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Heterochromatin differentiation in holocentric chromosomes of *Rhynchospora* (Cyperaceae)

André L.L. Vanzela[1] and Marcelo Guerra[2]

Abstract

Holocentric chromosomes of six species of *Rhynchospora, R. ciliata, R. pubera, R. riparia* and *R. barbata* (2n = 10), *R. nervosa* (2n = 30) and *R. globosa* (2n = 36), were stained with CMA_3/DAPI fluorochromes or treated with C-banding and sequentially stained with Giemsa or CMA_3/DAPI. Variability in banding pattern was found among the species studied. Heterochromatin was observed on terminal and interstitial chromosome regions, indicating that the holocentric chromosomes of *Rhynchospora* show a heterochromatin distribution pattern similar to those plant monocentric chromosomes.

INTRODUCTION

Heterochromatic sequence localization by banding techniques has contributed to our knowledge of chromosome organization, permitting discrimination between morphologically similar chromosomes, and aiding in establishing evolutionary relationships between closely related taxa. Few studies exist on the heterochromatin distribution pattern in holocentric chromosomes of plants. Initial research was done on the genus *Luzula* (Juncaceae) by Ray and Venketeswaram (1978) and Collet and Westerman (1984), who found interspersed among the euchromatin a variable number of C-bands, which fused during metaphase condensation. Thus, in prophase and prometaphase the band number was much larger than in metaphase. A similar situation was described for insect holocentric chromosomes (see Giles and Webb, 1972; Manicardi *et al.*, 1991; Manicardi and Gautam, 1994).

Other work has demonstrated that holocentric chromosomes can exhibit defined heterochromatic blocks in telomeric regions (Panzera *et al*., 1992) as well as in telomeric and interstitial regions, e.g., in the Heteroptera *Nezara viridula* (Camacho *et al*., 1985). A similar case was reported by Sheikh and Kondo (1995, 1996) for several species of *Drosera* (Droseraceae), where interstitial C-bands were found in a few chromosomes and CMA_3/DAPI bands preferentially in telomeric regions.

The holocentric nature of *Rhynchospora* chromosomes has been discussed (Vanzela *et al*., 1996; Luceño *et al.*, 1998). However, little information exists about the behavior and organization of the highly repetitive DNA in those chromosomes. The present article reports the heterochromatin distribution pattern in holocentric chromosomes of six Brazilian species of *Rhynchospora,* using three distinct chromosome banding methods.

MATERIAL AND METHODS

Six different species of the genus *Rhynchospora* (*R. ciliata, R. pubera, R. riparia, R. barbata, R. nervosa* and *R. globosa*) were studied. The voucher specimens collected in Northeastern and Southern Brazil are kept in the Universidade Federal de Pernambuco herbarium (Table I).

Root tips were pretreated with 2 mM 8-hydroxyquinolin for 24 h and further fixed in Carnoy (3:1, v/v) for 1-24 h. Tests were digested for 3 h in a mixture of 4% (v/v) cellulase and 40% (v/v) pectinase, and squashed in a drop of 45% acetic acid. The coverslip was removed in liquid nitrogen and, after three days, the chromosomes were treated in three different ways. In the first treatment, *R. ciliata* and *R. barbata* samples were directly stained with a drop of 0.5 mg/ml CMA_3 in McIlvaine buffer, pH 7.0/distilled water (1:1) and 2.5 mM $MgCl_2$ for 1 h, washed in distilled water and stained with a drop of 2 μg/ml DAPI in McIlvaine buffer, pH 7.0, for 30 min, as described by Schweizer (1976). In the second, *R. ciliata, R. barbata* and *R. nervosa* samples were treated according to the C-banding procedure (45% acetic acid at 60ºC for 10 min, 5% BaOH at room temperature for 10 min and 2xSSC, pH = 7.0, at 60ºC for 1 h and 20 min) and stained with 2% Giemsa, according to Schwarzacher *et al.* (1980). In the third treatment, *R. ciliata, R. pubera, R. riparia* and *R. globosa* samples were processed for C-banding, as mentioned above, and stained with CMA_3/DAPI (Barros e Silva and Guerra, 1998).

