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Characterisation of the low-chromosome number grass *Colpodium versicolor* (Stev.) Schmalh. (2n = 4) by molecular cytogenetics

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Abstract — A genome size of 2C = 235 Mbp and the chromosomal positions of the 5S and 45S rDNA were determined for the perennial, low-chromosome grass *Colpodium versicolor* (2n = 4). Based on taxonomical and cytological data we suggested previously that *C. versicolor* represents a putative donor of one of the two parental genomes of the allotetraploid grass *Zingeria trichopoda* (2n = 8). To test this hypothesis sequence comparison of the ITS1-5.8S-ITS2 -region (part of the 18S rDNA) and genomic in situ hybridization were performed. Although the ITS1-5.8S-ITS2 sequences of *C. versicolor* and *Z. trichopoda* are highly similar, genomic in situ hybridization of *Z. trichopoda* with labeled genomic DNA of *C. versicolor* did not result in a subgenome-specific labeling of *Z. trichopoda* chromosomes. Thus, the contemporary species *C. versicolor* is a closely related but not the direct ancestor of the allopolyploid species *Z. trichopoda*.

Key words: dysploid reduction, genome evolution, low-chromosome number species, *Poaceae*.

INTRODUCTION

The *Poaceae* is one of the most significant family of the flowering plants in agriculture (Hanelt 2001) and one of the largest and wide-distributed families. Variation in basic chromosome number, high incidence of polyploidy, frequent hybridization and wide range of variation in genome size are prominent features of grass genome evolution (Hilu 2004). The basic chromosome number from x = 2 to x = 18 in the *Poaceae* has generated somatic numbers that vary between 2n = 4 and 2n = 263 - 265 (de Wet 1987).

Unique four-chromosome species have been detected in the tribe *Poeae* of the *Pooideae* subfamily: *Zingeria biebersteiniana* (Claus) P. A. Smirn. (Tsvelev and Zhukova 1974) and *Colpodium versicolor* (Stev.) Schmalh. (Sokolovskaya and Probatova 1977). The areas of both taxa come close in the northern Caucasus region, though species obviously strongly differ in altitudinal distribution: meadows and steppes of lowlands (eastern part of the Lower Don region, right river banks of the Lower Volga) to northern foothills of the Great Caucasus (Kislovodsk) in *Zingeria biebersteiniana* versus alpine meadows and rocky slopes of the Caucasus region (Daghestan, Great Caucasus, Eastern and Southern Transcaucasus, Talysh), easternmost Anatolia, northern Iraq, and northwestern Iran, mostly above 3000 m, in *Colpodium versicolor* (Bor 1970; Tzvelev 1976; Davis 1985).

As currently known, the species mentioned beside *Brachycome dichromosomatica* C. R. Carter and *Haplopappus gracilis* (Nutt.) A. Gray of the family Asteraceae are the only plants with number of chromosomes 2n = 4 among 250 000 species of higher plants in the world (Takhtajan 1997). The only perennial species of these four is *C. versicolor.* This might suggest its more ancient origin because is widely believed that the angiosperm evolution mode was from perennials to annual forms and ephemera (Popov 1927; Takhtajan 1997). Based on genomic-mapping studies that used a synteny and circularisation approach of grass genomes (Moore *et al.* 1995) and karyotype analysis (Fuchs *et al.* 1995) a chromosome

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number reduction can be achieved via chromosomal rearrangements such as chromosome fusion and loss of active centromeres.

The goal of our study was to investigate the karyotype of *C. versicolor* and to test whether *C. versicolor* represents a putative donor of one of the two parental genomes of the allotetraploid grass *Zingeria trichopoda* (Boiss.) P. A. Smirn. (2n = 8). In our previous article it was shown that *Z. trichopoda* is of hybrid origin with one of the parental ancestors being close to the contemporary ephemerous grass *Z. biebersteiniana* (2n = 4). On the ground of taxonomical and cytological data we suggested previously that the second parent species forming *Z. trichopoda* was close to perennial alpine species *C. versicolor* (KOTSERUBA *et al.* 2003).

MATERIALS AND METHODS

Plant material and determination of genome size - The accessions of *Colpodium versicolor* were collected in Georgia, Great Caucasus (15 km southeast of Kazbegi, Khrebet Chaukhi, ca. 3 km southeast of Juta, alpine meadow, ca. 2950 m, leg. K. Pistrick et M. Akhalkatsi, 21.7. 2002) and in Armenia, Lesser Caucasus (Aragats mountain, near lake Qari, ca. 3250 m, leg. V. Kotseruba et I. Gabrielyan, 20. 8. 2003). Plant vouchers are deposited in the Herbarium Gatersleben (GAT) of the IPK.

The genomic DNA content of *C. versicolor* was determined by flow cytometry as described by BAROW and MEISTER (2002) with propidium iodide as base-independent DNA-specific fluorescent dye and *Pisum sativum* L. as reference plant.

