

Cytogenetic Characterization of Species and Hybrids of Orchids of *Cattleya* Genus

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Received April 20, 2016; accepted December 18, 2016

Summary This paper aims to study the cytogenetic characterization of *Cattleya nobilior* species and *C. bowringiana* and hybrids of orchids *C. violacea* “type”×*C. granulosa*, *C. violacea* “type”×*C. harrisoniae* “perola,” and *C. violacea* “type”×*C. nobilior* “amaliae.” *Cattleya nobilior* has $2n=2x=30$ metacentric chromosomes, with size ranging from 0.54 to 1.58 μm and an active NOR in the first chromosome pair, and *C. bowringiana* has $2n=2x=34$ metacentric chromosomes, from 0.38 to 1.46 μm of size and an active NOR in the first chromosome pair. As for the hybrids, *C. violacea* “type”×*C. harrisoniae* “pérola” has $2n=2x=32$ metacentric chromosomes, with size from 0.47 to 1.73 μm and an active NOR in the second chromosome pair, *C. violacea* “type”×*C. nobilior* “amaliae” has $2n=2x=66$ metacentric chromosomes, with size between 0.22 to 1.48 μm and active NORs in chromosome pairs 1 and 3, and *C. violacea* “type”×*C. granulosa* with $2n=2x=86$ metacentric chromosomes, with size from 0.26 to 1.56 μm and an active NOR in chromosome pairs 3 and 23.

Key words Orchidaceae, Chromosome number, NOR, *Cattleya*, Metaphase.

The *Cattleya* genus stands out for its importance to the global floriculture agribusiness by presenting many varieties of hybrids with lush flowers, high commercial and ornamental value with great morphological and chromosomal variation and high genetic diversity (Cruz *et al.* 2003). *Cattleya* has a natural recombination capacity which make hybridization mechanism possible and produce fertile descendants (Bernacci *et al.* 2005), and usually has a chromosomal variation with register from $2n=12$ to $2n=240$, more often $n=19$ or 20 (Felix and Guerra 2010). Cytogenetic studies are still considered incomplete by the scope of the Orchidaceae family. It is estimated that over 10% of known species have been analyzed chromosomally. However, the phylogenetic evolution of this family remains under discussion (Daviña *et al.* 2009, Penha *et al.* 2011).

This paper aims to study the cytogenetic characterization of *Cattleya* species and hybrids through mitotic chromosome analysis and identification of the nucleolus organizer regions (NORs).

Materials and methods

Root meristems of *C. nobilior*, *C. bowringiana* and *C. violacea* hybrid “type”×*C. nobilior* and *C. violacea* “type”×*C. harrisoniae* “perola” and *Cattleya violacea* “type”×*C. granulosa* after 90 d of *in vitro* cultivation were used to cytogenetic characterization. Meristems were immersed in a trifluralin solution with a concentration of 3 μM over 18 h at 4°C for blocking the cell cycle progression, after which they were washed in distilled water and fixed in methanol–acetic acid solution (PA) 3:1 at 4°C over 24 h. Meristems were allocated into micropipette tips containing 200 μL of Pectinase Sigma[®] enzyme at 3 μM for over 50 min and 36°C in a hot water bath.

After that, the materials were washed again and fixed in methanol–acetic acid (3:1) for over 24 h at 4°C. The slides were stained with 5% Giemsa for over 3 min, washed in distilled water and dried at about 23°C (Carvalho and Saraiva 1993).

Metaphase photo documentation was taken under a microscope (Leica ICC 50) coupled to a computer and LAZ EZ V1 7.0 software, and analyzed with the SXM Image program (Barrett 2002). Arms of each chromosome were measured in pixels and converted into micrometer scale. The relationship between the

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DOI: 10.1508/cytologia.82.137

arms (r =long arm/short arm), total haploid set length (THSL=absolute sum of the lengths of metaphase chromosomes of each species) and centromere index (CI=[short arm length/total length] \times 100) were estimated based on chromosomal measurements. Chromosomes were classified according to Guerra (1986) into four types: metacentric (M, r =1.00 to 1.49; CI=40.1 to 50.0), submetacentric (SM, r =1.50 to 2.99, CI=25.1 to 40.0), acrocentric (A, r =3.00 to 7, CI=0.01 to 25.0) and telocentric (T, r = ∞ , IC=0).

The centromere index (CI) is obtained by using the formula $CI=BC\times 100/T$, where "BC" is the short arm length and "T" the chromosomal length. For evaluating the karyotypic asymmetry, some indexes were estimated: TF (%), Rec, and SYi (Greilhuber and Speta 1976). TF is the ratio between the sum of the shorter arm lengths and of the haploid set lengths (Huziwara 1962); Rec index is the average ratio between chromosome length by the longer chromosome length; and SYi indicates the ratio between short arms length average and the long arms length average (Greilhuber and Speta 1976).

The Ag-NOR banding was performed after 20 d over the glass slide preparation (Funaki *et al.* 1975) using a solution (AgNO₃) of 50% after the glass slides have remained in a humid and dark chamber at 34°C for over 19 h. The coverslips were removed with a water jet and washed in running water for 2 min and in distilled water for 1 min.

