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Intra- and interspecific karyotypic variations of the genus *Senna* Mill. (Fabaceae, Caesalpinioideae)

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ABSTRACT

Although the chromosome number 2*n* = 28 predominates in most species of the genus *Senna*, variations are often observed, resulting from either polyploidy (2*n* = 42, 56, 112) or disploidy (2*n* = 22, 24, 26) events. To better understand the karyotypic variations in *Senna,* we examined heterochromatin patterns of 10 species of that genus using chromomycin A3 (CMA) and 4'6-diamidino-2-phenylindole (DAPI) staining, and reviewed information on the chromosome counts of 72 species of that genus. The CMA/DAPI banding patterns were relatively variable among the 10 species, both in terms of the numbers of bands (from two to 26) and their locations on the chromosomes (terminal or proximal regions). Our review indicated that 2*n* = 28 is the most common ploidy among species of *Senna*, although other numbers were observed, apparently due to polyploidy or disploidy events; polysomy and aneusomy were also observed. Karyotype variations appear to have contributed to the diversification and wide distribution of *Senna*.

Keywords: Chromosome number, CMA/DAPI, disploidy, Leguminosae, polyploidy

Introduction

Senna is one of the most diverse genera within the family Fabaceae, with approximately 350 species of trees, shrubs, and sub-shrubs distributed throughout the American, African, and Australian continents, with occurrences also in Asia and on Pacific islands (Irwin & Barneby 1982; Marazzi *et al*. 2006). Species occupy an extremely wide range of habitats, varying from humid forests, dry forests, rock outcrops, dry or cold deserts to anthropized areas (Irwin & Barneby 1982; Acharya *et al*. 2011).

Phylogenetic analyses have demonstrated that *Senna* is monophyletic and occupies a position near *Cassia senso stricto* and *Chamaecrista* (Marazzi *et al*. 2006; Acharya *et al*. 2011). Those three genera form the subtribe Cassiinae Irwin & Barneby, and they are morphologically distinguished in relation to characteristics of their androceu, corolla, floral architecture, bracteoles, and fruits. *Senna* has traditionally been divided into six sections: *Astroites*, *Chamaefistula*, *Paradictyon*, *Peiranisia*, *Psilorhegma*, and *Senna*, based on their floral morphologies and fruit and extrafloral nectary structures (Irwin & Barneby 1982), although examinations of the DNA sequences of different chloroplast gene regions (*rpS16*, *rpL16*, *matK*) have demonstrated that most of those sections are polyphyletic (Marazzi *et al*. 2006).

Chromosome counts are available for approximately 20 % of the species of *Senna*, with a predominance of 2*n* = 28, although there are also records of 2*n* = 22, 24 and 26 (Goldblatt 1981; Souza & Benko-Iseppon 2004; Biondo *et al*. 2005a; Matos *et al*. 2011; Resende *et al*. 2013; Rice *et al*. 2015); records of polyploidy, such as 2*n* = 42, 56 and 112 in *Senna rugosa* (Resende *et al*. 2014), 2*n* = 56 in *S. aversiflora*, and 2*n* = 52 and 104 in *S. gardneri* (Matos *et al*. 2011) have

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also been cited. The consistent record of $2n = 28$ for most species demonstrates that the basic number may be $x = 14$, while the other numbers ($x = 11$, 12 and 13) apparently reflect disploidy events (Goldblatt 1981).

Karyotypic analyses of representatives of *Senna* using fluorochromes have been relatively scarce, with the exception of work by Souza & Benko-Iseppon (2004). Those authors reported the occurrence of two chromosome pairs with terminal or subterminal $CMA^+/DAPI^-$ bands in most of the species analyzed, with the exception of a population of *Senna obtusifolia* (which demonstrated two chromosome pairs with terminal $DAPI^+/CMA^-$ bands). Studies of heterochromatin patterns can be important tools for understanding the taxonomic relationships between different plant species, especially those that are morphologically very similar (Pessoa *et al*. 2014; Almeida *et al*. 2016; Cordeiro *et al*. 2016).

