Chromosomal variability in Brazilian species of Anthurium Schott (Araceae): Heterochromatin, polyploidy, and B chromosomes

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Abstract

The genus Anthurium has a Neotropical distribution, with karyotype predominance of x = 15, although some species show diploidy or polyploid variations. The karyotypes of seven species and different populations of Anthurium were analyzed using fluorochrome CMA and DAPI staining. The karyotypes were composed of meta- and submetacentric chromosomes, with numbers varying from 2n = 30 to 2n = 60. Supernumerary euchromatic chromosomes were observed in A. affine, and supernumerary heterochromatic chromosomes were observed in A. gladiifolium and A. petrophilum. Polyploidy was recurrent in the Anthurium species analyzed, with records of 2n = 30 and 60 in different A. pentaphyllum populations. Fluorochrome staining revealed different CMA banding distributions between diploid and polyploid cytotypes of A. pentaphyllum, suggesting structural alteration events. Anthurium petrophilum, on the other hand, showed a more consistent banding profile, with 10 to 12 proximal CMA bands in the three populations analyzed. DAPI/CMA regions occurred exclusively in populations of A. gracile and A. pentaphyllum. The heterochromatic fraction in Anthurium was found to be quantitatively variable among species and populations, which may be related with adaptive aspects, different environmental conditions, or phylogenetic position.

Keywords: B chromosomes, CMA/DAPI, cytotaxonomy, Neotropical Araceae.

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Introduction

The genus Anthurium is a strictly Neotropical monophyletic group that occurs from Mexico to Argentina, and is included within the subfamily Pothoideae, tribe Pothieae (Croat, 1986; Coelho et al., 2009; Cusimano et al., 2011; Carlsen and Croat, 2013; Govaerts et al., 2016). This group comprises approximately 950 species (Boyce and Croat, 2013 onwards), 134 of which are known in Brazil (Coelho et al., 2011). The genus is monophyletic, with 18 clades that are easily distinguishable morphologically or geographically, and show low divergence in their trnG intron, trnH-psbA and trnC-ycf6 sequences, and in the CHS intron regions of their DNA, suggesting a rapid radiation of the group (Carlson and Croat, 2013).

Chromosomal records have been published for approximately 150 Anthurium species, with a predominance of 2n = 30 (88% of the species), but numbers vary from 2n = 24 to 2n = 124 (Sheffer and Croat, 1983; Petersen, 1989; Rice et al., 2015). The genus is notorious for the occurrence of intraspecific polyploidy, such as A. bellum Schott with 2n = 30, 90 (Sheffer and Croat, 1983; Cotias-de-Oliveira et al., 1999), A. pentaphyllum G.Don with 2n = 30, 60 (Cotias-de-Oliveira et al., 1999), and A. digitatum (Jacq.) G.Don with 2n = 30, 60 (Rice et al., 2015). Six species from southeastern and southern Brazil were analyzed and intraspecific polyploidy was observed in three of them, with the predominance of diploid cytotype, as in A. urvilleanum Schott and A. harrisii G.Don, or the predominance of tetraploid cytotype, as in A. intermedium Kunth (Viegas et al., 2006). B chromosomes also occur frequently and have been observed in both diploid and tetraploid samples. A population of A. urvilleanum, close to Parati, Rio de Janeiro State, Brazil (M. Nadruz, 1543), showed 2n = 30 + 0-2Bs, while another population within the same municipality (M. Na-
druz, 1394) showed $2n = 60 + 0-2Bs$ (Viégas et al., 2006). In spite of the frequent presence of B chromosomes in *Anthurium*, they are not easily distinguishable based on size and shape, or methodology employed.