Results and discussion

During interphase, the presence of a nucleolus per cell in *C. nobilior*, and *C. bowringiana* and in *C. violacea* was identified. Metaphases presented chromosomes with distinct chromatin condensation, well distributed in absence of overlaps. In hybrids, *C. violacea* "type" \times *C. granulosa* and *C. violacea* "type" \times *C. nobilior* "amaliae" two nucleoli per interphase nucleus were observed. *C. nobilior* revealed $2n=2x=30$ (Fig. 2A) and chromosomes over $1.58\pm 0.54\mu\text{m}$ long (Table 1), with simple NORs in small blocks at the centromeric region of the first chromosome pair (Fig. 1A). The species *C. bowringiana* exhibited $2n=2x=34$ chromosomes (Fig. 1B) with chromosomes over $1.46\pm 0.38\mu\text{m}$ long (Table 1), and simple NORs in small blocks in the middle region of the first

chromosome pair (Fig. 2B). Arditti (1992) reviewed several important cytological aspects in Orchidaceae family, and showed chromosome number variation with occurrence is $n=10$ and $n=20$. However, the frequent occurrence of $n=10$ in the family, makes it possible to infer $x=10$ as the basic number more likely (Dodson 1957, Felix and Guerra 2010).

The hybrid *C. violacea* "type" \times *C. nobilior* "amaliae" has $2n=2x=66$ chromosomes (Fig. 1D) that are $1.48\pm 0.22\mu\text{m}$ long (Table 1), and multiple NORs in

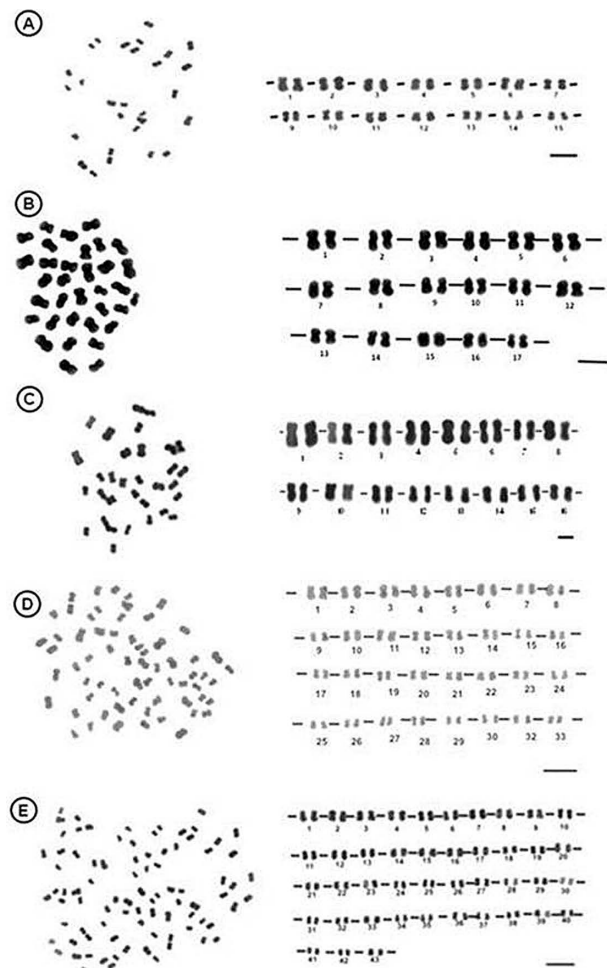


Fig. 1. Metaphases and karyotypes of orchid species and hybrids of *Cattleya* genus. (A) *C. nobilior* ($2n=30$). (B) *C. bowringiana* ($2n=34$). (C) *C. violacea* "type" \times *C. harrisoniae* "pérola" ($2n=32$). (D) *C. violacea* "type" \times *C. nobilior* "amaliae" ($2n=66$). (E) *C. violacea* "type" \times *C. granulosa* ($2n=86$). Bars= $5\mu\text{m}$.

Table 1. Diploid complement ($2n$), karyotype formulae (KF), chromosome size range (LC: Large chromosome to SC: Short chromosome), centromeric index (CI), total length of haploid set (TLH), Total Form index (TF%), Rec index, and Syi index for two species and three hybrids of *Cattleya*.

Species/hybrid	$2n$	KF	LC-SC (μm)	CI	TLH (μm)	TF%	Rec index	Syi index
<i>C. bowringiana</i>	34	17m	1.46-0.38	49.2	11.94	48.4	48.1	94.4
<i>C. nobilior</i>	30	15m	1.58-0.54	47.5	13.58	47.5	57.4	89.5
<i>C. violacea</i> \times <i>C. nobilior</i>	66	33m	1.48-0.22	48.79	19.48	49.12	41.1	96.7
<i>C. violacea</i> type \times <i>C. granulosa</i>	86	43m	1.56-0.26	46.18	25.02	46.40	36.9	87.1
<i>C. violacea</i> type \times <i>C. harrisoniae</i>	32	16m	1.73-0.47	47.95	13.06	47.8	39.9	90.7