In order to understand the role of karyotype differentiation and evolutionary trends in this genus, we analyzed the heterochromatin patterns of 10 species of *Senna* (Fabaceae, Caesalpinioideae) using the fluorochromes chromomycin A3 (CMA) and 4'6-diamidino-2- phenylindole (DAPI) and reviewed the chromosome numbers of 72 species of that genus (and performed first counts for two species) for evidence of polyploidy, disploidy, and intra- and interspecific variations.

Materials and methods

Taxonomic sampling

We examined variations in the chromosome numbers of 74 species of the genus *Senna* based on chromosome counts published in the literature and on work presented here. The species names, authors, and references are listed in Table 1. For those species whose counts were exclusively obtained from the Chromosome Counts Database (CCDB, Rice *et al*. 2015), we present only the predominant chromosome count.

Cytogenetic analyses were performed on ten species of the genus *Senna* growing in areas of Caatinga (dryland) and humid forest vegetation in the Agreste region of Paraíba State, northeastern Brazil, to determine their heterochromatin patterns. Prepared samples of collected specimens were deposited in the Professor Jayme Coelho de Moraes Herbarium (EAN) of the Federal University of Paraíba. Detailed information concerning the species and their main karyological features are listed in Table 2. Seeds from the collected species were sown to germinate in Petri dishes. After their roots had grown to approximately 1.5 cm in length, they were excised and treated as described below. At least 10 roots per species were analyzed.

Table 1. Chromosome records of species of the genus *Senna*, and their respective references.

Taxon Name	Cromosome number ($2n$)	Source
Senna acuruensis (Benth.) H.S.Irwin & Barneby var. *acuruensis*	28	Matos *et al*. 2011
S. alata (L.) Roxb.	28	Biondo *et al*. 2005a; Resende *et al*. 2013; Souza & Benko-Iseppon 2004; Present work
S. alexandrina Mill.	28	Rice *et al*. 2015
S. angulata (Vogel) H.S.Irwin & Barneby	26	Biondo *et al*. 2005a
S. *appendiculata* (Vogel) Wiersema	28	Rice *et al*. 2015
S. araucarietorum H.S.Irwin & Barneby	28	Biondo *et al*. 2005a
S. armata (S. Watson) H.S.Irwin & Barneby	28	Rice *et al*. 2015
S. artemisioides (Gaudich. ex DC.) Randell	28	Rice *et al*. 2015
S. artemisioides subsp. *circinnata* (Benth.) Randell	56	Rice *et al*. 2015
S. artemisioides subsp. *zygophylla* (Benth.) Randell	42	Rice *et al*. 2015
S. atomaria (L.) H.S.Irwin & Barneby	24	Rice *et al*. 2015
S. auriculata Roxb.	28	Rice *et al*. 2015
S. aversiflora (Herb.) H.S.Irwin & Barneby	28, 56	Matos *et al*. 2011; Present work
S. bicapsularis (L.) Roxb.	28	Rice *et al*. 2015
S. birostris (Vogel) H.S.Irwin & Barneby var. *hookeriana* (Hook.) H.S.Irwin & Barneby	28	Rice *et al*. 2015
S. bracteosa D.B.O.S.Cardoso & L.P.Queiroz	28	Matos *et al*. 2011
S. cana (Nees & Mart.) H.S.Irwin & Barneby	28	Rice *et al*. 2015; Matos *et al*. 2011
S. candolleana (Vogel) H.S.Irwin & Barneby	24	Rice *et al*. 2015
S. cernua (Balb.) H.S.Irwin & Barneby	28	Biondo *et al*. 2005a; Resende *et al*. 2013
S. chrysocarpa (Desv.) H.S.Irwin & Barneby	24	Rice *et al*. 2015
S. corymbosa (Lam.) H.S.Irwin & Barneby	28	Biondo *et al*. 2005a; Resende *et al*. 2013
S. didymobotrya (Fresen.) H.S.Irwin & Barneby	28	Rice *et al*. 2015
S. durangensis (Rose) H.S.Irwin & Barneby	28	Rice *et al*. 2015
S. × *floribunda* (Cav.) H.S.Irwin & Barneby	28	Rice *et al*. 2015
S. fruticosa (Mill.) H.S.Irwin & Barneby	28	Rice *et al*. 2015
S. gardneri (Benth.) H.S.Irwin & Barneby	26, 52, 104	Matos *et al*. 2011
S. gaudichaudii (Hook. & Arn.) H.S.Irwin & Barneby	28	Rice *et al*. 2015

Table 1. Cont.