The most complete study of B chromosomes in *Anthurium* was undertaken by Marutani and Kamemoto (1983), and included examining both somatic and meiotic cells in *A. warocqueanum* Moore. These authors observed that the numbers of B chromosomes in somatic cells in the species was constant ($2n = 30 + 3B$), although there were different associations during metaphase I of meiosis (one trivalent, one bivalent and one univalent, or three univalents), resulting in variable numbers of B chromosomes in selfed offspring (ranging from 0 to 6) and indicating their transmission from both male and female gametes. The diversity of B chromosomes in *Anthurium* was noted by Marutani et al. (1993), who reported them in *A. ochranthum*, *A. lindenianum*, and *A. garagaranum*. B chromosomes are commonly heterochromatic, although they appear euchromatic in some species (Camacho et al., 2000; Banaei-Moghaddam et al., 2014). No differential staining of *Anthurium* chromosomes has yet been undertaken, and the chromatin compositions of B chromosomes among its different species have not been examined. We therefore analyzed chromosome number variability and CMA/DAPI banding distributions in seven Brazilian species of *Anthurium* to identify interspecific variations and supernumerary chromosomes in different populations and cytotypes. The main objective of this work was to identify karyotype variability in Brazilian species of *Anthurium* to determine the importance of that variability to chromosome evolution in the genus.

Materials and Methods

Collections and botanical documentation

Seven species of *Anthurium* harvested in various regions of Brazil were investigated, including individuals from three different populations. Intraspecific variations were investigated in four of the seven species. Information concerning all of the samples and their respective collection localities, populations, and collectors are summarized in Table 1. Specimens were maintained alive in the experimental gardens of the Plant Cytogenetic Laboratory of the Department of Biological Sciences of the Agrarian Sciences Center at the Federal University of Paraíba (UFPB), Brazil. Exsiccates were deposited in the Prof. Jayme Coelho de Moraes Herbarium (EAN).

Chromosomal analyses

Root tips were pretreated with 0.2% colchicine for 24 h at 10 C, fixed in 3:1 ethanol – acetic acid (v:v) for 2 h at room temperature, and subsequently stored at -20 C until

| Genus/species       | Voucher     | Origin (city/state) | $2n$ | CMA bands | Figures |
|---------------------|-------------|---------------------|------|-----------|---------|
| *Anthurium affine*  | LPFelix 14635 | Queimadas, PB       | 30 + 1B | 2p | 1A       |
|                     | EMAlmeida 453  | Águas Belas, PE     | 30 | 1p | 1B       |
|                     | EMAlmeida 476  | Andarai, BA         | 30 | 2p | 1C       |
|                     |              |                     | 30 + 2B | 2p | 1D       |
| A. gladiifolium     | JPCastro 40   | Jacobina, BA        | 30 + 3B | 12p | 1F       |
| A. gracile Lindl.   | JPCastro 61   | Senhor do Bonfim, BA | 30 | 10p | 1G       |
| LPFelix 13662       |              | Mamanguape, PB      | 40 | 2p | 1H       |
| LPFelix 14865       |              | Peruíbe, SP         | 40 | 2p | 1I       |
| A. jilekii Schott*  | LPFelix 13761 | Taquaritinga do Norte, PE | 30 | 2p | 2A       |
| A. pentaphyllum G.Don| LPFelix 15074 | Meruoca, CE         | 30 | 13p | 2B       |
| LPFelix 13663       |              | Mamanguape, PB      | 60 | 5p | 2C       |
| LPFelix 14871       |              | Itabaiana, SE       | 60 | 3p | 2D       |
| A. petrophilum K.Krause* | LPFelix 12614 | Buíque, PE          | 30 | 10p | 2E       |
|                     | EMedeiros-Neto 22  | Brejo da Madre de Deus, PE | 30 | 12p | 2F       |
| SNascimento 150     |              | São João do Tigre, PB | 30 + 1B | 12p | 2G       |
| *Anthurium sp.*     | LPFelix 15273 | São Roque de Minas, MG | 30 | 2p | 2H       |

* First chromosome count for the species

PB = Paraíba, PE = Pernambuco, BA = Bahia, CE = Ceará, SE = Sergipe, MG = Minas Gerais State, Brazil. p = pericentromeric bands
Cytogenetics of Anthurium (Araceae) 637

analyzed. The material was then washed in distilled water and digested in an enzymatic solution containing 2% cellulase (Onozuka) and 20% pectinase (Sigma) (w/v) for 1 h at 37 °C. Slides were prepared using the squashing method in a drop of 45% acetic acid. Coverslips were subsequently removed in liquid nitrogen and samples were then air dried and kept for three days at room temperature (Guerra and Souza, 2002).

