



## Chromosomes of *Bromus auleticus* Trin. ex Nees (Poaceae)

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### Abstract

The chromosome number of 14 accessions of *Bromus auleticus* Trin. ex Nees, native of Rio Grande do Sul, was  $2n = 6x = 42$ , same ploidy level found in other South-American *Bromus* species. Its chromosomes were metacentric or submetacentric, ranging from ca. 4  $\mu\text{m}$  to ca. 8  $\mu\text{m}$  in length. Up to two satellite-bearing chromosome pairs were sometimes observed. However, as already reported for other species, the high symmetry and homogeneity of the karyotypes made it difficult to detect possible intraspecific differences.

*Key words:* *Bromus auleticus*, chromosome numbers, native forages.

Received: February 26, 2003; Accepted: May 29, 2003.

### Introduction

The genus *Bromus* comprises more than 100 annual and perennial species (Mabberley, 1997) distributed around the world, typically cool-season grasses, varying greatly in adaptation and use, and including some important forage and range species, such as the perennials *B. inermis*, *B. anomalus*, *B. pumpeianus*, *B. catharticus*, and the annuals *B. mollis* and *B. rigidus*, among others (Carlson and Newell, 1985).

Chromosome numbers vary from  $2n = 14$  to  $2n = 84$ , most of the species being diploid ( $2n = 14$ ) or tetraploid ( $2n = 28$ ) (Federov, 1969). Chromosome size may be very different among species (Armstrong, 1983; Schifino and Winge, 1983). Ploidy levels may vary within the same species, as in *B. diandrus* and *B. sterilis* (Oja and Laarmann, 2002). The chromosome complements consist mainly of chromosomes resembling each other in size and centromere position (Joachimik *et al.*, 2001). C-banding techniques (Joachimik *et al.*, 2001; Tuna, 2001) have shown that almost all the species analyzed present similar amounts of heterochromatin, mainly telomeric, albeit some small amounts of intercalary heterochromatin were detected. Therefore, distinction between different karyotypes is generally made based on the size of satellites (Armstrong, 1983; Joachimik *et al.*, 2001).

In Rio Grande do Sul, Southern Brazil, the genus is represented by the native *B. auleticus*, *B. brachyanthera*, *B. catharticus*, and the exotic *B. commutatus* and *B. mollis* species (Longhi, 1977). Among these, the perennial allogamous *B. auleticus*, popularly known as “cevadilha” or “cevadilha vacariana” (Araújo, 1971; Mohrdieck, 1973), occupies an outstanding position as a forage in the native pastures of that State. This species has called the attention of plant breeders and agronomists (Mohrdieck, 1993; Soares, 1999, among others), and some populations have already been characterized by isozyme and RAPD analysis (Yanaka, 2002). Cytological data on this species, however, is scarce. Only isolated chromosome counts in a few plants ( $2n = 42$ ) have been performed (Elliott, 1948 and 1949, in Federov, 1969; Bowden and Senn, 1962) and, to our knowledge, no other cytogenetic information on the species has been published.

This paper reports data on chromosome numbers and morphology of 14 natural populations of *B. auleticus* from the State of Rio Grande do Sul.

### Material and Methods

Seeds were collected at several locations of Rio Grande do Sul, Southern Brazil (ca. 33° to 28° S) (Table 1). Each sample from each collection site was considered as an accession. Seeds from 11 of these accessions were kindly provided by the germplasm bank of EMBRAPA-CPPSul (Empresa Brasileira de Pesquisa Agropecuária - Centro de Pesquisa de Pecuária dos Campos Sul-Brasileiros).

**Table 1** - List of the accessions of *Bromus auleticus* examined, number of individuals and cells analyzed per accession, and somatic chromosome numbers (2n).