Taxon Name	Cromosome number (2*n*)	Source
S. glutinosa (DC.) Randell	42	Rice *et al.* 2015
S. georgica H.S.Irwin & Barneby	26	Present work
S. hilariana (Benth.) H.S.Irwin & Barneby	28	Biondo *et al.* 2005a
S. hirsuta (L.) H.S.Irwin & Barneby	28	Biondo *et al.* 2005a
S. hirsuta (L.) H.S.Irwin & Barneby var. *hirta* Irwin & Barneby	24	Rice *et al.* 2015
S. holosericea (Fresen.) Greuter	28	Rice *et al.* 2015
S. insularis (Britton & Rose) H.S.Irwin & Barneby	28	Rice *et al.* 2015
S. italica Mill.	28	Rice *et al.* 2015
S. kurtzii (Harms) H.S.Irwin & Barneby	24	Rice *et al.* 2015
S. macranthera (DC. ex Collad.) H.S.Irwin & Barneby	26	Biondo *et al.* 2005a; Resende *et al.* 2013
S. martiana (Benth.) H.S.Irwin & Barneby	28	Matos *et al.* 2011; Present work
S. montana (B.Heyne ex Roth) V.Singh	28	Rice *et al.* 2015
S. multiglandulosa (Jacq.) H.S.Irwin & Barneby	24	Rice *et al.* 2015
S. multijuga (Rich.) H.S.Irwin & Barneby	24	Biondo *et al.* 2005a; Resende *et al.* 2013
S. neglecta (Vogel) H.S.Irwin & Barneby	28	Biondo *et al.* 2005a
S. nitida (Rich.) H.S.Irwin & Barneby	24	Rice *et al.* 2015
S. oblongifolia (Vogel) H.S.Irwin & Barneby	28	Biondo *et al.* 2005a
S. obtusifolia (L.) H.S.Irwin & Barneby	24, 26, 28	Biondo *et al.* 2005a; Souza & Benko-Iseppon 2004; Rice *et al.* 2015; Present work
S. occidentalis (L.) Link	24, 26, 28	Biondo *et al.* 2005a; Matos *et al.* 2011; Rice *et al.* 2015; Present work
S. odorata (R. Morris) Randell	28	Rice *et al.* 2015
S. pallida (Vahl) H.S.Irwin & Barneby	28	Rice *et al.* 2015
S. pendula (Willd.) H.S.Irwin & Barneby	28	Biondo *et al.* 2005a; Resende *et al.* 2013
S. petersiana (Bolle) Lock	28	Rice *et al.* 2015
S. pilifera (Vogel) H.S.Irwin & Barneby	22	Biondo *et al.* 2005a
S. planitiicola (Domin) Randell	28	Rice *et al.* 2015
S. pleurocarpa (F.Muell.) Randell	28	Matos *et al.* 2011
S. podocarpa (Guill. & Perrottet) Lock	16	Rice *et al.* 2015
S. polyantha (Collad.) H.S.Irwin & Barneby	28	Rice *et al.* 2015
S. quinquangulata (Rich.) H.S.Irwin & Barneby	26	Souza & Benko-Iseppon 2004
S. reticulata (Willd.) H.S.Irwin & Barneby	28	Souza & Benko-Iseppon 2004
S. rizzinii H.S.Irwin & Barneby	26	Present work
S. roemeriana (Scheele) H.S.Irwin & Barneby	28	Rice *et al.* 2015
S. rugosa (G. Don) H.S.Irwin & Barneby	42, 56, 112	Biondo *et al.* 2005a; Resende *et al.* 2014
S. septemtrionalis (Viv.) H.S.Irwin & Barneby	28	Biondo *et al.* 2005a
S. siamea (Lam.) H.S.Irwin & Barneby	28	Resende *et al.* 2013; Souza & Benko-Iseppon 2004; Matos *et al.* 2011; Present work
S. silvestris (Vell.) H.S.Irwin & Barneby	28	Souza & Benko-Iseppon 2004; Matos *et al.* 2011
S. silvestris (Vell.) H.S.Irwin & Barneby var. *bifaria* H.S.Irwin & Barneby	28	Resende *et al.* 2013
S. sophera (L.) Roxb.	28	Rice *et al.* 2015
S. spectabilis (DC.) H.S.Irwin & Barneby	26, 28	Rice *et al.* 2015; Resende *et al.* 2013
S. spectabilis (DC.) H.S.Irwin & Barneby var. *excelsa* (Schrader) H.S.Irwin & Barneby	28	Rice *et al.* 2015; Present work
S. splendida (Vogel) H.S.Irwin & Barneby	26	Biondo *et al.* 2005a; Resende *et al.* 2013; Present work
S. sulfurea (Collad.) H.S.Irwin & Barneby	28	Rice *et al.* 2015
S. surattensis (Burm.f.) H.S.Irwin & Barneby	32	Rice *et al.* 2015
S. timoriensis (DC.) H.S.Irwin & Barneby	28	Rice *et al.* 2015
S. tora (L.) Roxb.	26	Rice *et al.* 2015
S. tropica (Vell.) H.S.Irwin & Barneby	28	Biondo *et al.* 2005c
S. venusta (F.Muell.) Randell	28	Matos *et al.* 2011

