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Karyotype characterization and nuclear DNA content measurement in Bromeliaceae: State of the art and future perspectives

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ABSTRACT

In Bromeliaceae, cytogenetic and flow cytometry analyses have been performed to clarify systematic and evolutionary aspects. Karyotyping approaches have shown the relatively high chromosome number, similar morphology and small size of the chromosomes. These facts have prevented a correct chromosome counting and characterization. Authors have established a basic chromosome number of x = 25 for Bromeliaceae. Recently, one karyomorphological analysis revealed that x = 25 is no longer the basic chromosome number, whose genome may have a polyploid origin. Besides cytogenetic characterization, the 2C DNA content of bromeliads has been measured. Nuclear DNA content has varied from 2C = 0.60 to 2C = 3.34 picograms. Thus, in relation to most angiosperms, the 2C DNA content of Bromeliaceae species as well as their chromosome size can be considered relatively small. In spite of some advances, cytogenetic and flow cytometry data are extremely scarce in this group. In this context, this review reports the state of the art in karyotype characterization and nuclear DNA content measurement in Bromeliaceae, emphasizing the main problems and suggesting prospective solutions and ideas for future research.

Key words: allopolyploid, bromeliads, cytogenetics, flow cytometry, genome evolution.

INTRODUCTION

The Bromeliaceae family belongs to the order Poales (APG III 2009) and comprises about 58 genera and 3,170 species, distributed in tropical and subtropical regions of the American continent (Givnish et al. 2011). Approximately 50% of the bromeliad species are found in Brazil, occurring in Atlantic Rainforest regions, Caatinga, montane savannas – 'Campos Rupestres', semi-arid regions and tropical savanna – 'Cerrado' (Ceita et al. 2008). For this reason, the Brazil has been considered one of the most important biodiversity Bromeliaceae centers worldwide (Louzada et al. 2010).

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Economically, bromeliads have been used for food and fiber production, ornamental purposes and in natural medicine, with the use of the bromelin enzyme. This enzyme is present in the pineapple, *Ananas comosus* (Linnaeus) Merril, which is also widely explored in agriculture as a commercial fruit. Besides economic aspects, the bromeliads have assumed a substantial role in ecological features. Some species provide concentrated nectar to humming birds and furnish microhabitats for other vegetable species. Thus, the bromeliads have been highlighted as biodiversity enhancers (Versieux 2009, Favoreto et al. 2012).

Cytogenetic studies in Bromeliaceae have been reported, aiming to clarify systematic and

evolutionary aspects in the group (Favoreto et al. 2012). Initially, cytogenetic researches focused on chromosome counting (Lindschau 1933, Weiss 1965, Marchant 1967). On a second stage, they also reported on the morphology of Bromeliaceae chromosomes (Cotias-de-Oliveira et al. 2000, Palma-Silva et al. 2004, Bellintani et al. 2005, Gitai et al. 2005, Ceita et al. 2008).

Apart from cytogenetic studies, flow cytometry (FCM) analyses have also been performed to measure the nuclear DNA content and base composition (AT% and GC%) of different Bromeliaceae species, expanding the data about their genome. These analyses have contributed with information for systematic, evolution (Ebert and Till 1997, Ramirez-Morillo and Brown 2001) and genetic diversity studies (Sgorbati et al. 2004, Favoreto et al. 2012).

Despite previous cytogenetic and FCM studies, there is scarce data available for bromeliads, limiting inferences about the evolution of its karyotype. In particular, the most cytogenetic studies have only reported the basic chromosome number, x = 25. Considering all this, it is relevant to put together reported cytogenetic and FCM data and the problems being faced, as well as the next steps. Based on this approach, studies on basic chromosome number, ploidy and karyotype evolution in Bromeliaceae could be advanced. Given these aspects, this review was devoted to relating the cytogenetic and FCM data generated so far for this family, showing prospective solutions for old problems and raising new questions.

Cytogenetics of Bromeliaceae

At first, cytogenetic studies in Bromeliaceae intended to establish the chromosome number. In 1904, Billings initiated the chromosome analysis of this family, which became more significant after 1933, with studies by Lindschau on 50 species of different Bromeliaceae genera. Subsequently, determination of the chromosome number in some species was accomplished by Marchant (1967), Sharma and Ghosh (1971), McWilliams (1974), and Varadarajan and Brown (1985). These authors observed a wide diversity of chromosome numbers among species.

Lindschau (1933) proposed that Tillandsioideae has a basic chromosome number of x = 9, and Weiss (1965) found x = 8; both authors also reported the occurrence of species with different ploidy levels. Marchant (1967) studied 72 Bromeliaceae species, revealing the occurrence of 2n = 48, 50, 56, 64, 72, 94, 96, 100 and 126 chromosomes. Based on these results, the author reported that, with the exception of *Cryptanthus* (x = 17), Bromeliaceae present a basic chromosome number of x = 25. Brown and Gilmartin (1989) suggested that the number x = 25 could be derived from hybridization between paleo-diploid species with x = 8 and 9, followed by chromosome doubling generating a paleo-tetraploid showing x = 17. Subsequently, hybridization between the paleo-tetraploid and the paleo-diploid, with x = 8, could have resulted in an allohexaploid exhibiting x = 8 + 8 + 9 = 25, then considered the basic chromosome number for the Bromeliaceae family (Figure 1).