Fluorochrome staining followed the protocol described by Carvalho et al. (2005). Samples were stained with 10 µL chromomycin A3 (CMA) (0.1 mg/mL) and stored for 1 h in the dark, before staining with 10 µL of 2 µg/mL DAPI, then again stored in the dark for 30 min before mounting with glycerol/Mellvaine. The slides were aged for three days in the dark to stabilize the fluorochromes. Metaphases were photographed using an AxioCam MRM epifluorescence microscope (Zeiss) equipped with a video camera, utilizing Axiovision 4.8 software (Zeiss). Images were processed using Adobe Photoshop CS3 Software (Adobe Systems). Chromosome measurements were made using Image Tool 3.0 software (Brent et al., 2008). Chromosome morphology was determined using the centromeric index, following Guerra (1986a).

Results

Chromosome numbers and heterochromatin characteristics are summarized in Table 1. All species exhibited symmetrical karyotypes, with chromosomes varying from submetacentric to metacentric (Figures 1 and 2). Chromosome numbers varied from \( n = 30 \) to \( n = 60 \), with most species showing \( n = 30 \); \( n = 40 \) was observed in two populations of \( A. gracile \) (Figure 1H, I) and \( n = 60 \) in two populations of \( A. pentaphyllum \) (Figures 2C-D). Euchromatic B chromosomes were observed in a population of \( A. affine \) Schott from Queimadas, Paraíba State (Figure 1A), and in populations from Águas Belas, Pernambuco State (Figure 1C) and Andaraí, Bahia State (Figure 1E). Anthurium gladifolium Schott, on the other hand, showed three heterochromatic B chromosomes (Figure 1F), while the population of \( A. petrophilum \) K.Krause from São João do Tigre, Paraíba, showed a single heterochromatic B chromosome (Figure 2G). The species of Anthurium with B chromosomes analyzed here, their respective populations, and the frequency of B chromosomes in mitotic cells are presented in Table 2. None of the other species exhibited supernumerary chromosomes.

Staining with fluorochromes revealed from one to two proximal CMA'/DAPI bands on the short arm of \( A. affine \) (Figure 1A-E), in two populations of \( A. gracile \) Lindl. with \( n = 40 \) (Figure 1H-I), in \( A. jilekii \) Schott (Figure 2A), and in \( A. pentaphyllum \) sp. (Figure 2H). The population of \( A. gracile \) from Senhor do Bonfim, Bahia (\( n = 30 \)) showed up to 10 conspicuous proximal CMA bands (Figure 1G), while \( A. gladifolium \) showed up to 12 proximal bands (Figure 1F), and up to 13 CMA bands were seen in a diploid population of \( A. pentaphyllum \) (Figure 2B). The tetraploid populations of \( A. pentaphyllum \) examined, however, exhibited five proximal CMA bands in a population from Maman-guape, Paraíba (Figure 2C) and three bands in a population from Itabaiana, Sergipe (Figure 2D). Anthurium petrophilum, on the other hand, demonstrated a more consistent banding profile, with 10 to 12 proximal CMA bands in the three populations analyzed. DAPI'/CMA' bands were not clearly observed, except in the \( A. gracile \) population from Peruíbe (Figure 1I) and in \( A. pentaphyllum \) from Meruoca (Figure 2B), where the terminal regions of some chromosomes appeared stained with DAPI rather than with CMA, which were interpreted as DAPI'/CMA' regions.