Materials stained with 2% Giemsa were mounted with Entellan (Merck) and those stained with fluorochromes were mounted in glycerin:McIlvaine buffer (1:1, v/v) and 2.5 mM $MgCl_2$. Those stained with Giemsa were analyzed with an optical microscope, and photos were taken with Imagelink

[1]*Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, 05508-990 São Paulo, SP, Brasil.* [2]*Departamento de Botânica, CCB, Universidade Federal de Pernambuco, 50670-420 Recife, PE, Brasil. Send correspondence to A.L.L.V. E-mail: avanzela@hotmail.com*

HQ 25 ASA Kodak film. Those stained with fluorochromes were examined using epifluorescence microscopy and photographed with T-MAX 400 ASA Kodak film.

RESULTS

Three different species were analyzed with C-banding: *Rhynchospora ciliata* (2n = 10) showed the highest number of terminal and interstitial blocks in almost all chromosome pairs (Figure 1a), *R. barbata* (2n = 10) exhibited a small pair of interstitial dots in each chromosome, in addition to terminal blocks (Figure 1b), and *R. nervosa*, with 2n = 30, presented heterochromatic blocks in some terminal and interstitial regions (Figure 1c).

The CMA_3/DAPI staining revealed terminal and interstitial regions $CMA_3^+/DAPI^0$ (DAPI neutral) in *R. ciliata* prometaphases (Figure 2a and b), and $CMA_3^+/DAPI^-$ interstitial dots in *R. barbata* metaphases (Figure 2g). These signals corresponded to those obtained by C-banding.

The C-banding procedure, followed by CMA_3/DAPI staining, also revealed variations in number and type of heterochromatic sequences among the four analyzed species. *Rhynchospora pubera* (2n = 10) exhibited three chromosome pairs with CMA_3^+ terminal regions. One of these showed a larger and brighter signal, while the others showed weaker signals (Figure 2d and e). *R. riparia*, with 2n = 10, exhibited CMA_3^+ blocks in four chromosome pairs. In two of these, blocks were only terminal, while in the two other pairs, bands appeared in terminal and interstitial positions (Figure 2f). DAPI bands were not observed in these two species. *R. ciliata* (Figure 2c) showed a band pattern similar to that obtained using C-banding and CMA_3/DAPI staining. *R. globosa* was the only species that showed different patterns of CMA_3 and DAPI bands. $DAPI^+$ and CMA_3^+ signals occurred in different terminal or subterminal regions on most of the chromosomes (Figure 2h). Besides, CMA_3^+ bands always showed an appearance of small dots (Figure 2i), as in *R. barbata*. Interphasic nuclei showed randomly distributed CMA_3^+ and $DAPI^+$ blocks (Figure 2f, g, i, and h, respectively).

DISCUSSION

The CMA_3/DAPI staining, Giemsa C-banding and C-banding followed by CMA_3/DAPI staining revealed three different heterochromatin types in *Rhynchospora* holocentric chromosomes. The first type, characterized by $CMA_3^+/DAPI^0$ in *R. ciliata*, coincided with those obtained by Giemsa banding and was similar to that found in *Drosera puchella* and *D. scorpioides* holocentric chro-

Table I - Type and distribution of heterochromatin blocks on chromosomes of six species of *Rhynchospora*.

Species	Localities	UFP	2n	C-band	$CMA_3^+/DAPI^0$	$CMA_3^+/DAPI^-$	$CMA_3^-/DAPI^+$
R. ciliata	Recife-PE*	09337	10	T and I	T and I	T	---------
R. pubera	Recife-PE	11181	10	---------	---------	T	---------
R. riparia	Recife-PE	av26pe	10	---------	---------	T and I	---------
R. barbata	Gravatá-PE	av34pe	10	---------	---------	T and I	---------
R. nervosa	Ipojuca-PE	11136	30	T and I	---------	---------	---------
R. globosa	Tibagi-PR*	av410pr	36	---------	---------	T and I	T and I

*- PE and PR correspond to Brazilian States. UFP = Herbarium of the Universidade Federal de Pernambuco, Recife, PE, Brazil. T = Terminal bands. I = Interstitial bands.

