

Variability of the chromosomal number and meiotic behavior in populations of *Bidens pilosa* L. (Asteraceae) from southern Brazil

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Abstract — *Bidens pilosa* is a medicinal species from the Asteraceae family, with a wide geographical distribution, found all over Brazil. There are no reports on chromosomal studies from the Brazilian South. Mitotic chromosomes, meiotic behavior, and pollen viability were studied in 6 populations of *B. pilosa* from Rio Grande do Sul State, Brazil, using conventional staining with acetic orcein 2%. The populations include cytotypes with a variable number of chromosomes: $2n=36$, $2n=48$ and $2n=54$, indicating polyploidy and aneuploidy. The meiotic process was considered regular, even though irregular chromosomal pairing appeared, which is normal in cytotypes from a polyploid complex. The irregularities observed were mainly on univalent, trivalent, and tetravalent pairing associations. However, the pollen viability estimative was high.

Key words: *Bidens pilosa*, chromosome number, meiotic behavior.

INTRODUCTION

Bidens pilosa (Asteraceae family) is considered a weed on farms, but has an important phytotherapeutic value, used in alternative medicine as an anti-inflammatory, anti-leucocratic, as well as treating jaundice (Vasques *et al.* 1986). It's an annual plant, erect, with entire or lobed glabrous leaves, achene type fruit, with two or three apical aristae (Lorenzi 2000). The flowers (florets) are found in capitula surrounded by involucre bracts (Barroso 1991). The species presents an ample geographical distribution being found throughout practically all the Brazilian terrain (Kissmann and Groth 1992).

According to literature, the chromosomal evolution of the genus *Bidens* happens through mechanisms of polyploidy (Mariano 1999), and presents a basic number $x=12$ chromosomes (Barroso 1991). Grombone-Guaratini *et al.* (2006) found $2n=72$ chromosomes for *Bidens pilosa* in Brazilian populations from São Paulo State, and estimate that the variability found by Mariano and Marin-Morales (1999) can be explained by

misinterpretations in the identification of different species from the *Bidens* complex.

For the species *Bidens pilosa*, the chromosomal numbers $n=24$ (Robinson *et al.* 1981), $n=36$ (Aparício and Silvestre 1985; Banerjee 1971; Gupta and Gill 1983; Hill 1984; Pilz 1980; Ballard 1986; Gupta and Gill 1989; Keil *et al.* 1988), $n=48$ (Gill and Omigui 1989), $2n=24$ (Husaini and Iwo 1990), $n=36$ and $2n=72$ chromosomes (Nirmala and Rao 1981), $2n=48$ (Nirmala and Rao 1984 and 1986), $2n=72$ (Baltisberger 1990; Banerjee 1971; Gaddella 1982; Podlech 1986; Zhao *et al.* 1990; Paiva and Leitão 1989; Renard *et al.* 1983) have been described. Mariano (1999) studied this species in Brazilian populations from the southeastern region and from the state of Bahia, finding variations, of $2n=48, 68, 70$, and 72 chromosomes, whose cytotypes presented symmetrical karyotypes with a tendency to asymmetry. Nevertheless, in bibliography, cytogenetic data for this species from the Brazilian South were not found.

Due to the large chromosomal variation existent in the species, this paper aims to determine the number of chromosomes, meiotic behavior, and pollen viability of natural populations from the state of Rio Grande do Sul (RS). This study intends to provide data to understand the intraspecific variability within this species through cytogenetic characterization.

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MATERIALS AND METHODS

Botanic Material Sampling - Seeds and inflorescences were sampled from six natural populations around Rio Grande do Sul State, Brazil. From each population, an exsiccata was deposited in the Herbarium SMDB (Santa Maria Departamento de Biologia) at the Universidade Federal de Santa Maria (UFSM), RS, as shown in Table 1. The plants were taxonomically identified by Prof. Dr. Thais do Canto-Dorow.