Discussion

Numerical chromosome variations

Of the seven species analyzed here, our results confirmed previous counts for \( A. pentaphyllum \) with \( n = 30 \) and 60 (Cotias-de-Oliveira et al., 1999), \( A. gracile \) with \( n = 30 \) and 40 (Sheffer and Kamemoto, 1976; Guerra, 1986b), and \( A. gladifolium \) and \( A. affine \) with \( n = 30 \) (Sheffer and Kamemoto, 1976; Carvalheira et al., 1991; Cotias-de-Oliveira et al., 1999). The counts for \( A. jilekii \) and \( A. petrophilum \), both with \( n = 30 \), are new.

The chromosome number \( n = 30 \) is the most frequent in the genus Anthurium, although other chromosome numbers, such as \( n = 26, 28, 32, 36, \) and 40, also occur (Sheffer and Kamemoto, 1976; Sheffer and Croat, 1983; Viégas et al., 2006). Those variations may represent cases of ascending or descending diploidy or different euploidy series of \( n = 15 \). Similarly, reports of polyplody generally follow two distinct models (\( n = 30-60-90 \) and \( n = 28-56 \)) (Sheffer and Croat, 1983; Viégas et al., 2006). Among the polyplody species analyzed, \( A. pentaphyllum \) follows the 30-60-90 model, the most common in the genus (Sheffer and Croat, 1983). In the \( n = 30, 40 \) and 60 series reported for \( A. gracile \) (Sheffer and Croat, 1983; Guerra, 1986b; present work), however, \( n = 40 \) may have resulted from ascending or descending diploidy, although there are no intermediate chromosome numbers in the literature in support of those events.

Polyplody and diploidy are among the most important karyotype phenomena associated with the evolution of plant groups (Stebbins, 1971; Solits et al., 2014). Species showing diploidy and polyplody tend to be morphologically distinct from their parental diploids, and can present adaptations to different habitats and ecological niches (Madlung, 2013; Ramsey and Ramsey, 2014; Cordeiro et al., 2018; Scholthof et al., 2018). However, the occurrence of diploidy or polyplody in Anthurium does not appear to have any apparent ecological correlation or link to their geographic distributions, although they may have considerable effects on speciation within the genus, especially in the species \( A. scandens \) (Aubl.) Engl. (\( n = 24, 48, 84 \)), \( A. digitatum \) (Jacq.) G.Don (\( n = 26, 30, 36, 40, 60 \)), and \( A. bellum \) Schott (\( n = 28, 56, 90 \)) (Sheffer and Kamemoto, 1976; Rice et al., 2015).
Figure 1 - Metaphases of Anthurium species under CMA/DAPI staining. (A-E) Anthurium affine. (A) Queimadas-PB population with $2n = 30 + 1B$; (B-C) Águas Belas-PE population, with $2n = 30$ (B) and $2n = 30 + 2Bs$ (C); (D-E) Andaraí-BA population with $2n = 30$ (D) and $2n = 30 + 3Bs$ (E); (F) A. gladiifolium ($2n = 30 + 3Bs$); (G-I) A. gracile. Senhor do Bomfim-BA population with $2n = 30$ (G), Mamanguape-PB (H) and Peruíbe-SP population (I), both with $2n = 40$. Arrows in A, C, E and F show B chromosomes; arrow heads in B, C and I show minor CMA bands; inserts in A and D highlight chromosomes with CMA bands; inserts in I show chromosomes with DAPI+/CMA0 blocks. Bar in I is equivalent to 10 μm.
The basic number \( x = 15 \) appears as the most probable for *Anthurium* based on the wide occurrence of \( 2n = 30 \) in the genus (Marchant, 1973). Sheffer and Kamemoto (1976) and Sheffer and Croat (1983) suggested \( x = 12 \) as the basic