Accession	Collection site	Number of individuals	Number of cells	2n
OvGoMgGu 64 <sup>a</sup>	Livramento, RS	5	16	42
OvGoMgGu 66 <sup>a</sup>	Livramento, RS	8	17	42
OvGoMgGu 69 <sup>a</sup>	Livramento, RS	7	19	42
OvGoMgGu 80 <sup>a</sup>	Uruguaiiana, RS	8	12	42
OvGoMgGu 97 <sup>a</sup>	Itaara, RS	5	12	42
GoRg 121 <sup>a</sup>	Júlio de Castilhos, RS	6	21	42
GoRg 126 <sup>a</sup>	Vacaria, RS	3	3	42
GoOv 59 <sup>a</sup>	Pinheiro Machado, RS	5	16	42
GoOv 62 <sup>a</sup>	Dom Pedrito, RS	8	22	42
GoLe 110 <sup>a</sup>	Livramento, RS	10	29	42
OvGoGu 107 <sup>a</sup>	Cruz Alta, RS	9	20	42
Santiago	Santiago, RS	4	10	42
Santana Livramento	Santana do Livramento, RS	7	10	42
Quaraí	Quaraí, RS	5	13	42

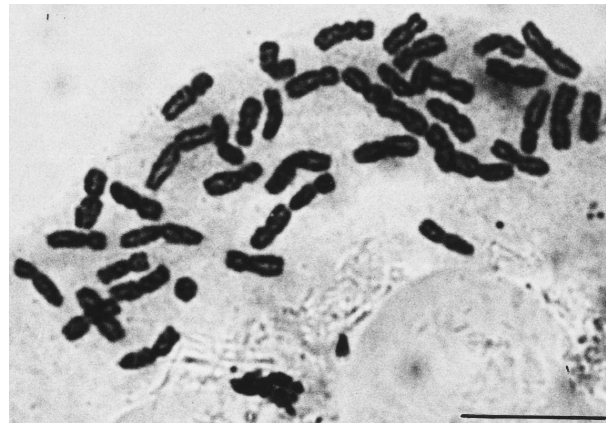
<sup>a</sup>accession from the germplasm bank of EMBRAPA-CPP Sul.

For somatic chromosome counts, seeds were germinated in Petri dishes with moistened filter paper, and the 1-1.5 mm long roots were pre-treated with a saturated solution of paradichlorobenzene (PDB) for 18-20 h at 4 °C, fixed in 3:1 ethanol-acetic acid for 24 h, and stored in 70% ethanol below 0 °C until required. For slide preparation, root tips were hydrolyzed with HCl 1 N for 10 to 15 min at 60 °C, stained with Feulgen for *ca.* 2 h, and squashed in a drop of acetic carmine. Semi-permanent slides were examined by light microscopy. Only intact and well-spread metaphases were analyzed. Each root tip was considered as one individual.

## Results and Discussion

All the 220 cells of the 90 analyzed individuals of the 14 accessions presented 2n = 42 chromosomes (Table 1, Figure 1). The ploidy level of *B. auleticus* (hexaploid) is the same as in other native South-American species, such as *B. bonariensis*, *B. brevis*, *B. parodii*, *B. brachyanthera* var. *uruguayensis*, *B. catharticus* (Schifino and Winge, 1983; Naranjo, 1985).

Chromosome size ranged from *ca.* 4µm to *ca.* 8µm, and they were all either metacentric or submetacentric. Detailed karyotypic analyses were not performed, but chromosome morphology was very similar to that reported for several Eurasian and North-American *Bromus* species (Schulz-Schaeffer and Markarian, 1957; Armstrong, 1983; Joachimiak *et al.*, 2001; Tuna *et al.*, 2001), as well as for the South-American species *B. catharticus* (Schifino and Winge, 1983; Naranjo, 1985), *B. brevis*, *B. parodii*, and *B. bonariensis* (Naranjo, 1985).



**Figure 1** - Metaphase plate of *Bromus auleticus* (2n = 42) (one chromosome on the lower left side of the cell is folded). Scale bar equal to 10 µm.

Up to two satellite-bearing chromosome pairs could be seen in a few cells of some accessions, but in most cells the satellites were very difficult to distinguish. Armstrong (1982) already pointed out the difficulty in detecting satellites when they are small or when the chromosomes are very condensed. Naranjo (1985), reviewing data from the literature, commented that there might be actual differences in the number of satellite-bearing chromosomes (or in the number of these chromosomes expressed) among different *Bromus* individuals or species, but that the high symmetry and homogeneity of the karyotypes could also make it difficult to detect possible differences.

A pilot FISH (fluorescent in situ hybridization) test performed in one *B. auleticus* accession (data not shown) suggested the presence of up to fourteen 45 S rDNA sites.

*Bromus* chromosomes are a good material for cytogenetic analysis, regarding for example their size, however the little success obtained so far in detecting variability with conventional staining and C-banding techniques suggests that more advanced techniques should be employed.

