C. dactylon* var. *aridus* (Harlan et al., 1969). *C. dactylon* var. *polevansii* is described as strictly tetraploid ($2n = 4x = 36$), and is found only near Barberspan in South Africa (Harlan et al., 1970b). However, plants of this species evaluated in the present study (PI 289750-10) had $2n = 5x = 45$ chromosomes. In a previous classification, *C. dactylon* var. *polevansii* was classified as a species (*C. polevansii*) by Stent in 1927. In a study by Anderson et al. (2009), *C. polevansii* plants were identified as triploid, tetraploid, and pentaploid; the tetraploid and pentaploid plants were from Barberspan and the triploid plants were from Delareyville, South Africa. Pentaploids, such as accession IP 289750-10, can occur through crosses between hexaploid and tetraploid cytotypes (Wu, 2011).

Regarding DNA quantification, the mean coefficient of variation obtained by flow cytometry was approximately 0.58%, which demonstrates the quality of the results and the reliability of the DNA content estimates, because values below 2% are considered high quality (Marie and Brown, 1993). The nuclear DNA contents obtained for the accessions analyzed in this study were similar to the values reported by Wu et al. (2006), Kang et al. (2008), and Gulsen et al. (2009). The variation in DNA found between the tetraploid accessions *C. dactylon* var. *dactylon* (PI 291151-20) and *Cynodon* sp (PI 224148-28) is common, as there are differences in DNA content between genotypes, populations, lineages, and cultivars within a species (Dolezel, 1997). This variation in the amount of DNA can be explained by variations in genome size between species within the same genus, or even within the same species.

Diploid accessions of *C. nlemfuensis* (ERX 7) and *C. incompletus* var. *hirsutus* (PI 29175301 PL 15) had the lowest DNA content and the greatest TLHL, because this measure is obtained from the average of the lengths of the homologous chromosomes, and does not take into account the number of chromosomes and ploidy of a species. Analyses of the karyotypes (Figure 2) revealed that these two accessions had larger chromosomes than the other accessions, as demonstrated by the TLHL. The TLHL can be influenced by the level of chromosome condensation, which explains a difference between the nuclear DNA content and the TLHL.

Regarding chromosome morphology, there were only submetacentric chromosomes in the diploid *C. incompletus* var. *hirsutus* (PI 29175301 PL 15), which ranged from 1.7 to 6.2 μm in length. There were only metacentric chromosomes in the other accessions/species. The only previous study using karyotypic data was conducted by Thomas and Murray (1978) for seven accessions of *C. dactylon*. The authors reported the occurrence of metacentric and submetacentric chromosomes, but those chromosomes had a total length of between 1 and 2 μm .

The parameters proposed by Stebbins (1971) pooled the accessions into three groups, and the parameters of Zarco (1986) and Peruzzi and Eroğlu (2013) grouped the accessions into two groups. The results obtained using the parameters of Stebbins (1971), Zarco (1986), and Peruzzi and Eroğlu (2013) to infer karyotype symmetry were similar, because both the scatter plot analysis and the classification of Stebbins (1971) showed that the karyotype of *C. incompletus* var. *hirsutus* (PI 29175301 PL 15) tended to be more asymmetric than the polyploids, which were a more homogeneous group. The karyotype of the diploid *C. nlemfuensis* (ERX 7) was the most symmetrical.

Symmetrical karyotypes mainly consist of metacentric and submetacentric chromosomes that are approximately the same size. Generally, species that are considered primitive in a group have symmetrical karyotypes with large numbers of chromosomes, whereas species with more asymmetric karyotypes are more derived. Asymmetry may increase due to the accumulation of differences in the relative sizes of chromosomes in the complement, making the karyotype more heterogeneous. Chromosomes can change their morphologies by rearrangements, such as pericentric inversions, uneven translocations, fissions, and centric fusions, which increase asymmetry. Therefore, karyotype variability can be used to identify evolutionary, chromosomal mechanisms in plants (Stebbins, 1958; Paszko, 2006; Techio et al., 2010).

The karyotype of the diploid accession *C. incompletus* var. *hirsutus* (PI 29175301 PL 15) consisted only of submetacentric chromosomes, and was the species with the most asymmetrical karyotype. Karyotype asymmetry increases as the centromere is gradually displaced from the middle to the end position (Zuo and Yuan, 2011). In *C. nlemfuensis* (ERX 7), which was also diploid but with a symmetrical karyotype, the chromosomes were metacentric and there was a gradual decrease in size, with little variation in chromosome length.

Our results demonstrate that *Cynodon* has variations in ploidy level but very similar karyotypes. Taxonomically, this variation in ploidy can generate numerous questions, as polyploids can be considered cytotypes with different ploidy levels or chromosomal races of the same species (Schifino-Wittmann, 2004). Chromosome counting, the construction and comparison of karyotypes, and the determination of ploidy level are very important tools in taxonomy. In this study, with the use of conventional cytogenetics, it was possible to identify a new chromosome number for *C. transvaalensis*, and reveal that accession PI 291755-24, identified as *Cynodon* sp, had a hybrid origin. However, the combination of karyotype and DNA content data was inadequate in accurately discriminating between all of the *Cynodon* accessions; for example, the accessions PI 224148-28 and PI 224147-13 were both identified as *Cynodon* sp and had the same chromosome number and morphology. There is, therefore, a need to investigate new cytogenetic markers in order to elucidate karyotype differences and separate accessions and species.

Regarding the accessions' relationships, the methodologies used for chromosome asymmetry showed that *Cynodon* sp (PI 291755-24) was closely linked to *C. dactylon* var. *polevansii* (PI 289750-10), while the other accessions that were identified as *Cynodon* sp (PI 224148-28 and PI 224147-13) were closer to each other. This observation can be extrapolated

to *C. dactylon* var. *dactylon* (PI 291151-20) and *C. transvaalensis* (PI 289923-09), which had the same chromosome number, ploidy, DNA content, and karyotype formula. The diploid accessions *C. incompletus* var. *hirsutus* (PI 29175301 PL 15) and *C. nlemfuensis* (ERX 7), which had the same chromosome number, ploidy level, and DNA content, differed in karyotype formula and chromosomal asymmetry. *C. nlemfuensis* (ERX7) was close to the triploid *Cynodon* sp (PI 291755-24) and the pentaploid *C. dactylon* var. *polevansii* (PI 289750-10).

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

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