Fluorescence in situ hybridization (FISH) and chromosome image analysis - Genomic DNA of *Z. biebersteiniana* and *C. versicolor* were used as genomic *in situ* hybridisation probes, the *Arabidopsis thaliana* (L.) Heynh. derived clones pCT4.2 (CAMPELL *et. al.* 1992) and a BAC clone (EMBL accession number AF167571) were used as 5S rDNA and 45S rDNA-specific *in situ* hybridization probes, respectively. Preparation of mitotic chromosomes was performed as described by SAUNDERS and HOUBEN (2001). Labelling of probes and FISH were carried out as described by HOUBEN *et al.* (2001) and KOTSERUBA *et al.* (2003). Epifluorescence signals were recorded electronically with a cooled CCD-camera. The image manipulations were performed with the program Adobe Photoshop. The morphology of chromosomes was analyzed by using the image analysis system "VideoTest-Karyo" (PUNINA *et al.* 1999).

Characterization of ITS1-5.8S-ITS2 sequences - Preparation of genomic DNA and PCR to amplify the ITS1-5.8S-ITS2 region was performed as described by KOTSERUBA *et al.* (2003). PCR products were ligated into the vector pGEM-T Easy (Promega) and sequenced. Sequences obtained in this study (*C. versicolor* from Georgia, sequence accession number AJ867445; and from Armenia, accession number AJ867446) were analysed for similarity to known sequences using the BLAST package on the National Centre for Biotechnology Information server (http://www.ncbi.nim.nih.gov/BLAST) and compared with each other using analysis tools (sequence alignment method ClustalV) of the program DNA STAR.

RESULTS AND DISCUSSION

To investigate the positions of ribosomal genes in *C. versicolor*, *in situ* hybridization experiments with differently labelled probes of 5S and 45S rDNA were performed on somatic chromosomes. FISH revealed one chromosomal site each for 45S rDNA and 5S rDNA on chromosome types 1 and 2, respectively (Fig. 1a, b). The data on chromosome size measurements and the physical positions of rDNA loci are summarized in an idiogram (Fig. 1d). No karyotype differences were noticed between *C. versicolor* plants been collected in Georgia or Armenia. Notably, the chromosome morphology and chromosomal position of 5S and 45S rDNA in *C. versicolor* are similar to the chromosomes of the second subgenome of *Z. trichopoda* (which we will call hereby C-subgenome). The genomic DNA content of *C. versicolor* determined by flow-cytometry was 2C=2.4 pg (2352 Mbp).

The ITS1-5.8S-ITS2 -region, which is part of the 18S rDNA was determined for *C. versicolor*. Genes encoding the processed rRNA species are evolutionarily among the most conserved sequences, whereas the internal transcribed spacers (ITS1 and ITS2) evolve rapidly and show a high degree of sequence variation and therefore are suitable for comparative studies (BALDWIN 1992). Sequences obtained from three clones of *C. versicolor* coming from Armenia and Georgia were aligned and consensus sequences were deduced. Sequence heterogeneity among the individual clones within the species was low, resulting from