### Acknowledgments

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the post-doctoral fellowship granted to the G.E.M., and Dr. Klecius Ellera Gomes and MSc José Carlos de Oliveira from EMBRAPA-CPPSul for supplying the seeds.

### References

- Araújo AA (1971) Principais gramíneas do Rio Grande do Sul. Sulina, Porto Alegre, 255 pp.
- Armstrong KC (1982) Hybrids between the tetraploids of *Bromus inermis* and *B. pumellianus*. Can J Bot 60:476-482.
- Armstrong KC (1983) The relationship between some Eurasian and American species of *Bromus* section Pnigma as determined by the karyotypes of some F1 hybrids. Can J Bot 61:700-707.
- Bowden WM and Senn HA (1962) Chromosome numbers in 28 grass genera from South America. Can J Bot 40:1115-1124.
- Carlson IT and Newell LC (1985) Smooth Bromegrass. In: Heath ME, Barnes RF and Metcalfe DS (eds) Forages. 4th ed. Iowa State University Press, Ames, pp 198-206.
- Elliott, FC (1948) Cross-fertility and cytogenetics of selected Bromopsis section Members within the genus *Bromus* L. Iowa State Coll J Sci 24:44-45 in: Federov AA (1969) Chromosome Numbers of Flowering Plants. Russian Academy of Sciences, Leningrad, 926 pp.
- Elliott, FC (1949) The cytology and fertility relations of *Bromus inermis* and some of its relatives. Agron Jour 41:298-303 in: Federov AA (1969) Chromosome Numbers of Flowering Plants. Russian Academy of Sciences, Leningrad, 926 pp.
- Federov AA (1969) Chromosome Numbers of Flowering Plants. Russian Academy of Sciences, Leningrad, 926 pp.
- Joachimiak A, Kula A, Sliwiska E and Sobieszczanska A (2001) C-banding and nuclear DNA amount in six *Bromus* species. Acta Biol Cracov Ser Bot 43:105-115.
- Longhi, HM (1977) O gênero *Bromus* L. (Gramineae) no Rio Grande do Sul. In: Trabalhos do XXVI Congresso Nacional de Botânica, Academia Brasileira de Ciências, Rio de Janeiro, pp 333-342.
- Mabberley DJ (1997) The Plant-Book. Cambridge University Press, Cambridge, 858 pp.
- Mohrdieck KH (1993) Formações campestres do Rio Grande do Sul. In: Federacite (ed), Campo nativo - melhoramento e manejo, Caramurú, Porto Alegre, pp 11-23.
- Naranjo CA (1985) Estudios citogeneticos, bioquimicos y sistematicos en algunas especies americanas del genero *Bromus* (Gramineae). PhD Thesis, Universidade de Buenos Aires, Buenos Aires.
- Oja T and Laarmann H (2002) Comparative study of the ploidy series *Bromus sterilis*, *B. diandrus* and *B. rigidus* (Poaceae) based on chromosome numbers, morphology and isozymes. Plant Biol 4:484-491.
- Schifino MT and Winge H (1983) Karyotypes and nuclear DNA content of species of the *Briza* complex and some other genera of Poaeae (Gramineae). Rev Bras Genet 6:245-259.
- Schulz-Schaeffer J and Markarian D (1957) Cytologische Untersuchungen in der Gattung *Bromus* L. II. Untersuchungen an Wurzelspitzen zur Klärung der Chromosomenmorphologie bei den Untergattungen Ceratochloa und Bromus. Zeitsch Pflanzenz 37:299-316.
- Soares GS (1999) Caracterização agrônômica e melhoramento genético de uma população de cevadilha vacariana (*Bromus auleticus* Trinius ex Nees). MSc Dissertation, Universidade Federal do Rio Grande do Sul, Porto Alegre.
- Tuna M, Gill KS and Vogel KP (2001) Karyotype and C-banding patterns of mitotic chromosomes in diploid bromegrass (*Bromus riparius* Rehm). Crop Sci 41:831-834.
- Yanaka FY (2002) Caracterização molecular e isoenzimática de acessos de *Bromus auleticus* Trinius ex Nees. MSc Dissertation, Universidade Federal do Rio Grande do Sul, Porto Alegre.

Editor: Yatiyo Yonenaga-Yassuda