Cytogenetic and CMA/DAPI banding pattern analyses

To analyze heterochromatin patterns, the root tips were pretreated with 0.002 M 8-hydroxyquinoline (8-HQ) for 24 hours, fixed in 3:1 (v/v) absolute ethanol/glacial acetic acid for 30 minutes, and then stored in a freezer. For slide preparation, the roots were digested in a solution of 2 % cellulase and 20 % pectinase at 37 °C for 40 minutes.

Table 2. Principal karyological information concerning species of the genus *Senna*. Legend: T = terminal region of the chromosome, P = proximal region, H = heteromorphic band.

Species	Vouchers	2n	Median size	Heterochromatin patterns
Senna alata	JMPC - 1068	28	1.49 µm	6T $CMA^+/DAPI^-$
S. aversiflora	JMPC - 1066	56	1.96 µm	4T $CMA^+/DAPI^-$
*S. georgica**	JMPC - 1067	26	2.11 µm	4T $CMA^+/DAPI^-$, 22P $CMA^+/DAPI^-$
S. martiana	JMPC - 1070	28	2.00 µm	4T $CMA^+/DAPI^-$
S. obtusifolia	JMPC - 1146	26	2.78 µm	2T $CMA^+/DAPI^-$, 24P $CMA^+/DAPI^-$
S. occidentalis	JMPC - 1072	28	2.25 µm	2T $CMA^+/DAPI^-$
*S. rizzinii**	JMPC - 1069	26	2.19 µm	4T $CMA^+/DAPI^-$, 22P $CMA^+/DAPI^-$
S. siamea	JMPC - 1071	28	2.01 µm	6T $CMA^+/DAPI^-$, 1PH $CMA^+/DAPI^-$
S. spectabilis var. *excelsa*	JMPC - 1073	28	2.14 µm	6T $CMA^+/DAPI^-$, 2P $CMA^+/DAPI^-$
S. splendida	JMPC - 1074	26	2.49 µm	4T $CMA^+/DAPI^-$, 1PH $CMA^+/DAPI^-$

* First chromosome count for the species

The root tips were squashed in 45 % acetic acid, the slides frozen in liquid nitrogen to remove the coverslip. Slides were aged for three days and then stained for one hour with 10 µL of CMA (0.1 mg/mL) followed by staining with 10 µL DAPI (1 µg/mL) for 30 min., with subsequent mounting in glycerin/McIlvaine buffer (pH 7.0) (1:1, v/v). Slides were held for three days in the dark to stabilize the fluorochromes (Guerra & Souza 2002). Metaphases were recorded using a Zeiss microscope equipped with a Axio Cam MRC5 video camera, using Axiovision 4.8 software. At least three slides were analyzed for each species, generally photographing 10 cells per slide. Chromosome measurements were performed using Image Tool version 3.0 software (Donald *et al*. 2008). Chromosome morphologies were characterized using the centromeric index, following Guerra (1986). The images were edited using Adobe Photoshop CS3 Extended Version 10.0 software.