On a second stage of cytogenetic studies, besides determining the chromosome number, researchers also characterized the chromosomes of bromeliads morphologically. Several authors reported a chromosome number of 2n = 50 for most of the species analyzed, with the exception of the genus *Cryptanthus*, 2n = 34, and some polyploid species with 2n = 100, 150 or 160. Moreover, morphometric studies revealed the relatively small size of the bromeliad chromosomes (Cotias-de-Oliveira et al. 2000, 2004, Palma-Silva et al. 2004, Bellintani et al. 2005, Gitai et al. 2005, Ceita et al. 2008).

Initially, Cotias-de-Oliveira et al. (2000) determined the chromosome number of 14 Bromeliaceae species showing 2n = 50 and three polyploid species with 2n = 100 (*Orthophytum burle-marxii* LB Smith & R. W. Read) or 2n = 150 (*Bromelia laciniosa* Martius ex Schultes f. and *Orthophytum*

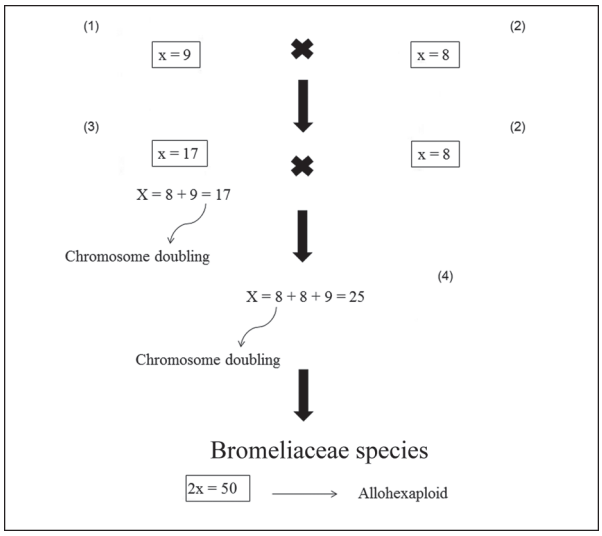

Figure 1 - Schematic representation, which was adapted from Brown and Gilmartin (1989), of the evolutionary process that culminated in the basic number of x = 25 chromosomes for the Bromeliaceae family. First, hybridization between paleo-diploid species with x = 9 (1) and 8 (2), followed by chromosome doubling generating a paleo-tetraploid with x = 17 (3). Subsequently, hybridization between the paleo-tetraploid (3) and the paleo-diploid with x = 8 (2) may have resulted in an allohexaploid with x = 8 + 8 + 9 = 25 (4).

maracasense L. B. Smith). These authors also observed that the chromosome size of most species ranged from 0.23 µm (chromosome 25) to 1.08 µm (chromosome 1). Similarly, Cotias-de-Oliveira et al. (2004) reported a chromosome number of 2n = 50 for 23 species and 2n = 100 for two species, *Orthophytum albopictum* Philcox and *Neoglaziovia variegata* (Arruda da Camara) Mez. Moreover, these authors reported a total chromosome size ranging from 0.36 µm (chromosome 25) to 1.20 µm (chromosome 1).

Analyzing chromosomal features of Bromeliaceae, Gitai et al. (2005) reported cytological information and chromosome counting of 15 taxa, referring to 19 genera of this family. The basic number x = 25 was confirmed, and the occurrence of polyploidy was detected in two species. The species *Deinacanthon urbanianum* (Nees) Mez had 2n = 160, while for *Bromelia laciniosa* Martius ex Schultes f. a number of 2n = 150 was found. The full size of the chromosomes ranging from 0.50 µm (chromosome 25) to 2.72 µm (chromosome 1) was observed in different species. Ceita et al. (2008) studied the chromosome number of 18 Bromeliaceae species, finding mostly 2n = 50, except for *Cryptanthus*, with 2n = 34. *Cryptanthus* chromosomes ranged from 0.71 µm (chromosome 17) to 1.25 µm (chromosome 1) in length, while other species, showing 2n = 50, they ranged from 0.25 µm (chromosome 25) to 1.5 µm (chromosome 1).

As summarized in Table I, 2n = 50 prevails in the family. However, cytogenetic studies have shown some variations regarding chromosome number in *Cryptanthus* genus and some species, as well as discrepancies between the data obtained by analysis of mitotic and meiotic cells. The large number and small size of the chromosomes may have contributed to erroneous counts, which were based on prometaphases or metaphase chromosomes with overlappings, or else due to the possible presence of B chromosomes (Nunes et al. 2013).

The occurrence of relatively small chromosomes has been considered an obstacle for an accurate cytogenetic characterization of most plant species (Carvalho et al. 2008). The chromosome discrimination in Bromeliaceae species has been regarded as particularly laborious, due to their relatively small size and subtle morphological differences (Cotias-de-Oliveira et al. 2000, Palma-Silva et al. 2004, Gitai et al. 2005, Ceita et al. 2008).

Distinct researches have reported chromosome counts in Bromeliaceae based on prophase chromosomes or interphase nuclei, which present blocks of heterochromatin. This kind of chromatin organization, observed before prometaphase, results in chromosomes morphologically elongated and unsuitable for morphometric measurement and karyogram assembly.