Chromosomal Number - For germination, the seeds were placed in Petri dishes with filter paper damped with distilled water. These were incubated at a temperature around 28-31°C, 12 hours light and 12 hours dark, in the Plant Cytogenetics Laboratory at UFSM. When the rootlets were 1cm, they were collected and pre-treated with paradichlorobenzene for 10 hours at room temperature. Afterwards, the rootlets were fixed with Carnoy 3:1 (ethanol/ acetic acid) for 24 hours at room temperature and stocked in ethanol 70% under refrigeration. For slide preparation, the rootlets were washed with distilled water, hydrolyzed in HCl 1N for 4 min, and then washed again. The slide was prepared by taking away the merismatic region, which was squashed with acetic orcein 2%, adapted from Guerra and Souza (2002). The cells which presented good chromosomal distribution were photographed, and chromosomes counted.

Meiotic analysis and pollen viability - For meiosis analysis, the inflorescences collected in the field were immediately fixed in Carnoy 3:1 for 24 hours at room temperature, being transferred to

ethanol 70% and stored under refrigeration. The slides for analysis were prepared using the technique of squashing the anthers and colored with acetic orcein 2% (Guerra and Souza 2002). All the phases of meiosis were analyzed, but special emphasis was given to those chromosomal associations in diakinesis and/or metaphase I, and chromosomal segregation in anaphases and telophases I and II. The cells that did not present meiotic configurations with bivalent chromosomes were considered irregular, and other irregularities were also considered like tardiness and bridges during anaphase and irregular chromosomal disjunction in the subsequent phases of metaphases I and II. The meiotic index was calculated according to Love (1949), considering normal those tetrads with four equal cells and abnormal those with other formations, like triads and dyads. Around 250-400 tetrads were examined per population.

To estimate the viability of pollen grains, the slides were prepared for meiotic analysis, counting 400 pollen grains per population. The pollen grains considered viable were those colored, and unviable, those not-colored (Fig. 1).

RESULTS

The results obtained by microsporogenesis analysis and chromosome counting are presented in Table 1.

Observing the somatic cells from root point, *Bidens pilosa* populations presented variations

Table 1 — Populations, registry number, microsporogenesis analysis, meiotic index, pollen fertility, and number of chromosomes in *Bidens pilosa*.

Population of <i>Bidens pilosa</i>	Registry number	Diakinesis / Metaphase I	Anaphase I/ Telophase I	Metaphase II	Anaphase II/ Telophase II	Meiotic index (%)	Pollen viability (%)	Number of chromosomes
Camobi - Santa Maria	10054	52 ^a	53	-	108 ^b	88.05	95.25	2n= 54
Espumoso	10082	3	114 ^c	6	191 ^d	86.26	96	2n= 36
Ibirubá	10085	-	-	-	-	-	-	2n= 36
Botanical Gardens - UFSM	10079	74 ^e	78 ^f	-	135 ^{gh}	88.17	94	-
Silveira Martins	10053	69	94	20	152 ^h	88.95	98.5	2n= 36
UFSM	10080	59 ⁱ	86	20	71 ^j	88.73	94	2n= 48

a- bridge phase II; b- 2 cells with triad in telophase II; c- 9 irregular diakinesis; d- 9 cells with triad in telophase II.