Results

Chromosome Numbers in Senna

The chromosome numbers of 72 species of the genus *Senna* were reviewed, and first counts were made for two species: *Senna georgica* ($2n = 26$) and *S. rizzinii* ($2n = 26$). Among them, 51 species (68.9 %) showed $2n = 28$; 11 species (14.8 %) showed $2n = 26$; 10 species showed $2n = 24$; three species showed $2n = 56$; and three others showed $2n = 42$; the numbers $2n = 112$, 104, 32, 22 and 16 were recorded in only one species each (Tab. 1).

Cytogenetic Analyses and CMA/DAPI Banding

Among the 10 species analyzed here, *Senna alata*, *S. martiana*, *S. occidentalis*, *S. siamea,* and *S. spectabilis* var. *excelsa* showed $2n = 28$, while *S. georgica*, *S. obtusifolia*, *S. rizzinii,* and *S. splendida* showed $2n = 26$ (Fig. 1). Polyploidy was identified in *S. aversiflora*, with $2n = 56$ (Fig. 1B). The predominant chromosome morphology was metacentric to submetacentric, with the mean sizes of the karyotypes varying from 1.49 µm in *S. alata* to 2.78 µm in *S. obtusifolia* (Tab. 2).

CMA/DAPI banding analysis showed an occurrence of GC-rich base pairs ($CMA^+/DAPI^-$) preferentially located in the terminal chromosome regions of the chromosomes, corresponding to Nucleolar Organizing Regions (NORs). Heterochromatic bands located in proximal regions were observed in some species, such as *S. obtusifolia*, *S. georgica*, and *S. rizzinii*. There were also differences band numbers, which varied between two and six bands in the terminal chromosome regions, and between one and 24 bands in proximal regions (Fig. 1, Tab. 2). Two species, *S. siamea* and *S. splendida*, showed heteromorphic bands, with only one of the homologous chromosomes of each species showing $CMA^+/DAPI^-$ bands in their proximal region (Fig. 1H, J, arrows).

Discussion

The chromosome numbers recorded here for species of the genus *Senna* confirmed previous counts reported in the literature (Biondo *et al*. 2005a; Resende *et al*. 2013; Souza & Benko-Iseppon 2004), with new records for *S. georgica* ($2n = 26$) and *S. rizzinii* ($2n = 26$).

The consistent records of $2n = 28$ in most species of *Senna* subjected to karyological analysis, especially in the basal clades (Marazzi *et al*. 2006), allied to that same number in diverse species of closely related genera (such as *Cassia*, *Apuleia* and *Delonix* [Biondo *et al*. 2005b; Rice *et al*. 2015]), confirms $x = 14$ as the basic ancestral number of *Senna*,