As displayed in Figure 2 (a – d), different chromatin compaction levels could be observed in cytogenetic analyses. However, interphase

TABLE I
2C nuclear DNA content, base composition (AT% and GC%) and chromosome number (2n) of Bromeliaceae species.

Genus	Species	2C value (pg)	AT%	GC%	Reference for 2C value and AT%/GC%	2n chromosome number	Reference for Chromosome number
Aechmea	*A. nudicaulis var. cuspidata* Baker	0.780	64.88	35.12	Favoreto et al. (2012)	-	-
	A. ramosa Martius ex Schultes f.	1.370	61.37	38.63	Favoreto et al. (2012)	-	-
	A. calyculata (Morren) Baker	-	-	-	-	50	Palma Silva et al. (2004)
	A. gamosepala Wittmack	-	-	-	-	50	Palma Silva et al. (2004)
	A. aquilega (Salisbury) Grisebach	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
	A. blanchetiana (Baker) L. B. Smith Salvador	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
	A. conifera L. B. Smith Baixa Gree	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
	A. fulgens Brongniart	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
	A. miniata (Beer) hortus ex Baker b *var. discolor* (Beer) Beer	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	A. bromeliifolia (Rudge) Baker	-	-	-	-	50	Gitaí et al. (2005)
	A. caudata Lindman	-	-	-	-	50	Ceita et al. (2008)
	A. recurvata (Klotzsch) L. B. Smith	-	-	-	-	50	Ceita et al. (2008)
	A. correia-araujoi Pereira & Moutinho	-	-	-	-	50	Ceita et al. (2008)
	A. pineliana (Brongniart ex Planchon) Baker	-	-	-	-	50	Ceita et al. (2008)
	A. marauensis Leme	-	-	-	-	50	Ceita et al. (2008)
	A. bicolor L. B. Smith	-	-	-	-	50	Ceita et al. (2008)
	A. comata (Gaudichaud) Baker	-	-	-	-	50	Ceita et al. (2008)
Alcantarea	*A. nahoumi* (Leme) R. Grant	-	-	-	-	50	Ceita et al. (2008)
	A. imperialis (Carrière) Harms	-	-	-	-	50	Ceita et al. (2008)
Ananas	*A. macrodontes* E. Morren	1.000	60.76	39.24	Favoreto et al. (2012)	-	-
	A. bracteatus (Lindley) Schultes f.	0.920	-	-	Arumuganathan and Earle (1991)	-	-
	A. comosus Linnaeus Merril	1.090	-	-	Arumuganathan and Earle (1991)	50	Gitaí et al. (2005)
	A. lucidus Miller	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
	A. nanus (L. B. Smith) L. B. Smith	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
Ayensua	*A. uaipanensis* L. B. Smith	0.860	-	-	Ebert and Till (1997)	50/100	Gitaí et al. (2005)
Billbergia	*B. nutans* H. Wendle ex Regel	0.752	-	-	Ramírez-Morillo and Brown (2001)	-	-
	B. horrida Regel	0.770	64.65	35.35	Favoreto et al. (2012)	-	-
	B. euphemiae E. Morren	0.890	62.59	37.42	Favoreto et al. (2012)	50	Cotias-de-Oliveira et al. (2004)
	B. tweedieana Baker	0.950	66.05	33.95	Favoreto et al. (2012)	-	-

TABLE I (continuation)

Genus	Species	2C value (pg)	AT%	GC%	Reference for 2C value and AT%/GC%	2n chromosome number	Reference for Chromosome number
Billbergia	*B. chlorosticta* Saunders Hortus	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
	B. morelii Brongniart	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
Brocchinia	*B. acuminata* L. B. Smith	0.760	-	-	Ebert and Till (1997)	-	-
	B. tatei L. B. Smith	0.780	-	-	Ebert and Till (1997)	-	-
Bromelia	*B. antiacantha* Bertoloni	0.810	61.57	38.43	Favoreto et al. (2012)	-	-
	B. laciniosa Mart. ex Schultes	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
	B. plumieri (E. Morren) L. B. Smith	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
Canistropsis	*C. simulans* (Pereira & Leme) Leme	-	-	-	-	50	Bellintani et al. (2005)
	C. seidelii (L. B. Smith A Reitz) Leme	-	-	-	-	50	Bellintani et al. (2005)
	C. microps (Pereira & Leme) Leme	-	-	-	-	50	Bellintani et al. (2005)
	C. bilbergioides (Schul.)	1.000	62.64	37.36	Favoreto et al. (2012)	50	Bellintani et al. (2005)
Canistrum	*C. fosterianum* L. B. Smith	-	-	-	-	50	Bellintani et al. (2005)
Catopsis	*C. floribunda* L. B. Smith	-	-	-	-	50	Gitaí et al. (2005)
Cryptanthus	*C. bahianus* L. B. Smith	0.750	-	-	Ramírez-Morillo and Brown (2001)	34	Cotias-de-Oliveira et al. (2000)
	C. schwackeanus Mez	0.710	-	-	Ramírez-Morillo and Brown (2001)	34	Ramírez-Morillo and Brown (2001)
	C. beuckeri E. Morren	1.458	-	-	Ramírez-Morillo and Brown (2001)	34	Bellintani et al. (2005)
	C. acaulis (Lindley) Beer	1.380	-	-	Ramírez-Morillo and Brown (2001)	34	Ramírez-Morillo and Brown (2001)
	Cryptanthus sp.	1.330	61.23	38.78	Favoreto et al. (2012)	-	-
	C. warren-loosei Leme	-	-	-	-	34	Ceita et al. (2008)
	C. maritimus L. B. Smith	-	-	-	-	34	Ceita et al. (2008)
	C. lyman-smith Leme	-	-	-	-	34	Bellintani et al. (2005)
	C. vexatus Leme	-	-	-	-	34	Bellintani et al. (2005)
	C. bromelioides Otto & F. Dietr.	-	-	-	-	34	Sharma and Ghosh (1971)
	C. praetextus E. Morren ex Baker	-	-	-	-	34	Sharma and Ghosh (1971)
	C. zonatus (Visiani) Beer	-	-	-	-	36	Lindschau (1933)
Deinacanthon	*D. urbanianum* (Mez) Mez	-	-	-	-	50	Gitaí et al. (2005)
Deuterocohnia	*D. longipetala* (Baker) Mez	0.740	-	-	Ebert and Till (1997)	-	-
	D. schreiteri Castellanos	0.800	-	-	Ebert and Till (1997)	-	-
	D. lorentziana (Mez) Spencer & L. B. Smith	-	-	-	-	50	Gitaí et al. (2005)
Dyckia	*D. estevesii* Rauh	1.600	-	-	Ebert and Till (1997)	-	-