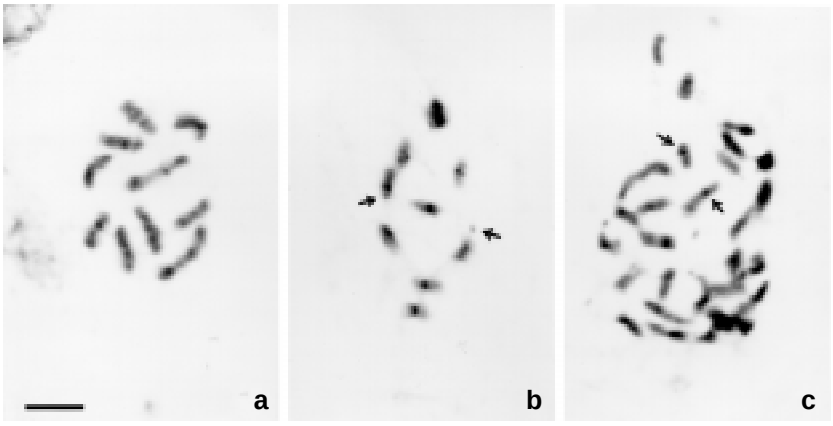

Figure 1 - Giemsa C-banding in mitotic chromosomes of *Rhynchospora*. **a**) *R. ciliata* with 2n = 10. **b**) *R. barbata* with 2n = 10. Arrows indicate terminal bands. **c**) *R. nervosa* with 2n = 30. Arrows indicate terminal and interstitial bands. Bar represents 5 μm.