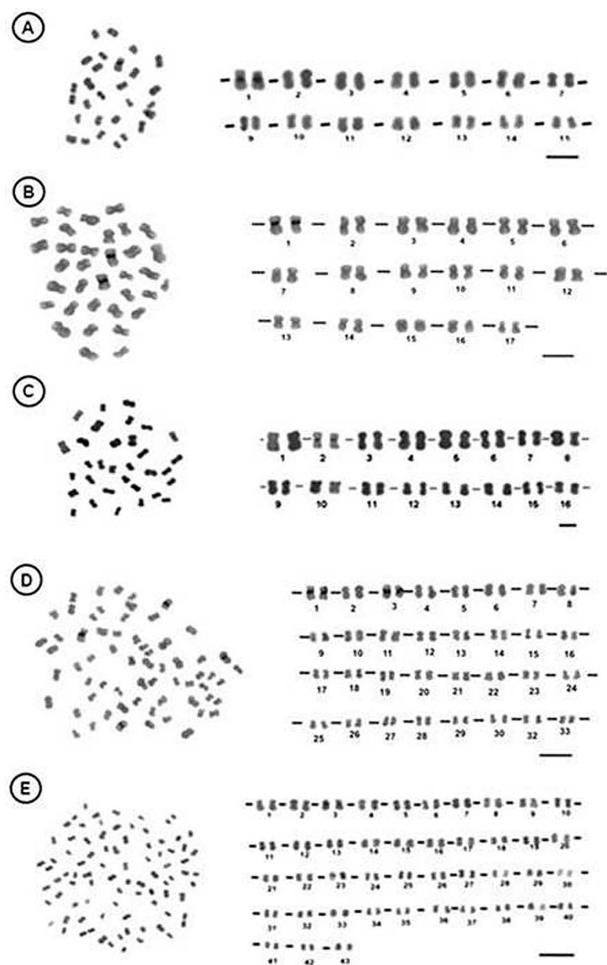


Fig. 2. Nucleolar Organizer Regions (NORs) in species and hybrids of *Cattleya* orchid genus. (A) *C. nobilior* NORs in the first chromosome pair. (B) *C. bowringiana* NORs in the first chromosome pair. (C) *C. violacea* "type" × *C. harrisoniae* "pérola" NORs in the second chromosome pair. (D) *C. violacea* "type" × *C. nobilior* "amaliae" NORs in the first and third chromosome pairs. (E) *C. violacea* "type" × *C. granulosa* NORs in the first and twenty-third chromosome pair. Bars = 5 μm.

small blocks in the middle of the first and third chromosome pairs (Fig. 2D). *C. violacea* "type" × *C. harrisoniae* "pérola" has $2n=2x=32$ (Fig. 1C), with chromosome length over $1.73 \pm 0.47 \mu\text{m}$ (Table 1), and NORs show a simple shape in small blocks in the middle region of the second chromosome pair (Fig. 2C). *C. violacea* "type" × *C. granulosa* has $2n=2x=86$ chromosomes and $1.56 \pm 0.26 \mu\text{m}$ (Table 1), presenting NORs in the third and 33rd chromosome pairs (Table 1). The intense hybridization in orchids may explain the chromosome number variations found in these studied species and hybrids. According to Loveless and Hamrick (1984), joint perspective in mutation, migration, selection and genetic drift, and other processes, cause the change in the number and structure of the orchid species chromosome set, and the chromosomal variation is quite high, presenting $2n=12$ to $2n=240$ (Felix and Guerra 2010). The hybrid *C. violacea* "type" × *C. granulosa* presents $2n=2x=86$

chromosomes (Fig. 1E) and length over $1.56 \pm 0.26 \mu\text{m}$ (Table 1).

Multiple NORs were found in small blocks in the centromeric regions in pairs 3 and 23 (Fig. 2E). By Ag-NOR banding, it was possible to identify proteins with transcriptional activity of rDNA genes by silver stain in the *C. nobilior* and *C. bowringiana* and hybrid *C. violacea* "type" × *C. harrisoniae* "pérola" *C. violacea* "type" × *C. granulosa* and *C. violacea* "type" × *C. nobilior* "amaliae" chromosomes. The studied orchids karyotypes show different characteristics in the conservation of the NORs.

The rated karyotype symmetry observed the estimated TF at 46.4% in *C. violacea* × *C. granulosa*, and 49.1% in *C. violacea* × *C. nobilior*. According to Huzi-wara (1962), the TF% can vary from zero (asymmetric karyotype) to 50% (symmetric karyotype). The SYi index ranges from zero (asymmetric) to 1 or 100% (symmetrical) (Peruzzi and Eroğlu 2013). In this study, the SYi ranged from 87.1 to 96.7, and Rec ranged from 36.9 (*C. violacea* × *C. granulosa*) to 57.4 (*C. nobilior*). The results suggest that species and hybrids have symmetrical karyotypes. A symmetrical karyotype is characterized mainly by the presence of metacentric and submetacentric chromosomes with similar sizes, which in orchids indicate primitive evolutionary characteristics (Begum and Alam 2005, Paszko 2006, Peruzzi *et al.* 2009).

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