TABLE I (continuation)

Genus	Species	2C value (pg)	AT%	GC%	Reference for 2C value and AT%/ GC%	2n chromosome number	Reference for Chromosome number
Dyckia	*D. floribunda* Grisebach	1.580	-	-	Ebert and Till (1997)	-	-
	D. platyphylla L. B. Smith	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	D. saxatilis Mez	-	-	-	-	50	Gitaí et al. (2005)
Encholirium	*E. irwinii* L. B. Smith	1.740	-	-	Ebert and Till (1997)	-	-
	Encholirium sp.	1.740	-	-	Ebert and Till (1997)	-	-
	E. spectabile Mart. ex Schult.f.	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
Fascicularia	*F. bicolor* (Ruiz & Pavon) Mez ssp. *bicolor* E.C. Nelson & Zizka	-	-	-	-	50	Gitaí et al. (2005)
	F. bicolor (Ruiz & Pavon) Mez ssp. *canaliculata* E.C. Nelson & Zizka	-	-	-	-	50	Gitaí et al. (2005)
Fosterella	*F. penduliflora* (C.H. Wright) L.B. Smith	1.860	-	-	Ebert and Till (1997)	-	-
	F. villosula (Harms) L. B. Smith	1.860	-	-	Ebert and Till (1997)	-	-
Greigia	*G*. spec. nov. (*aff. G. mulfordii var. micrantha*)	-	-	-	-	50	Gitaí et al. (2005)
	G. sphacelata (Ruiz & Pavon) Regel	-	-	-	-	50	Gitaí et al. (2005)
Hechtia	*H. epigyna* Harms	0.960	-	-	Ebert and Till (1997)	-	-
	H. matudae L. B. Smith	0.940	-	-	Ebert and Till (1997)	-	-
Hohenbergia	*H. catingae* Ule var. catingae	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
	H. littoralis L. B. Smith	-	-	-	-	50	Cotias-de-Oliveira et al. (2000) Cotias-de-Oliveira et al. (2000)
	H. stellata Schultes Baixa Gree *H. blanchetti (Baker)* E. Morren ex Mez	-	-	-	-	50 50	Ceita et al. (2008)
	H. pennae Pereira	-	-	-	-	50	Bellintani et al. (2005)
	H. castelanosii L. B. Smith a Read	-	-	-	-	50	Bellintani et al. (2005)
	H. correia-araujoi Pereira & Moutinho	-	-	-	-	50	Pereira & Moutinho
	H. aff. utriculosa Ule	-	-	-	-	50	Cotias-de-Oliveira et al. (2000)
Navia	*N. splendens* L. B. Smith	1.420	-	-	Ebert and Till (1997)	-	-
Neoglaziovia	*N. variegata* (Arruda de Camara) Mez	-	-	-	-	100	Cotias-de-Oliveira et al. (2000)
Neoregelia	*N. aff. Simulans* L. B. Smith	0.980	62.76	37.24	Favoreto et al. (2012)	-	-
	N. carcharodon (Baker) L. B. Smith	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	N.hoehneana L. B. Smith	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	N. laevis (Mez) L. B. Smith	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)

TABLE I (continuation)