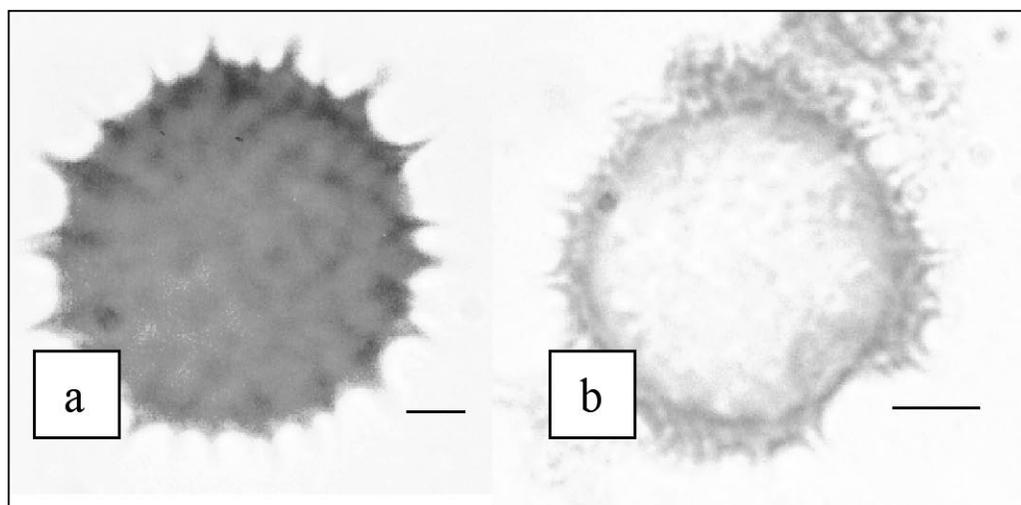


Fig. 1 — Flow cytometric analyses of *A. halimus* L.: Sidi Bouzid population. Leaf samples were chopped and then stained with propidium iodide using tomato (2c nuclear DNA content 1.96 pg) as internal standard.

in the number of chromosomes (Table 1, Fig. 2a,b,c), where $2n=36$, 48, and 54 chromosomes was found.

Through microsporogenesis analysis, the populations present regular meiotic behavior during meiosis I and II (Table I, Fig. 2e, g), with some irregularities, like the presence of trivalents and univalents in diakinesis (Fig. 2d), univalent chromosomes in metaphase I, anaphasic bridges (Fig. 2f), and triads in telophase II.

The meiotic index was high, with a small variation within the populations from 86.26 to 88.95% (Table 1), where normal tetrads were found with four cells (Fig. 2g), and abnormal with three (Fig. 2h) and two cells.

In all populations, high pollen viability was observed (Table 1), from 94 to 98.5%, even though unviable pollen grains were found (Fig. 1b) in all populations.

DISCUSSION

The number of chromosomes in *Bidens pilosa* varies greatly, which was also observed in this study. Barroso (1991), while describing this species, cited as a basic number, $x=12$. Mariano (1999), studying Brazilian populations found $2n=48$ and $2n=72$ chromosomes, indicating that polyploidy is an important evolutionary process for the genus *Bidens*. Besides these, Mariano (1999) also found $2n=68$ and $2n=70$ chromosomes, considered diploid cytotypes, suggesting a secondary evolution by aneuploidy and also found $x=12$, as the basic number.

For populations from southern Brazil that were analyzed in this study, a chromosomal variation of $2n=36$ and $2n=54$ chromosomes (Table 1) were found. Bibliographical data also bring numeric variation for this species from $2n=24$ (Gill and Omoigui 1989; Husaini and Iwo 1990) to $2n=96$ (Gill and Omoigui 1989), where $2n=72$ chromosomes is the most found cytotype (Mariano 1999). Variations among species and populations with the same number of chromosomes are considered important for the generation of new forms of recombination, which influence the variability of natural populations in the adaptive process (Rees and Dale 1974).

The studies by Grombone-Guaratini *et al.* (2006) showed that *Bidens pilosa* has $2n=72$ chromosomes, and believe that there variation found by Mariano and Marin-Morales (1999) does not exist, based on a possible confusion in identifying the species. We emphasize that there is no doubt in the identification of the species in our study (Table 1) and that the large variation in the number of chromosomes was found, obtained through mitotic metaphases of the analysis of the meiotic behavior in *Bidens pilosa* (Table 1). Therefore, it is very clear that there is an existence of different cytotypes in Brazilian populations, especially from the South of Brazil.

The genus *Mikania* (Asteraceae), also presents variations in its chromosomal number, with $2n=36$ and 42 for diploid cytotypes, and $2n=72$ chromosomes for the tetraploids in the species *M. micrantha* Kunth. (Maffei *et al.* 1999).