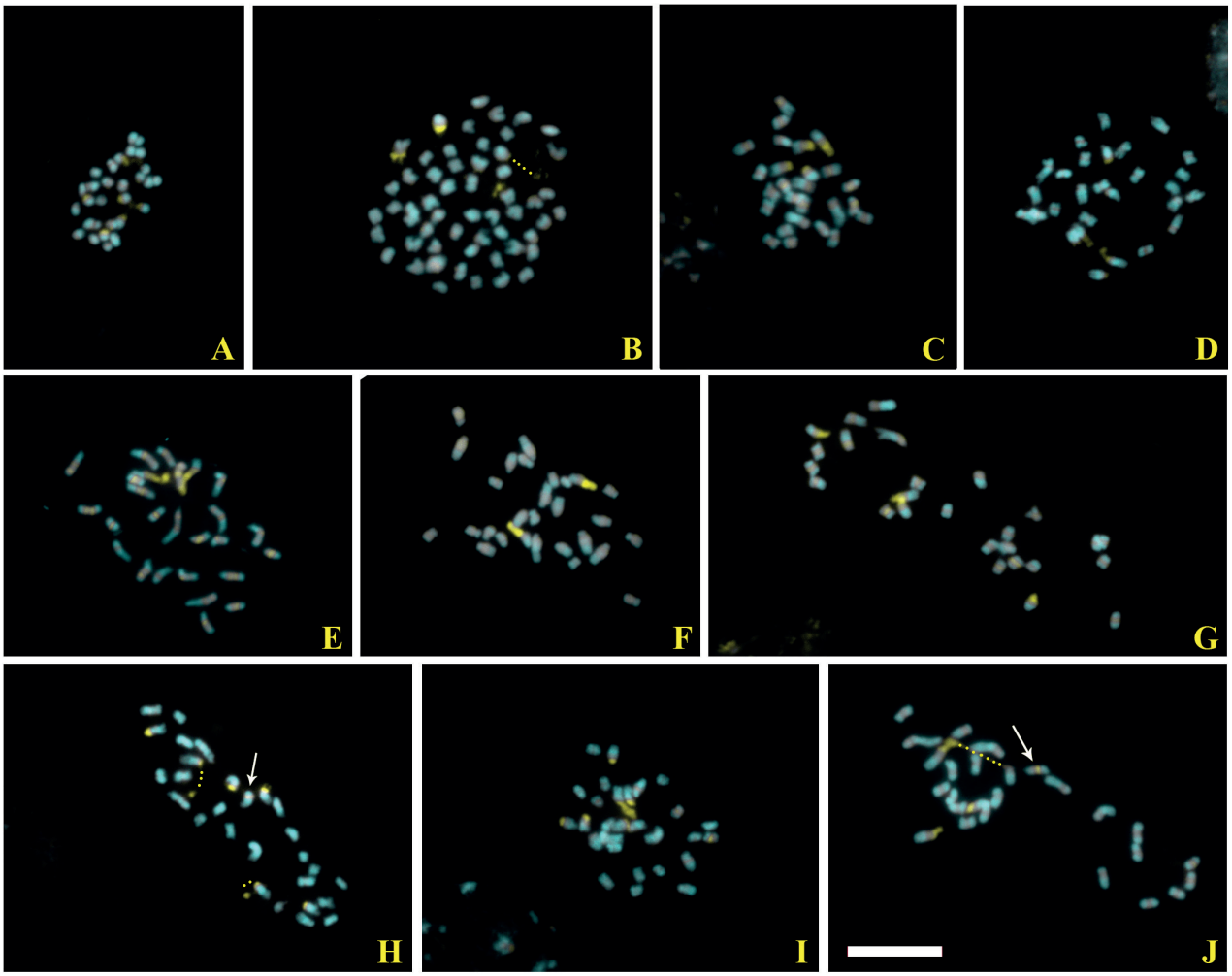

Figure 1. Mitotic metaphases and CMA$^+$/DAPI$^-$ bands (yellow) in species of the genus *Senna*. **A.** *Senna alata* (2*n* = 28); **B.** *S. aversiflora* (2*n* = 56); **C.** *S. georgica* (2*n* = 26); **D.** *S. martiana* (2*n* = 28); **E.** *S. obtusifolia* (2*n* = 26); **F.** *S. occidentalis* (2*n* = 28); **G.** *S. rizzinii* (2*n* = 26); **H.** *S. siamea* (2*n* = 28); **I.** *S. spectabilis* var. *excelsa* (2*n* = 28); **J.** *S. splendida* (2*n* = 26). The scale bar in J corresponds to 10 μm. Arrows in H and J indicate chromosomes with heteromorphic CMA$^+$/DAPI$^-$ bands.

corroborating the positions of various authors (Goldblatt 1981; Biondo *et al*. 2005a).