Genus	Species	2C value (pg)	AT%	GC%	Reference for 2C value and AT%/GC%	2n chromosome number	Reference for Chromosome number
Neoregelia	*N. johannis* (Carrière) L. B. Smith	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	N. wilsoniana M.B. Foster	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	N. rutilans E. Morren	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	N. longiflorum Ule	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	N. procerum Lindman	-	-	-	-	50	Ceita et al. (2008)
	N. tenebrosa Leme	-	-	-	-	50	Ceita et al. (2008)
	N. leucophoea (Baker) L. B. Smith	-	-	-	-	50	Bellintani et al. (2005)
	N. longisepala Pereira & Penna	-	-	-	-	50	Pereira & Penna
	N. cruenta (R. Graham) L. B. Smith	-	-	-	-	50	Bellintani et al. (2005)
	N. carolinae (Beer) L. B. Smith	-	-	-	-	50	Bellintani et al. (2005)
	N. bahiana (Ule) L. B. Smith	-	-	-	-	50	Bellintani et al. (2005)
Nidularium	*N. lyman-smithii* Leme	-	-	-	-	50	Ceita et al. (2008)
	N. scheremetiewii Regel	-	-	-	-	50	Ceita et al. (2008)
	N. innocentii var. innocentii Lemaire	-	-	-	-	50	Ceita et al. (2008)
	N. innocentii x Neoregelia johannis	-	-	-	-	50	Ceita et al. (2008)
Ochagavia	*O. elegans* R. Philippi	-	-	-	-	50	Gitaí et al. (2005)
	O.litoralis (Phil.) Zizka, Trumpler & Zoellner	-	-	-	-	50	Gitaí et al. (2005)
Orthophytum	*O. saxicola* (Ule) L. B. Smith	0.640	-	-	Ramírez-Morillo and Brown (2001)	50	Ramírez-Morillo and Brown (2001)
	O. maracasense L. B. Smith	-	-	-	-	150	Cotias-de-Oliveira et al. (2000)
	O. burle-marxii L. B. Smith & Rangel	-	-	-	-	100	Cotias-de-Oliveira et al. (2000)
	O. albopictum Philcox	-	-	-	-	100	Louzada et al. (2010)
	O. amoenum (Ule) L. B. Smith	-	-	-	-	100	Louzada et al. (2010)
	O. disjunctum L. B. Smith	-	-	-	-	50	Louzada et al. (2010)
	O. hatschbachii Leme	-	-	-	-	50	Louzada et al. (2010)
	O. mucugense Wand.& A. A. Conc.	-	-	-	-	50	Louzada et al. (2010)
	O. supthutii E. Gross & Barthlott	-	-	-	-	50	Louzada et al. (2010)
	O. ophiuroides Louzada & We	-	-	-	-	50	Louzada et al. (2010)
	O. vagans M. B. Foster	-	-	-	-	50	Louzada et al. (2010)
Pitcairnia	*P.flammea* (L. B. Smith) L. B. Smith	1.44	64.28	35.72	Nunes et al. (2013)	50	Nunes et al. (2013)
	P. feliciana (A. Chevalier) Harms & Mildbraed	0.600	-	-	Ebert and Till (1997)	-	-

TABLE I (continuation)

Genus	Species	2C value (pg)	AT%	GC%	Reference for 2C value and AT%/GC%	2n chromosome number	Reference for Chromosome number
	P. heterophylla (Lindley) Beer	0.880	-	-	Ebert and Till (1997)	-	-
	P. prolifera Rauh	0.840	-	-	Ebert and Till (1997)	-	-
	P. cardenasii L. B. Smith	1.020	-	-	Ebert and Till (1997)	-	-
	P. rectiflora Rauh	1.200	-	-	Ebert and Till (1997)	-	-
	P. sceptrigera Mez	1.200	-	-	Ebert and Till (1997)	-	-
	P. piepenbringii Rauh & E. Gross	1.200	-	-	Ebert and Till (1997)	-	-
	P. poeppigiana Mez	1.200	-	-	Ebert and Till (1997)	-	-
	P. atrorubens (Beer) Baker	1.200	-	-	Ebert and Till (1997)	50	Gitaí et al. (2005)
	P. chiapensis Mirea	1.220	-	-	Ebert and Till (1997)	-	--
	P. spicata (Lamarck) Mez	1.220	-	-	Ebert and Till (1997)	-	-
	P. aphelandriflora L. B. Smith	1.240	-	-	Ebert and Till (1997)	-	-
	P. pomacochae Rauh	1.240	-	-	Ebert and Till (1997)	-	-
	P. aureobrunnea Rauh	1.120	-	-	Ebert and Till (1997)	-	-
	P. yaupi-bajaensis Rauh	1.120	-	-	Ebert and Till (1997)	-	-
Pitcairnia	*P. riparia* Mez	1.140	-	-	Ebert and Till (1997)	-	-
	P. halophila L. B. Smith	1.080	-	-	Ebert and Till (1997)	-	-
	P. micotrinensis R.W. Read	1.100	-	-	Ebert and Till (1997)	-	-
	P. tabuliformis Linden	1.100	-	-	Ebert and Till (1997)	-	-
	P. angustifolia Soleer in Aiton	1.060	-	-	Ebert and Till (1997)	-	-
	P. albomarginata L. B. Smith	1.280	-	-	Ebert and Till (1997)	-	-
	P. heerdeae E. Gross & Rauh	1.180	-	-	Ebert and Till (1997)	-	-
	P. palmoides Mez & Sodiro	1.180	-	-	Ebert and Till (1997)	-	-
	P. paraguayensis L. B. Smith	1.360	-	-	Ebert and Till (1997)	-	-
	P. venezuelana L.B. Smith & Steyermark	1.360	-	-	Ebert and Till (1997)	-	-
	P. villetaensis Rauh	1.260	-	-	Ebert and Till (1997)	-	-
	P. andrena Linden	1.300	-	-	Ebert and Till (1997)	-	-
	P. schultzei Harms	1.360	-	-	Ebert and Till (1997)	-	-
	P.grafii Rauh	1.340	-	-	Ebert and Till (1997)	-	-
Portea	*P. silveirae* Mez	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	P. greiflora Philcox	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	P. mirabilis (Mez) L. B. Smith	0.880	-	-	Ebert and Till (1997)	50	Gitaí et al. (2005)
Puya	*P. stenothyrsa* (Baker) Mez	0.940	-	-	Ebert and Till (1997)	-	-
	P. raimondii Harms	1.130	-	-	Sgorbati et al. (2004)	-	-
Quesnelia	*Q. arvenis* (Vellozo) Mez	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	Q. edmundoi L. B. Smith b var. *rubrobracteata* E. Pereira	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	V. racinae L. B. Smith	1.190	60.26	39.74	Favoreto et al. (2012)	-	-
	V. scalaris E. Morren	1.110	60.77	39.23	Favoreto et al. (2012)	-	-
Vriesea	*V. carinata* Wawra	-	-	-	-	50	Palma Silva et al. (2004)
	V. erytrodactylon (E. Morren) E. Morren ex. Mez	-	-	-	-	50	Palma Silva et al. (2004)
	V. flammea L. B. Smith	-	-	-	-	50	Palma Silva et al. (2004)