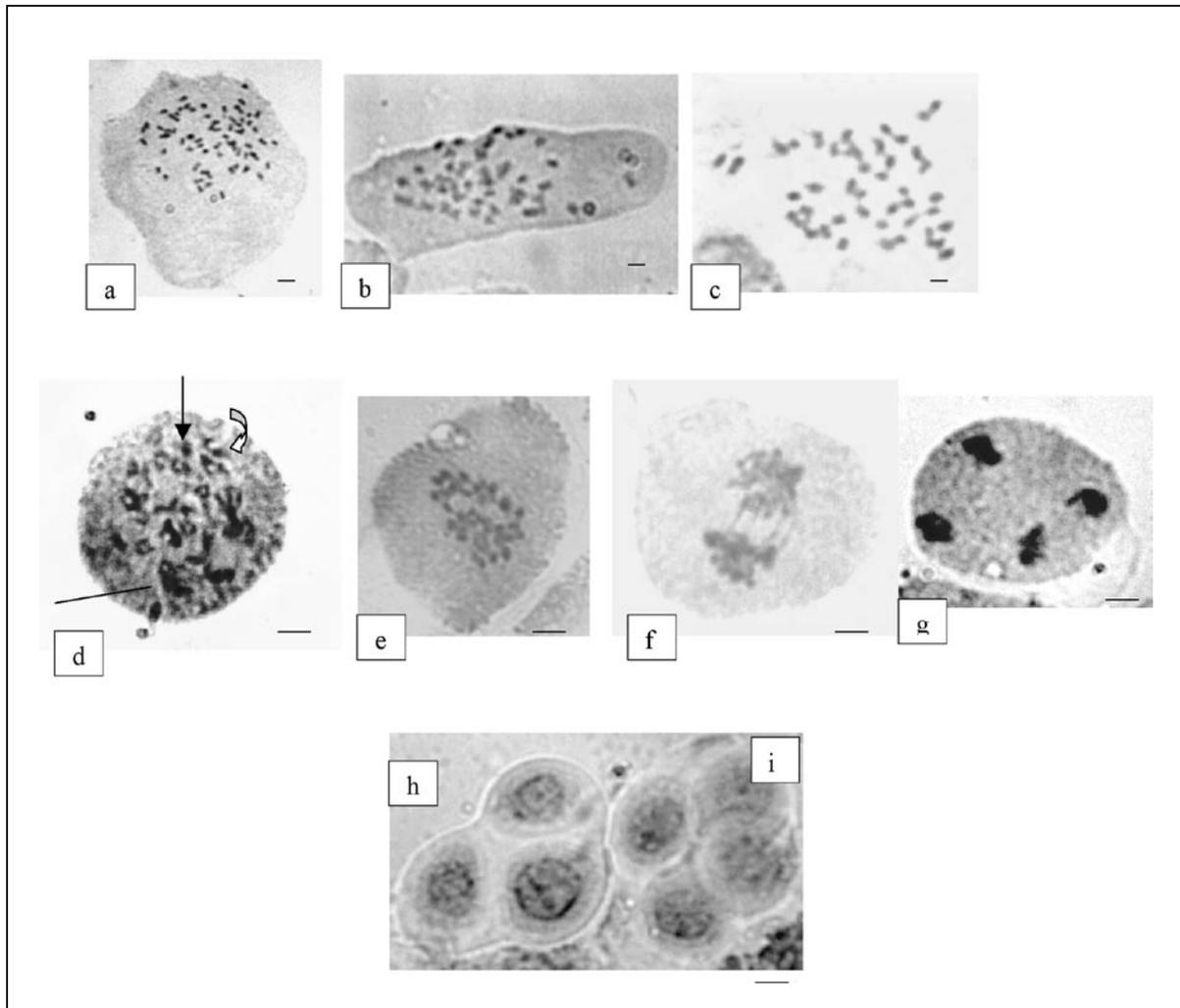


Fig. 2 — Mitotic and Meiotic chromosomes from natural populations of *Bidens pilosa* from RS, Brazil. a) Population UFSM, Santa Maria, $2n=48$ chromosomes. Scale = $4.5\mu\text{m}$; b) Population Espumoso, $2n=36$ chromosomes. Scale = $3\mu\text{m}$; c) Population Ibirubá, $2n=36$ chromosomes. Scale = $2.5\mu\text{m}$; d) Population JB UFSM, diakinesis (16 II + 02 IV + 04 I); e) Population Camobi, metaphase regular (24 II); f) Population Espumoso, anaphasic bridge; g) Population Silveira Martins, Telophase II regular; h) Population Silveira Martins, Triad (irregularity); and i) Tetrad. Bar = $4\mu\text{m}$ (Arrow indicating univalent chromosome, line indicating tetravalent chromosome, thick arrow indicating bivalent).