Some species, such as *S. occidentalis* and *S. obtusifolia*, demonstrated intraspecific karyotypic variations, with karyotypes of 2*n* = 24, 26 and 28 (Chaulagain & Sakya 2002; Biondo *et al*. 2005a; Rice *et al*. 2015). Diverse karyological phenomena may be involved in the intraspecific variations observed in different plant groups, especially neopolyploidy (species with diploid and polyploid cytotypes) and disploidy (centric fusions and fissions) (Guerra 2008). Additionally, polysomy and aneusomy (intra-individual variations in somatic chromosome numbers caused by polyploidy or aneuploidy respectively [Nirmala & Rao 1996; Rodrigues *et al*. 2009]) have likely occurred in certain species of *Senna* (Chaulagain & Sakya 2002; Matos *et al*. 2011), contributing to chromosome number variability in the genus. Intraspecific variations in chromosome numbers are quite common in other plant groups, such as *Epidendrum secundum* (Orchidaceae) (Assis *et al*. 2013), *Rutidosis leptorrhynchoides* (Asteraceae) (Murray & Young 2001) and *Zephyranthes sylvatica* (Amaryllidaceae) (Felix *et al*. 2008). In those cases, intraspecific variations could be related to factors such as distance or geographic isolation, together with hybridization in natural populations. Intraspecific variations were also observed in *Senna spectabilis*, with records of 2*n* = 26 and 28 (Resende *et al*. 2013; Rice *et al*. 2015), although those variations appear to be distinct at the variety level, as *S. spectabilis* var. *excelsa* shows 2*n* = 28 in all of its karyotypic descriptions (including the present work), while *S. spectabilis* var. *spectabilis* generally shows 2*n* = 26 (Rice *et al*. 2015).

Chromosome records of the genus *Senna* also demonstrated variations in ploidy levels, especially for *S. rugosa* (2*n* = 42, 56 and 112; Resende *et al*. 2014), *S. aversiflora* (2*n* = 56; Matos *et al*. 2011; present work) and *S. gardneri* (2*n* = 52 and 104, Matos *et al*. 2011). Those

variations could be a result of autopolyploidy, because hybridizations within the genus *Senna* are rarely viable, even among species having different morphotypes (see, for example, Holman & Playford 2000).

In addition to intraspecific and ploidy-level variations, interspecific chromosome variations were also observed in *Senna*. Although $2n = 28$ predominated (68.9 % of the species), there are significant numbers of records of $2n = 26$ (14.8 %) and $2n = 24$ (13.5 %) among its species (Goldblatt 1981; Souza & Benko-Iseppon 2004; Biondo *et al*. 2005a; Matos *et al*. 2011; Resende *et al*. 2013; Rice *et al*. 2015). Interspecific variations were observed in the present work, with five species showing $2n = 28$ but four species showing $2n = 26$. These numbers most likely represent disploidy events during the evolution of the genus, although they are generally treated as random phenomena (Goldblatt 1981; Biondo *et al*. 2005a; Resende *et al*. 2013).

Phylogenetic analyses undertaken by Marazzi *et al*. (2006) demonstrated that the genus *Senna* can be divided into seven monophyletic clades. Chromosome count comparisons with the results of those phylogenetic analyses can facilitate our understanding of interspecific variations. The prevalence of $2n = 28$ is observed in essentially all of the seven clades of *Senna*, especially in the most primitive and most derived clades. Most variations ($x = 11$, 13, 21 and 28), on the other hand, occur in clade IV. That clade corresponds to the monophyletic series *Bacillaris* (section *Chamaefistula*), which comprises approximately 50 species of shrubs and small trees whose leaves are exclusively composed of two pairs of folioles (Irwin & Barneby 1982; Marazzi *et al*. 2006). Most chromosome records of $x = 13$ occur in clade IVb. Phylogenetic analyses point to a large polytomy among the species that compose that clade, indicating its probable recent radiation (Marazzi *et al*. 2006); the clade probably experienced a disploidy event ($x = 13$) in a common ancestor, with most of the descendent species then conserving that cytological characteristic. Other variations, such as $x = 12$ in *S. atomaria* and $x = 13$ in *S. spectabilis* (clade III), $x = 12$ in *S. multijuga* (clade VI), and $x = 12$ in *S. hirsuta* var. *hirta* (clade VIIb) were also apparently important for the diversification of the genus, although with lesser evolutive significance in terms of their respective clades.