TABLE I (continuation)

Genus	Species	2C value (pg)	AT%	GC%	Reference for 2C value and AT%/GC%	2n chromosome number	Reference for Chromosome number
Vriesea	*V. friburgensis* Mez	-	-	-	-	50	Palma Silva et al. (2004)
	V. guttata Linden e Ere	-	-	-	-	50	Palma Silva et al. (2004)
	V. incurvata Gaudchaud	-	-	-	-	50	Palma Silva et al. (2004)
	V. platynema Gaudchaud	-	-	-	-	50	Palma Silva et al. (2004)
	V. platzmannii E. Morren	-	-	-	-	50	Palma Silva et al. (2004)
	V. psittacina (Hooker) Lindley	-	-	-	-	50	Palma Silva et al. (2004)
	V. fosteriana L. B. Smith	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	V. picta (Mez et Wercklé) L. B. Smith et Pittendr.	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	V. botafogensis Mez	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
	V. saundersii (Carrière) E. Morren ex Mez	-	-	-	-	50	Cotias-de-Oliveira et al. (2004)
Tillandsia	*T. stricta* Soleer	1.200	61.94	38.06	Favoreto et al. (2012)	-	-
	T. cyanea Linden ex K. Koch	2.200	-	-	Zonneveld et al. (2005)	-	-
	T. usneoides (Linnaeus) Linnaeus	2.500	-	-	Zonneveld et al. (2005)	-	-
	T. loliacea Martius ex Schultes f.	3.340	60.75	39.25	Favoreto et al. (2012)	-	-
	T. dodsonii L. B. Smith	-	-	-	-	50	Ceita et al. (2008)
Wittrockia	*W. gigantea* (Baker) Leme	-	-	-	-	160	Gitaí et al. (2005)
	W. spiralipetala Leme	-	-	-	-	50	Bellintani et al. (2005)

and prophase chromosomes (Figure 2a, c), which present low chromatin compaction level, are inadequate for morphometric measurements, as they prevent correct karyomorphological analyses. As a result thereof, erroneous chromosome counts and characterization are made, generating incorrect data from the karyotype study.

Recently, Nunes et al. (2013) established a protocol for obtaining metaphase chromosomes of *Pitcairnia flammea* (L. B. Smith) L. B. Smith. The authors used the technique of cell dissociation of enzymatically macerated roots and subsequent air drying of the slides. These procedures provided chromosomes with well-defined primary constrictions, few overlaps, deformations or cytoplasmic chromatin fragments (Figure 2d). Based on these results, the authors were able to perform morphometric analyses, pairing of homologous chromosomes and assembly of a Bromeliaceae karyogram. Analyzing the karyogram of *P. flammea*, they detected the presence of grouped pairs of cytogenetically identical chromosomes. Thus, the authors inferred that the basic number for