Figure 2 - Metaphases of *Anthurium* species under CMA/DAPI staining. (A) *Anthurium jilekii* with \( 2n = 30 \); (B-D) *A. pentaphyllum*, Meruoca-Ce population with \( 2n = 30 \) (B), Mamanguape-PB (C) and Itabaiana-SE populations (D), both with \( 2n = 60 \); (E-G) *A. petrophilum*, Buíque-PE population (E) and Brejo da Madre de Deus-PE population (F), with \( 2n = 30 \) and São João do Tigre-PE population (G) with \( 2n = 30 + 1B \); H. *Anthurium* sp. (\( 2n = 30 \)). Arrows in G show B chromosomes; arrow heads in C show chromosomes with CMA bands. Bar in H is equivalent to 10 \( \mu m \).
Anthurium numbers to the karyotypic evolution of Anthurium flexile (Carlsen and Croat, 2013). Anthurium occupies a derived position in the genus (Carlsen and Kamemoto, 1976), and NORs of one or two chromosomes. B chromosomes composed principally of GC-rich heterochromatin. Anthurium affine is characterized by having only small quantities of GC-rich heterochromatin, which are observed only in the A. petrophilum species and in NORs (Lamb et al., 2007). Heterochromatin is most frequently located in the subtelomeric and pericentromeric regions of plant chromosomes and in NORs (Lamb et al., 2007). Heterochromatin distribution appears to be relatively variable among different species and populations of Anthurium. Heterochromatin is most frequently located in the subtelomeric and pericentromeric regions of plant chromosomes and in NORs (Lamb et al., 2007). Heterochromatin associated with NORs in plants frequently appears as CMA-/DAPI− bands (Guerra, 2000). Those sequences can be differentially amplified, forming characteristic patterns useful in differentiating between the karyotypes of closely related taxa, such as in Citrus (Patterson and Givnish, 2003), suggesting that the occurrence of B chromosomes in different plants reflects different causes.

The presence of B chromosomes can produce phenotypic effects at the level of individuals, especially related to vigor, fertility and fecundity, increased germination vigor or speed, or the appearance of morphological traits (leaf striping in maize, for example) (Camacho et al., 2000; Levin et al., 2005). One well-accepted hypothesis is their derivation from A chromosomes (Jones and Houben, 2003; Houben et al., 2013). In that sense, it is reasonable to suppose that GC-rich heterochromatin regions of the A chromosomes of A. gladiifolium and A. petrophilum were incorporated into (and amplified in) their B chromosomes.

The occurrence of B chromosomes in Anthurium, as well as other groups of plants, seems to be independent phenomena (Camacho et al., 2000; Levin et al., 2005), without any clear effects above the species level. Phylogenetic analyses corroborate that hypothesis, as one can see in Anthurium species that have B chromosomes, but are placed in different clades (see the phylogenetic hypothesis proposed by Carlsen and Croat, 2013). As in Anthurium, the occurrences of B chromosomes in Picea A.Dietr. (Pinaceae) do not show clear phylogenetic relationships (Lockwood et al., 2013). All of the species of Calochortus Pursh (Liliaceae) that have B chromosomes (D’Ambrosio et al., 2017), on the other hand, are in the same clade (Subsection Venusii, Patterson and Givnish, 2003), suggesting that the occurrence of B chromosomes in different plants reflects different causes.

### Table 2 - Anthurium species with B chromosomes and their frequencies of occurrence in mitotic cells.

| Species          | Origin (city/state) | Total number of analyzed cells | 2n     | Frequency absolute (relative) |
|------------------|---------------------|--------------------------------|--------|------------------------------|
| Anthurium affine | Quelimandas, PB     | 13                             | 30 + 1B| 13 (100%)                    |
|                  | Águas Belas, PE     | 15                             | 30     | 9 (60%)                      |
|                  | 30 + 2B             | 6 (40%)                        |        |                              |
|                  | Andarai, BA         | 30                             | 30     | 11 (36.7%)                   |
|                  | 30 + 3B             | 19 (63.3%)                     |        |                              |
| A. gladiifolium  | Jacobina, BA        | 31                             | 30 + 3B| 31 (100%)                    |
| A. petrophilum   | São João do Tigre, PB| 35                             | 30 + 1B| 35 (100%)                    |