OLIVEIRA *et al.* (2004) underwent a study with *Stevia rebaudiana* (Bertoni) Bertoni (Asteraceae), finding $2n=22$ chromosomes for diploid accessions, $2n=33$ for triploids, and $2n=44$ for tetraploids. In diakinesis, associations of 11_{II} , 11_{III} , and 11_{IV} were found for the diploid, triploid, and tetraploid accessions, respectively, suggesting autopolyploidy origin. The variance analyses revealed a positive correlation between the ploidy level and morphological characters for *S. rebaudiana*. A better adaptation of individuals and an increase of organ and cell size are generally associated to polyploidy (Guerra 1988).

In our study, regular bivalent chromosomal associations and irregular univalent, trivalent, and multivalent, were found in diakinesis and/or metaphase I (Fig. 2d). Irregularities were also found, like anaphasic bridges (Fig. 2f), formation of triads in telophase II, as well as irregular tetrads and dyads. Even with the irregularities, the meiosis was regular, having a high meiotic index and high pollen viability (Table 1).

In other Asteraceae, like species from the genus *Senecio*, LOPES *et al.* (2002) observed the formation of polyvalents with a low pollen viability (40.6%). For the genus *Cousinia*, Sheidai *et al.*

(2006) suggest that the studied species present two basic numbers ($x=12$ and 13), given these indicate aneuploidy probably due to chromosomal reduction during evolution of the genus *Cousinia* (Susana *et al.* 2003). In *Achyrocline alata* (Kunth.) DC, a haploid chromosomal complement $n=14$ was reported, with a basic number of $x=7$ (Giangualiani 1976; Amat 1998), differing from that found for *Achyrocline satureioides* (Lam.) DC, which was $2n=24$ chromosomes (Pereira *et al.* 2006).

In another microsporogenesis study, an autohexaploid of *Eupatorium laevigatum* Lam. (Asteraceae) revealed abnormalities in chromosomal pairing, which resulted in forming univalents, bivalents, trivalents, and polyvalents, with the consequent formation of chromosome retarding, unbalanced nuclei, micronuclei, and sterile pollen (Bertasso-Borges and Coleman 2005).

Cytogenetics in Brazil is more focused on cultivated plants with some kind of economic interest. There is, however, an increasing interest for other species, both of medicinal and ornamental value (Guerra 1990; Lovatto and Battistin 1997; Pedrosa *et al.* 1999; Pagliarini 2000). In this sense, the study by Lunardi *et al.* (2004) stands out, with the medicinal species *Maytenus ilicifolia* Mart. ex Reissek (Celastraceae), which presents a large intraspecific variation for its number of chromosomes ($n=32$, 35 , and 40), regular meiosis, high mitotic indexes, and high pollen viability.

The intraspecific variability is highly evident in this polyploidy complex of *Bidens pilosa*, and considering the basic number as $x=12$, described by Barroso (1991), found also by Mariano (1999), it can be considered that the studied populations are triploid ($2n=36=3x$) and tetraploid ($2n=48=4x$), even though one is aneuploid ($2n=54=4x+6$).

Our results demonstrate that the populations from southern Brazil present variations in their number of chromosomes $2n=36$ to $2n=54$ chromosomes, the meiosis was regular, with a few irregularities during meiosis I and II, high meiotic index, and high pollen viability. From these data, it can be suggested that these studied populations, outside of being polyploid, are also aneuploid. Probably more studies with this species would better clarify the intraspecific variation existent in the *Bidens* complex.

Acknowledgments — We thank Prof. Dr. Thais Scotti do Canto-Dorow for the taxonomic identification of *Bidens pilosa* L. and Prof. Luiz Augusto Salles das Neves for the collaboration in germinating the seeds of this species.

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Received October 15th 2007; accepted December 20th 2007