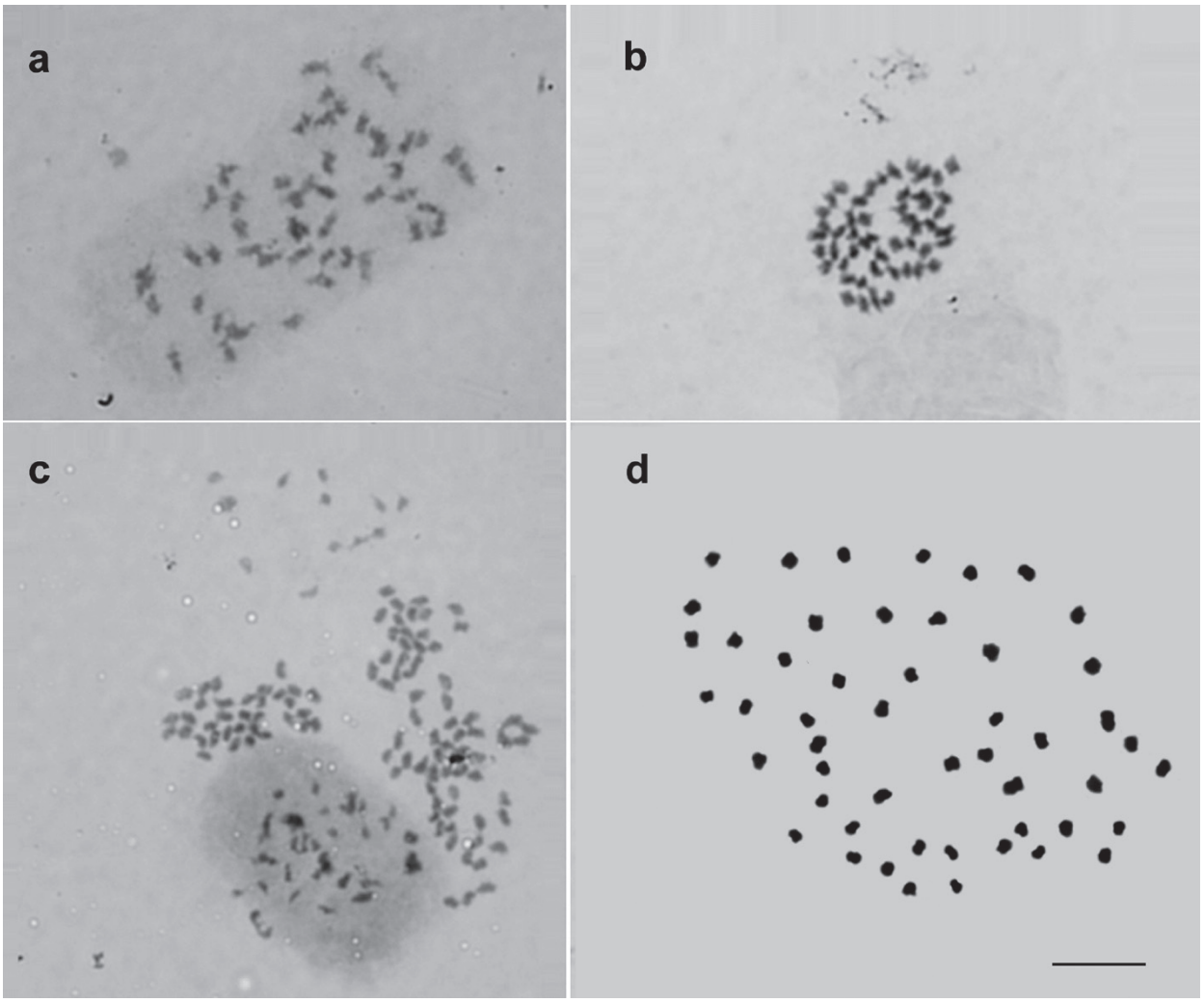

Figure 2 - Cytogenetic preparations of *Pitcairnia flammea* (L. B. Smith) L. B. Smith with chromosomes at different levels of chromatin compaction. **(a)** Late prophase with 2n = 50 chromosomes. **(b)** Early prometaphase exhibiting 2n = 50 chromosomes. **(c)** Overlapping interphase nuclei, prophase and prometaphase chromosomes. **(d)** Metaphase displaying 2n = 50 chromosomes, all submetacentric. This adequate cytogenetic preparation showed well-spread chromosomes, with well-defined primary constriction, without chromatin damage and cytoplasmic background.

the Bromeliaceae family is not x = 25 chromosomes. Furthermore, the presence of an isolated chromosome (number 1) led to evidence of an allopolyploid origin for the genome of *P. flammea*.

Despite the advances made in cytogenetic studies, these analyses in Bromeliaceae have covered 10% of all species of the group. This fact is due especially to the relatively high number, generally 2n = 50, and the small total size of the chromosomes. Therefore, more cytogenetic studies in bromeliads have to be conducted aiming to expand the knowledge about their karyotype. For this end, karyotype researches must be allied to molecular cytogenetics, which can evidence the evolution of DNA sequences present in the chromosomes.

FCM in Bromeliaceae

FCM analyses have been used to estimate the nuclear DNA content and base composition (AT% and GC%) of a few Bromeliaceae species

(Favoreto et al. 2012). The determination of these values has contributed to studies on systematic and evolution (Ebert and Till 1997, Ramirez-Morillo and Brown 2001, Nunes et al. 2013), genetic diversity and reproductive biology (Sgorbati et al. 2004, Zonneveld et al. 2005, Favoreto et al. 2012) of bromeliads species.

Applied to Bromeliaceae species, FCM analyses have primarily aimed to determine their 2C nuclear DNA content in picograms (pg). Using this application, Arumuganathan and Earle (1991) reported, for the first time, the 2C nuclear value of two bromeliad species: *Ananas bracteatus* (Lindley) Schultes f. presented 2C = 0.920 pg, and *A. comosus* (Linnaeus) Merrill showed 2C = 1.090 pg.

Also applying FCM, Ebert and Till (1997) established the nuclear genome size of 47 species distributed in ten genera of the subfamily Pitcairnioideae. The values varied from 2C = 0.600 pg for *Pitcairnia* L'Heritier to 2C = 1.860 pg for *Fosterella* L. B. Smith (Table I).