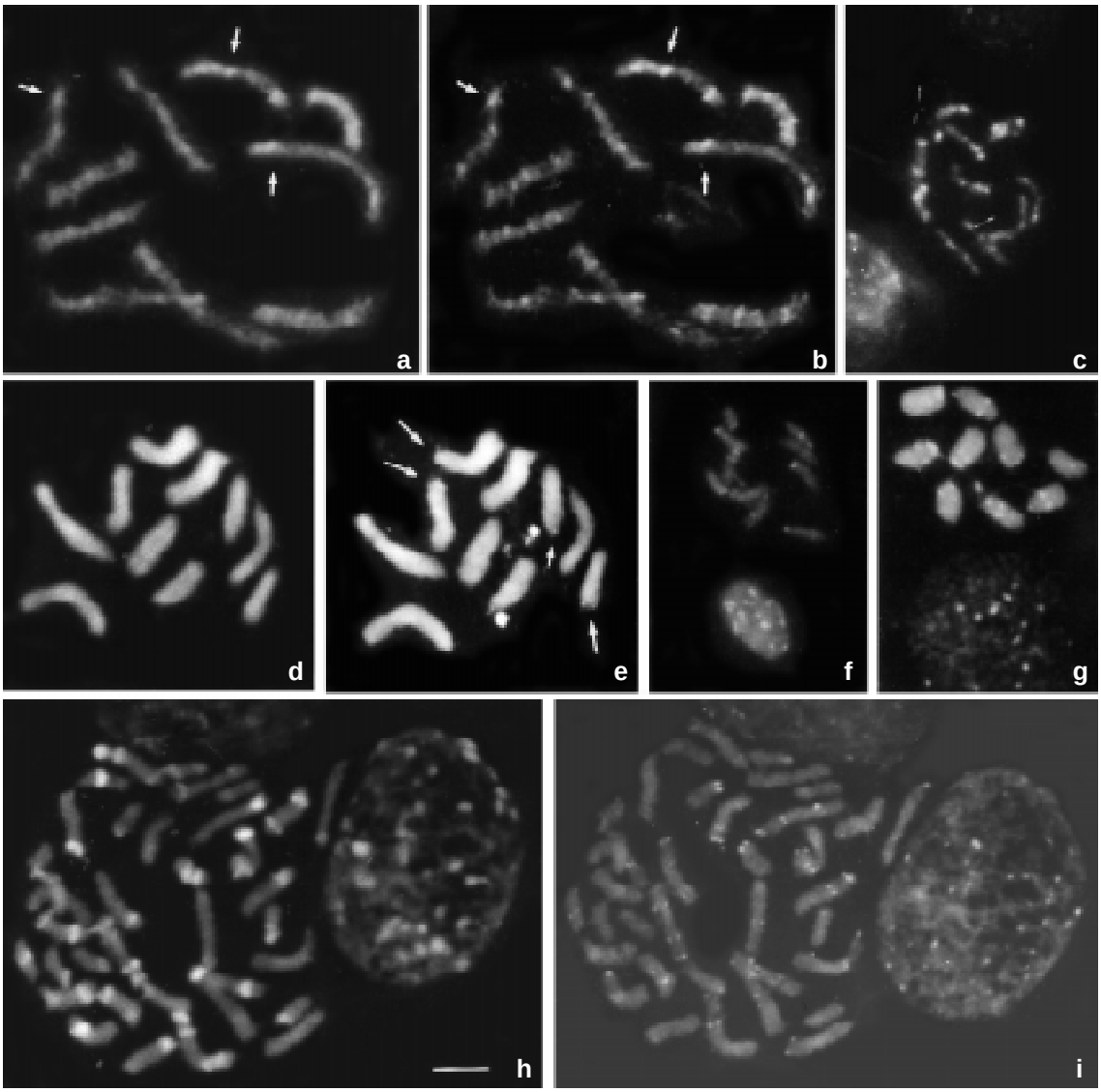

Figure 2 - CMA_3/DAPI banding in mitotic chromosomes of *Rhynchospora.* **a** and **b**) Prometaphase in *R. ciliata* (2n =1 0) directly stained with DAPI and CMA_3, respectively. Arrows indicate blocks $DAPI^0/CMA_3^+$. **c**) Chromosomes of *R. ciliata* stained with CMA_3 after C-banding procedure. **d** and **e**) Mitotic metaphase in *R. pubera* with 2n=10. Arrows show CMA_3^+ blocks. **f**) Mitotic chromosomes in *R. riparia* (2n = 10) stained with CMA_3 after C-banding procedure. **g**) Mitotic chromosomes in *R. barbata* (2n = 10) stained with CMA_3. **h**) Mitotic chromosomes of *R. globosa* (2n = 36) stained with DAPI. **i**) Mitotic chromosomes of *R. globosa* stained with CMA_3. Bar represents 5 μm.

mosomes (Sheikh and Kondo, 1996). These regions could correspond to heterogeneous heterochromatin sites, where GC-rich segments are intercalated with AT-rich ones, as described by Guerra (1989) and Cuellar *et al.* (1996). The second type, $CMA_3^+/DAPI^-$ bands present in most of the species, and the third type, $CMA_3^-/DAPI^+$ blocks, found only in *R. globosa*, seem to correspond to GC-rich and AT-rich DNA sequences, respectively (Schweizer, 1976).

Comparative band pattern analysis in these six species showed that size and location of heterochromatic segments were very variable. Heterochromatin may occur as large or small blocks (*R. pubera* and *R. riparia*), as few or multiple blocks along the chromosomes (*R. barbata* and *R. globosa*) and in terminal and/or interstitial regions. This heterochromatin segment distribution pattern resembles that observed in some plant groups (see Schweizer and Ehrendorfer, 1983). The data presented here differ from those observed for *Luzula* holocentric chromosomes (Ray and Venketeswaram, 1978; Collet and Westerman, 1984).

The variation observed in the holocentric chromosome banding patterns of *Rhynchospora* could be explained by the presence of different initiation and amplification heterochromatin sites (Peacock *et al*., 1981), possibly with dispersion by gene conversion or unequal crossing-over (Schweizer and Ehrendorfer, 1983) or by an equilocal dispersion mechanism similar to that proposed by Schweizer and Loidl (1987), for monocentric chromosomes.

Terminal CMA_3 bands were observed in one or more chromosomes of all species studied. At least in *R. ciliata* and *R. pubera*, the CMA_3 blocks seem to correspond in position to rDNA sites described by Vanzela *et al*. (1998) through *in situ* hybridization. Therefore, some or all CMA_3 terminal bands observed in *R. riparia*, *R. barbata* and *R. globosa* could possibly be related to the NOR. Similar results were found for *Drosera* (Sheikh and Kondo, 1995).

These results suggest that no single typical pattern of heterochromatic segment distribution is present on holocentric chromosomes of *Rhynchospora*, in contrast with the conclusion presented by Collet and Westerman (1984) for *Luzula* holocentric chromosomes, i.e., "highly-repeated DNA is not localized in single blocks, but is interspersed amongst the euchromatin". Band pattern variations may be more related to structural rearrangements, as in monocentric chromosomes (Deumling and Greilhuber, 1982; Schweizer and Ehrendorfer, 1983), rather than reflecting kinetochore organization on the chromosomes.

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RESUMO

Cromossomos holocêntricos de seis espécies de *Rhynchospora (R. ciliata, R. pubera, R. riparia* e *R. barbata* (2n = 10), *R. nervosa* (2n = 30) and *R. globosa* (2n = 36)) foram corados com os fluorocromos CMA_3/DAPI ou tratados para bandeamento C e seqüencialmente corados com Giemsa ou CMA_3/DAPI. Variabilidade no padrão de bandas foi encontrada entre as espécies estudadas. A heterocromatina foi observada em regiões terminais e intersticiais dos cromossomos, indicando que os cromossomos holocêntricos de *Rhynchospora* mostram um padrão de distribuição de heterocromatina similar àqueles dos cromossomos monocêntricos de plantas.

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