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Research Article

Localization of 45S rDNA and telomeric sites on holocentric chromosomes of *Rhynchospora tenuis* Link (Cyperaceae)

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Abstract

Rhynchospora tenuis Link (Cyperaceae) is a weed widely distributed in Brazil that presents a small number of holocentric chromosomes (2n = 4) with some autopolyploid populations (2n = 8). The haploid number n = 2 is considered as a derivative of the base number x = 5. 45S rDNA probes and telomeric DNA were hybridized in both chromosome races of *R. tenuis*, looking for indications of chromosome fusions. The results showed that hybridization sites of the telomeric probe were restricted to end chromosome regions whereas rDNA sites were terminally located. The chromosome race with n = 4 exhibited a doubled number of sites, with similar size and location to the hybridized sequences, confirming its autopolyploid origin. Furthermore, the terminal location of the single 45S rDNA site in the 2n = 4 race suggested that disploid reduction in *Rhynchospora,* from n = 5 to n = 2, was followed by elimination or reorganization events, keeping the terminal distribution of these sites, as in an others species of the genus.

Key words: Cyperaceae, FISH, holocentric chromosomes, 45S rDNA, telomere sequences.

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Introduction

Cyperaceae is the most studied plant family with holocentric chromosomes. This family presents variation in chromosome numbers attributed to agmatoploidy and symploidy, which lead to the large series of disploid chromosome numbers (Wahl, 1940; Faulkner, 1972; Luceño and Castroviejo, 1991; Luceño and Guerra, 1997). However, in some Cyperaceae representatives, such as in the genus *Rhynchospora*, the polyploidy instead of agmatoploidy/symploidy seems to play the most important role in their karyotype evolution (Luceño *et al.*, 1998; Vanzela *et al.*, 2000). The chromosome numbers variation reported for the *Rhynchospora* species ranged from 2n = 4 in *R. tenuis* to 2n = 50 in *R. rosemariana*. According to Vanzela *et al.* (2000), 25 of the 50 *Rhynchospora* species are polyploids, and x = 5 is the most probable basic number of the genus.

Vanzela *et al.* (1996) reported two natural chromosome races in *R. tenuis*, a diploid with 2n = 2x = 4, probably derivates from chromosome fusions (n = 5 to n = 2) and another with 2n = 4x = 8, that probably arose by autopolyploidy. Karyotypes with low chromosome numbers usually represent a derivative feature and constitute excellent material for chromosome organization studies. This paper reports the chromosome localization of 45S rDNA and telomeric sequences by fluorescent *in situ* hybridization (FISH) in both chromosome races of *R. tenuis*. Data are discussed on the basis of the evolutionary mechanisms of these sites, and in the involvement of these DNA segments after chromosome reduction and subsequent autopolyploidy.

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Materials and Methods

Representatives of *R. tenuis* were collected in Ipojuca, Pernambuco, northeast Brazil. The vouchers are deposited at the herbarium UFP of the Federal University of Pernambuco. Chromosome analysis was performed on root tips pretreated with 2 mM 8-hydroxyquinolin for 24 h, fixed in ethanol:acetic acid (3:1, v:v) solution for 12 h and stored in fixative at -20 °C. Samples were digested in 4% cellulase plus 40% pectinase at 37 °C for 3 h and squashed

in a drop of 45% acetic acid. The cover slips were removed in liquid nitrogen and the slides were air dried and stored in the freezer until the FISH.