In relation to heterochromatin patterns, significant differences were observed among most of the species of the genus *Senna* analyzed, whether in terms of the numbers of bands (2-26) or their localizations on the chromosomes (terminal or proximal). Only *S. georgica* and *S. rizzinii* demonstrated the same banding pattern (four terminal $CMA^+/DAPI^-$ and 22 proximal $CMA^+/DAPI^-$ bands). *Senna martiana* and *S. aversiflora* demonstrated the same numbers of bands (four terminal $CMA^+/DAPI^-$ bands), although the latter demonstrated a polyploid karyotype ($2n = 52$). Variations in heterochromatic banding patterns are quite common in plant groups and can vary among species, populations, or even individuals (Guerra 2000; Dobigny *et al*. 2004). Those variations can reflect the results of various cytological phenomena, especially satellite DNA amplification (Guerra 2000; Silva *et al*. 2010; Ribeiro *et al*. 2016). Differences in heterochromatic banding patterns are quite useful for cytotaxonomic characterizations of plant species, especially among those having karyotypes formed by morphologically similar chromosomes that are numerically stable (Guerra 2000; Scaldaferro *et al*. 2012; Cordeiro *et al*. 2016). The observed differences in the CMA/DAPI banding patterns in *Senna* ratified the effectiveness of that technique for facilitating karyological differentiation of its species.

Among the 10 species analyzed, only three (*S. alata*, *S. obtusifolia,* and *S. siamea*) had previously been examined in terms of their CMA/DAPI banding patterns (Souza & Benko-Iseppon 2004). Those species did, however, demonstrate differences when compared to the analyses performed here. *Senna alata*, *S. obtusifolia,* and *S. siamea* had previously been reported to have two terminal $CMA^+/DAPI^-$ bands (Souza & Benko-Iseppon 2004), while we found those same species to have six terminal $CMA^+/DAPI^-$ bands, two terminal $CMA^+/DAPI^-$ + 24 proximal $CMA^+/DAPI^-$ bands, and six terminal $CMA^+/DAPI^-$ bands plus one proximal heteromorphic band respectively. Differences in heterochromatin patterns within the same species can occur in distinct populations, as was previously reported by Souza & Benko-Iseppon (2004) in *S. obtusifolia*; those authors found one population having two terminal $CMA^+/DAPI^-$ bands and another showing two terminal $DAPI^+/CMA^-$ bands. Variations in the patterns of heterochromatic bands in different individuals of the same species are often observed in plants, including *Oziroë argentinensis* (Dematteis *et al*. 2006), *Capsicum* (Scaldaferro *et al*. 2012), *Allium pulchellum* (Vosa 1996), and *Pinus nigra* (Bogunić *et al*. 2011). Variations in heterochromatin bands between individuals of the same species appear to be an intraspecific cytological characteristic of the genus *Senna* that is worthy of further investigation, calling for the analysis of distinct populations from different ecosystems and different geographic regions.

The present study allowed us to put forward the following general considerations: a) CMA/DAPI banding patterns in the genus *Senna* are quite useful for cytotaxonomically differentiating its species, although possible variations between different populations of a given species will need to be closely examined; b) although most species of *Senna* show $2n = 28$, records of polyploidy ($2n = 52$, 56, 104 and 112) and disploidy ($2n = 22$, 24 and 26) were observed in numerous species of that genus; c) intraspecific variations observed in certain species, such as *S. obtusifolia* and *S. occidentalis*, appeared to be result of cytological phenomena such as disploidy, or polysomy and aneusomy.

The karyotypic variations observed in *Senna*, whether interspecific or intraspecific, probably contributed to the diversification of that genus, making it one of the most

representative taxa of the Leguminosae in many different regions of the world.

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