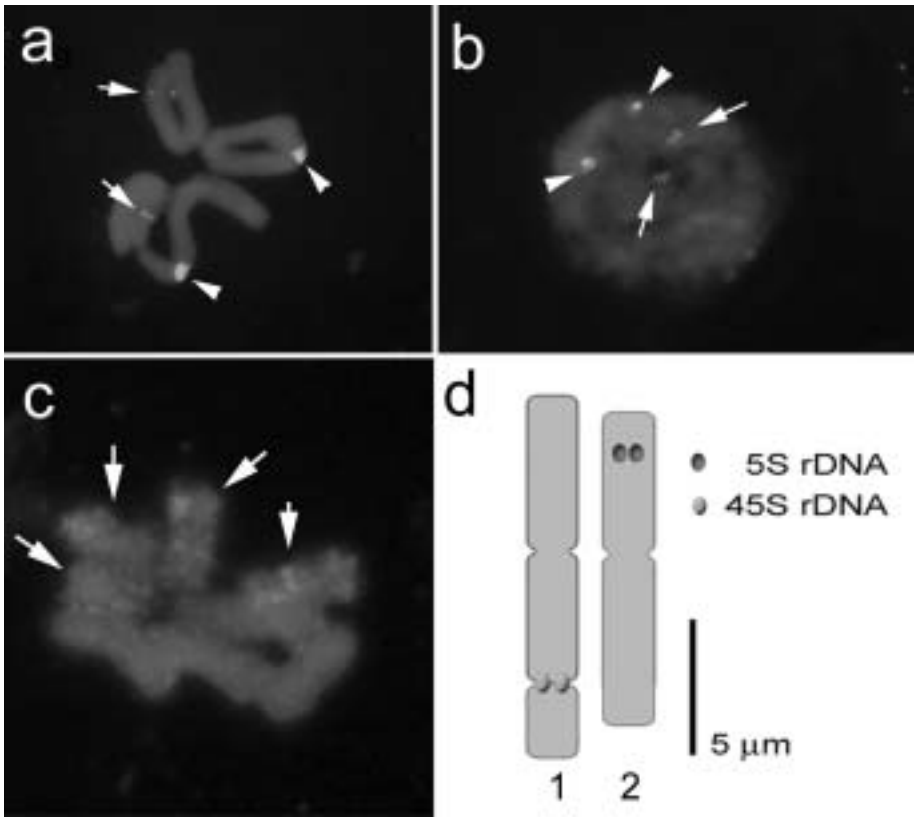

Fig. 1 — Somatic chromosomes of the grasses *Colpodium versicolor* and *Zingeria trichopoda* after DAPI-staining and fluorescence *in situ* hybridization. (a) Prometaphase and (b) interphase nuclei of *C. versicolor* after FISH with labelled 5S rDNA (red signals, arrows) and 45S rDNA (green signals, arrowheads), (c) Z. *trichopoda* chromosomes after genomic *in situ* hybridisation with labelled DNA of *C. versicolor.* Note the pronounced hybridisation of the C-subgenome-specific chromosomes (arrowed). (d) Idiogram of the haploid chromosome set of *C. versicolor.*

either real variation or PCR and/or sequencing errors. However, there were minor invariable differences in the ITS1-5.8S-ITS2 region between the Armenia- and Georgia-derived sequences. Within 665 base pair positions 2 base substitutions were detected. Database searches for similarity to known sequences with both ITS2-consensus sequences revealed 97% and 96% identity to the ITS2 regions from *Z. trichopoda* (accession number AJ428835) and *Z. biebersteiniana* (accession number AJ428836), respectively. Notably the ITS1-5.8S-ITS2-sequence of *C. versicolor* shows more similarity to *Z. trichopoda*, than the ITS1-5.8S-ITS2-sequence of *Z. biebersteiniana* to the same sequence of *Z. trichopoda* (Fig. 2). However, we expect other grass species with even higher sequence similarity, when ITS1-5.8S-ITS2 sequence informations are becoming available from other *Poeae*.

In order to extend our analysis whether *C. versicolor* took part in formation of *Z. trichopoda* genomic *in situ* hybridization (GISH) was carried out on mitotic chromosomes of *Z. trichopoda*. After GISH using labeled genomic DNA of *C. versicolor* together with 1- to 60-fold excess of unlabeled DNA of *Z. trichopoda* and/or *Z. bieber-*

Table 1 — Morphometric data of mitotic prometaphase chromosomes of *Colpodium versicolor*

Chromosome Nr.	Length of arms (µm)			Centromeric index		Morphological type of chromosome and rDNA loci
	short, p	long, q	Total (L)	p/L, %	AR=q/p	
I	5.8	7.9	13.7	42.3	1.36	Metacentric+SC (45s rDNA)
II	5.0	5.8	10.8	46.3	1.16	Metacentric +5s rDNA

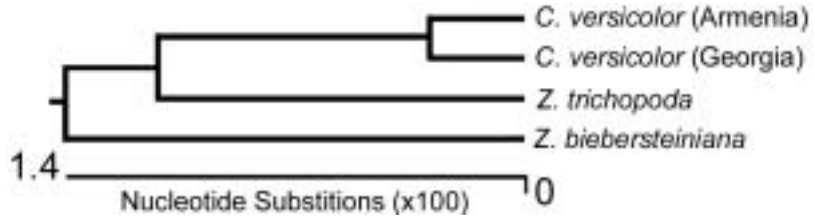

Fig. 2 — Unrooted phylogenetic tree to view evolutionary relationships predicted from the multiple sequence alignment of the ITS1-5.8S-ITS2 region of *C. versicolor*, *Z. biebersteiniana* and *Z. trichopoda*. The length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events.

steiniana a disperse labeling of all chromosomes was revealed. However, stronger hybridization was revealed in heterochromatic regions of the C-subgenome (Fig. 1c, arrowed). Therefore it is unlikely that the contemporary species *C. versicolor* represents the second subgenome donor of the allopolyploid grass *Z. trichopoda.* Alternatively, but less likely, after allopolyploidization *C. versicolor*-type repetitive sequences spread throughout the polyploid genome to a greater extent than ancestral genome sequences of *Z. biebersteiniana*. Such colonization and subsequencent homogenization process of genome-specific repetitive sequences has been reported for the allotetraploid wheat *Triticum dicoccoides* (Körn. ex Asch. et Graebn.) Schweinf, and might reflect a general tendency in speciation and stabilization of allopolyploid genomes (BELYAYEV *et al*. 2000).

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