ancestral number due to records of 2n = 24 and 48 in species of the section Tetraspermium Schott. Molecular phylogenetic data nonetheless suggest that the section Tetraspermium occupies a derived position in the genus (Carlsen and Croat, 2013). Anthurium flexile Schott, with 2n = 60 (Sheffer and Kamemoto, 1976), and A. eldemiioides Standl. with 2n = 30 (Petersen, 1989) have been considered the most basal species (Carlsen and Croat, 2013), in support of x = 15 as the basic number of Anthurium. However, the hypothesis of x = 12 cannot be discarded offhand, as species of the genus Pothis L. (a sister group to Anthurium) show 2n = 24 and 26 (Rice et al., 2015), suggesting a relationship of those numbers to the karyotypic evolution of Anthurium.

### B chromosomes

Of the 153 species of the genus Anthurium with known chromosome numbers, B chromosomes have been identified in 20 (approximately 13%). Among the species found to have B chromosomes, there are records for A. affin (Cotias-de-Oliveira et al., 1999) and the new occurrences in A. gladiifolium (30+3Bs) and A. petrophilum (30+1B). However, the occurrence of B chromosomes in the genus may be underestimated, whereas the numbers of B chromosomes may have been interpreted as intraspecific diploidy variation in chromosome numbers. For example, A. obtusum (Engl.) Grayum with 2n = 24, 30, A. durandii Engl. with 2n = 28, 30 (Sheffer and Croat, 1983), and A. conspicuum Sodiro with 2n = 28, 32 (Rice et al., 2015) may reflect B chromosomes interpreted as A chromosomes.

The B chromosomes of Anthurium, besides varying in number, can also vary in their origin and chromatin composition. Anthurium affine is distinct from other species because its B chromosomes were euchromatic, while A. gladiifolium and A. petrophilum show B chromosomes composed principally of GC-rich heterochromatin. Anthurium affine is characterized by having only small quantities of GC-rich heterochromatin, which are observed only in the NORs of one or two chromosomes. Anthurium gladiifolium and A. petrophilum, on the other hand, show large CMA bands in the pericentromeric regions of five to six chromosome pairs. Although the origins of B chromosomes are not yet certain, one well-accepted hypotheses is their derivation from A chromosomes (Jones and Houben, 2003; Houben et al., 2013). In that sense, it is reasonable to suppose...
2007), *Ameroglossum* Eb. Fisch., S. Vogel & A.V.Lopes (Almeida et al., 2016), and *Vigna* Savi (Shamuralatpam et al., 2014). Differential amplification of heterochromatin was observed in all of the species analyzed in the present work, especially in *A. gracile*, which exhibited from 2 to 10 CMA+ bands in different populations.

The phenomena responsible for variation in the heterochromatic portions of different plant species are not well known. The diverse CMA banding patterns observed in genera such as *Caesalpinia* L. *sensu lato* (Fabaceae) appear to be related to geographic distribution, ecological niches, and the phylogenetic relationships between the species (Van-Lume et al., 2017). The heterochromatic fraction in *Anthurium* is quantitatively variable among species and populations, and may be related to adaptive aspects, reflecting environmental or phylogenetic factors in those taxa. Corroborating this hypothesis, the population of *A. gracile* from Senhor do Bonfim in the semi-arid region of Bahia showed large numbers of CMA bands (10) when compared to populations from the humid coastal areas of Paraíba and São Paulo (each with only one pair of bands). Chromosome studies involving larger numbers of species and populations, in conjunction with evolutionary phylogenetic methodologies, could aid in understanding the karyotypic diversity observed in *Anthurium*, one of the most diversified groups of Neotropical monocotyledons.

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Conflict of interest

The authors whose names are listed immediately below certify that they have no conflict of interest related to the article entitled “Chromosomal variability in Brazilian species of *Anthurium* Schott (Araceae): Heterochromatin, polyploidy, and B chromosomes”.

Author contributions

SN analyzed cytogenetically all species and wrote the initial draft of the main text; MANC identified all species analyzed; JMPC analyzed the data and critically reviewed the final version of the manuscript; LPF conceived and designed the study. All authors read and approved the final version.

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