Ramirez-Morillo and Brown (2001) measured the nuclear genome size of *Cryptanthus* species, with 2n = 34 or 36 chromosomes, and other bromeliads showing 2n = 50 chromosomes. The highest value of nuclear genome size was found for *C. beuckeri* E. Morren (2C = 1.458 pg), and the lowest for *C. schwackeanus* Mez (2C = 0.710 pg). In relation to other species, *Orthophytum saxicola* (Ule) L. B. Smith showed the lowest DNA content, estimated at 2C = 0.640 pg (Table I).

Sgorbati et al. (2004) used FCM to study the genetic diversity and reproductive biology of *Puya raimondii* Harms. These authors examined relationships between populations of this species and their reproductive mechanism for obtaining subsidies to delineate conservation strategies. Therefore, the 2C value of *P. raimondii* was estimated (2C = 1.130 pg), and the embryo was found to have a relative DNA content equivalent to 2C and 3C of the endosperm. This data revealed the sexual reproduction system in this species.

Zonneveld et al. (2005) measured the nuclear genome size of two bromeliad species, *Tillandsia cyanea* Linden ex K. Koch (2C = 2.200 pg) and *Tillandsia usneoides* (Linnaeus) Linnaeus (2C = 2.500 pg). In current analyses, Favoreto et al. (2012) reported the nuclear DNA content and base composition (AT%) of 14 Bromeliaceae species, which ranged from 2C = 0.770 pg, for *Billbergia horrida* Regel, and 2C = 3.340 pg, for *Tillandsia loliaceae* Martius ex Schultes f. The base composition was AT = 60.26% for *Vriesea racinae* L. B. Smith and AT = 66.05% for *Billbergia tweedieana* Baker (Table I).

Nunes et al. (2013) reported the 2C value (1.440 pg) and base composition (AT = 64.28% and GC = 35.72%) of *P. flammea*. According to these authors, the nuclear DNA content of *P. flammea* can be considered relatively small compared to the nuclear 2C value of most angiosperms. As stated by Bennett and Leitch (2011), the nuclear DNA content of angiosperms ranges from an equivalent minimum value of 2C = 0.1296 pg for *Genlisea margaretae* Hutch to a maximum value of 2C = 304.46 pg for *Paris japonica* (Franch. & Sav.) Franch.

Different studies have reported the nuclear DNA content for various Bromeliaceae species (Table I). Similarly to *P. flammea* (Nunes et al. 2013), these nuclear 2C values are considered relatively small, characterizing a constant in the bromeliad group. Thus, regarding a possible reconstruction of the ancestral nuclear genome (2C = 3.700 pg) for the monocot group, a significant decrease in the DNA content of bromeliads can be observed throughout their genomic evolution.

Despite concerted efforts towards the 2C value estimation for the Bromeliaceae family, FCM analyses are scarce, and only 2.3% of bromeliads have had their genome size established (Table I).

Perspectives and Conclusion

Cytogenetic studies in Bromeliaceae have evolved with the establishment of a concise number of 2n = 50 chromosomes for most of the analyzed species.

Additionally, karyological analysis of *P. flammea* allowed initial inferences on the karyotype evolution of bromeliads. Thus, as a starting point for further research, it should be assumed that x = 25 might not be the basic Bromeliaceae chromosome number, a fact that could be the subject of new cytogenetic studies.

Bromeliaceae FCM approaches are incipient, since only few species have already had their 2C nuclear DNA content estimated this way. FCM provides fast and reliable analyses, playing an important role in genetic diversity and systematic studies and in assisting cytogenetic research, by enabling immediate DNA ploidy level determination. Considering these facts, not to expand FCM studies means to delay the gain of Bromeliaceae genome knowledge. Therefore, more different bromeliads species ought to have their 2C value determined, with the aim of supporting karyotype studies.

In conclusion, in order to reach a robust result regarding the process of bromeliad karyotype evolution, it is time to initiate a combination of classical and molecular cytogenetics of Bromeliaceae species, allied with a greater number of FCM analyses.

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RESUMO

Análises citogenéticas e de citometria de fluxo em Bromeliaceae têm sido realizadas para esclarecer aspectos de sistemática e evolução. O cariótipo das bromeliáceas apresenta cromossomos em número relativamente elevado, morfologicamente similares e com tamanho pequeno. Esses aspectos têm dificultado a determinação correta do número e da caracterização de cromossomos. Os autores estabeleceram um número básico de cromossomos de x = 25 para Bromeliaceae. Recentemente, uma análise cariomorfológica revelou que x = 25, já não é o número básico de cromossomos de Bromeliaceae, cujo genoma pode ter origem poliploide. Além da caracterização citogenética, o conteúdo 2C de DNA de Bromeliaceae tem sido mensurado. O conteúdo de DNA nuclear tem variado de 2C = 0,60 a 2C 3,34 picogramas. Portanto, em comparação com a maioria das angiospermas, o conteúdo 2C de DNA das espécies de Bromeliaceae e o tamanho de seus cromossomos podem ser considerados relativamente pequenos. Apesar de alguns avanços, os dados citogenéticos e de citometria de fluxo são extremamente escassos nesse táxon. Nesse contexto, a presente revisão reporta o estado da arte no que se refere à caracterização do cariótipo e o mensuramento do conteúdo de DNA nuclear em Bromeliaceae, enfatizando os principais problemas e sugerindo soluções potenciais e ideias para pesquisas futuras.

Palavras-chave: alopoliploide, bromélias, citogenética, citometria de fluxo, evolução do genoma.

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