In situ hybridization was carried out using the p*Ta*71 probe containing the 18S-5.8S-26S rDNA sequence isolated from wheat (Gerlach and Bedbrook, 1979) and the p*LT*11 probe containing the TTTAGG telomeric insert of pAtT4 from *Arabidopsis thaliana* (Richards and Ausubell, 1988). The rDNA probe was labeled with digoxigenin-11-dUTP by nick translation and the telomeric probe with rodamine-11-dUTP by PCR. Preparations were incubated in RNase (100 µg/mL), post-fixed in 4% (w/v) paraformaldehyde, dehydrated in a 70%-100% graded ethanol series and air dried. The probe (20-100 ng) mixture was denaturated at 70 °C for 10 min and the chromosome denaturation/hybridization was done at 80 °C for 7 min using an MJ Research thermal cycler, and 37 °C overnight in a humid chamber. Post hybridization washes were done as described by Cuadrado and Jouve (1994). The probe labeled with rodamine (red, 510-540 nm) was directly observed and the probe labeled with digoxigenin was detected with anti-digoxigenin-FITC conjugate (green, 420-490 nm). Chromosomes were counterstained with 2 µg/mL DAPI (blue, 340-380 nm). Slides were mounted in Vectashield antifade (Vector Labs.) and examined with a Zeiss Axiophot epifluorescence microscope (UV radiation). Photographs were taken with Fuji Color 400 ISO.

Results and Discussion

The diploid karyotype of *R. tenuis* exhibited one larger and one smaller chromosome pair, whereas the tetraploid race displayed two larger and two smaller pairs, neither with any primary constrictions, as previously reported (Vanzela *et al*., 1996).

The results obtained with *in situ* hybridization of the 45S rDNA probe revealed dispersed bright signals at interphase nuclei (Figure 1A) and in one of the terminal regions of all the smaller chromosomes in both races (Figures 1A and 1B). No hybridization signals were detected at interstitial positions. In eight other *Rhynchospora* species hybridized with 45S rDNA probe, the signals were always dispersed, terminal, and mostly numerous (Vanzela *et al.*,

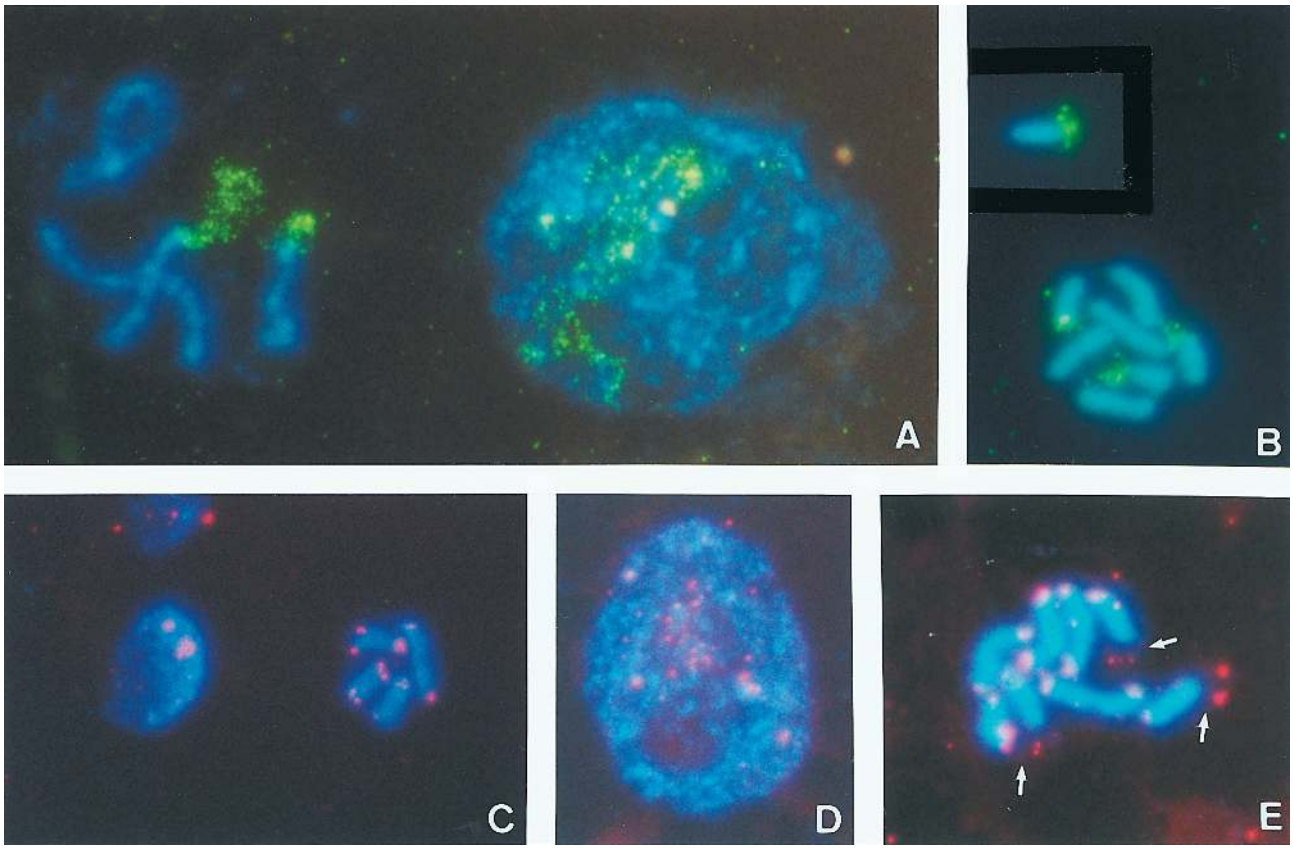

Figure 1 - FISH with probes of 45S rDNA and telomere in *Rhynchospora tenuis* chromosomes. A) Nuclei and prometaphase of 2n = 4 race hybridized with 45S rDNA. B) Metaphase of 2n = 8 race hybridized with 45S rDNA. Observe the diffused state of hybridization signals (FITC) on the chromosomes. Chromosome inside box corresponds to distant chromosome and that was a replacement of the original complement. C) Nuclei and metaphase of 2n = 4 race hybridized with telomeric probe (rodamine). D) Interphase nuclei of 2n = 8 race hybridized with telomeric probe. Observe the dispersed red signals. E) Metaphase of 2n = 8 race metaphase hybridized with telomeric probe. Arrows point to telomeric sites distant from the DAPI stained chromosomes.

1998). The presence of a single pair of terminally located rDNA block in the 2n = 4 suggests that if there were other rDNA sites they were lost during the chromosome fusion process, or were reorganized "de novo" into a single site at the terminal region of the smallest chromosome pair. Similarly, during the *Brachyscome lineariloba* karyotype evolution, a composite species with 2n = 4, there were changes in heterochromatic patterns as well as in NOR localization and activity (Watanabe *et al.* 1975).

The hybridization of the telomeric probe revealed dispersed bright signals at interphase nuclei (Figures 1C and D) and small signals on the chromosome extremities of both races (see arrows in the Figure 1E). Interstitial hybridization sites of the telomeric probe were not detected. It is generally believed that telomeres are necessary for chromosome stability and integrity and the elimination of telomeres is a prerequisite for the chromosome fusions (Slijepcevic *et al.*, 1997). Cox *et al.* (1993) detected no interstitial telomeric DNA sites in other plant karyotypes with monocentric chromosomes that were supposed to originate by chromosome fusion. Likewise, Fuchs *et al.* (1995) were not able to find telomeric probe in the holocentric chromosomes of *Luzula luzuloides* (Juncaceae), which probably was derived from chromosome fusion. Nevertheless, interstitial telomeric DNA sites have been found in chromosomes of *Phaseolus* (Guerra and Kenton, 1996).

The telomeric DNA hybridization site on the end of smaller chromosome pairs appeared always separated from the DAPI stained chromosome (see arrows in the Figures 1C and 1E). These chromosome ends are probably the ones that bear the rDNA site decondensed during the metaphase and negatively stained with DAPI. Because the telomeric sites are located after the rDNA site they appeared as distant signals of the DAPI stained chromosomes.

The results obtained in this study corroborate the proposal of autopolyploid origin for the chromosome race with 2n = 8 (Vanzela *et al.*, 1996), since its monoploid complement was identical to the diploid race for number and location of the 45S rDNA sites. On the other hand, the reduction in the number of telomere DNA sites as well as rDNA sites followed the fusion of the basic chromosome complement of the genus (n = 5 to n = 2). It suggests that sequence elimination may play an important role in karyotype adjustment not only after polyploidization (Eckardt, 2001) but also after chromosome fusions.

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