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Chromosomal studies of three species of *Bidens* (L.) (Asteraceae)

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Abstract — The chromosomal numbers of three species of *Bidens* were determined based on an analysis of somatic metaphases. The species studied were *Bidens pilosa* L., *Bidens subalternans* DC and *Bidens alba* (L.) DC, all of which belong to the *Bidens pilosa* species complex. The three species have a similar morphology: *Bidens pilosa* and *B. subalternans* are widely distributed in agricultural areas, in disturbed habitats and along roadsides, whereas *B. alba* occurs only along the seacoast. *Bidens pilosa* has 2*n*=72 and *B. subalternans* and *B. alba* has 2*n*=48. These numbers agreed with the basic chromosomal number of *n*=12 reported for the tribe Heliantheae and show that the number of metaphase chromosomes can be used to distinguish *Bidens pilosa* from the other two species of the *B. pilosa* complex in southeastern Brazil.

Key words: Asteraceae, *Bidens*, chromosomes, Heliantheae, weed species.

INTRODUCTION

Three species of *Bidens* (*B. pilosa*, *B. subalternans* and *B. alba*) form the *Bidens pilosa* complex in southeastern Brazil. *Bidens pilosa* L. (Asteraceae) is a cosmopolitan, subtropical and tropical weed (Ballard 1986). The habitats occupied by this invasive species range from agricultural areas to disturbed sites and roadsides. According to Sherff (1937), *Bidens pilosa* contains six varieties, three of which occur in Brazil: var. *pilo*sa, var. *minor* and var. *radiata.* Morphological characters are commonly used to assess relationships among species, but the extensive morphological variation within and among *B. pilosa* populations has resulted in imprecise and controversial taxonomic characterization of this species.

Bidens subalternans occurs from Uruguay and central Argentina to northern and western Brazil (Sherff 1937; Cabrera 1974), with three varieties (var. *subalternans*, var. *simulans* and var. *unipinata*) present in Brazil (Sherff 1937). Sherff (1937) reported a South American specimen of *Bidens* that had the general aspect of *B. pilosa,* but with the fruiting heads arranged as in the closely related *B. subalternans*. Based on the ample morphological differences found among *B. pilosa* populations, Leitao Filho *et al.* (1975) considered *B. subalternans* as a synonym of *B. pilosa.*

Recently, Moraes (1997) collected a different *Bidens* species on the Brazilian seacoast that had the general mophological aspect of *B. alba*, a North and Central American species (Ballard 1986). Using morphological characters, Magenta (1998) subsequently showed that most of the voucher specimens identified as *Bidens pilosa* belonged to *B. subalternans* and *B. alba*.

Chromosomal numbers have been used to support the taxonomic identification of species, especially when they are associated with morphological differences (Solbrig 1977). Historically, chromosomal numbers have been used to define generic and infrageneric taxa in Asteraceae (Sunderberg *et al.* 1986; Dematteis 1998; Dematteis and Fernández 2000; Vallès *et al.* 2001). Studies of some genera of Asteraceae have suggested that polyploidy is the most frequent chromosomal change within and among populations and species in South America (Dematteis and Fernándes 2000). Some phenotypic characteristics of polyploids, such as the greater size and vigor of seeds, may enable them to tolerate a wider

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range of environmental conditions and to colonize new habitats (STEBBINS 1971).

BALLARD (1986) used chromosomal counts of North and Central American *Bidens* species to demonstrate that *B. pilosa* is a complex containing three species: *B. alba* (2*n*=48), *B. odorata* (2*n*=24) and *B. pilosa* (2*n*=36). With few exceptions, cytogenetic studies of the genus *Bidens* have been restricted to chromosomal counts (SOLBRIG *et al.* 1972; POWELL *et al.* 1974; TURNER *et al.* 1979; SUNDERBERG *et al.* 1986).

In this paper, we report the chromosomal numbers and other taxonomic information for ten populations of *Bidens* as part of an investigation aimed at increasing our understanding of this complex in Brazil.

MATERIALS AND METHODS

Three *Bidens* species collected in the state of São Paulo were analyzed: *B. alba* from Cubatão (23° 50' S, 47° 23' W) (UEC 129.381) and Guarujá (23° 59' S, 46° 15' W) (UEC 128.916), *B. pilosa* from Campinas (22° 58' S, 47° 04' W) (UEC 128.914), Itirapina (22° 14' S, 47° 49' W) (UEC 128.913), Ribeirão Preto (21° 12' S, 47° 46' W) (UEC 128.910) and Santa Bárbara do Oeste (22° 45' S, 47° 23' W) (UEC 128.911), and *B. subalternans* from Campinas (22° 58' S, 47° 04' W) (UEC 128.918), Itirapina (22° 14' S, 47° 49' W) (UEC 128.908), Ribeirão Preto (21° 12' S, 47° 46' W) (UEC 128.907) and Santa Bárbara do Oeste (22° 45' S, 47° 23' W) (UEC 128.980).

Mitotic metaphase cells from the root-tips of germinated cypselas were used for the chromosomal counts. The rootlets were pretreated with 0.002 M 8-hydroxyquinoline for 5 h at 14°-15°C then fixed in Carnoy solution (ethanol: acetic acid, 3:1, v/v) for 24 h and stored in 70% alcohol at -20°C. The material was hydrolyzed in 5 N HCl at room temperature for 25 min and washed in distilled water. The root-tips were squashed in a drop of 45% acetic acid and dipped in liquid nitrogen to remove the cover slip. The slides were subsequently dried at room temperature and then stained with 2% Giemsa for 20 min and mounted in Entellan (GUERRA 1983). The best metaphases were photographed with an Olympus BX50 photomicroscope using Agfa Pan APX film (25 ASA).

Voucher specimens were deposited in the herbarium of the Department of Botany of the State University of Campinas (UEC). The collected material was identified based on literature reports (SHERFF 1937; MAGENTA 1998). Material from various herbaria (CEPLAC, Herbarium Rizzo, Herbarium Sérgio Tavares, HUEFS, IPA, SP and SPF) and natural populations (from the states of Minas Gerais, Bahia and Pernambuco) was also examined.

To assess the existence of published chromosomal counts for the species studied, we also used the following indexes of plant chromosomal numbers: MOORE (1972; 1973; 1977), GOLDBLAT (1981; 1984; 1985; 1988) and GOLDBLAT and JOHNSON (1990; 1994; 1996).

RESULTS AND DISCUSSION

The chromosomes of *B. alba*, *B. pilosa* and *B. subalternans* were small (< 5 μm) and of similar morphology (centromeres in the central region of the chromosomes). The number of somatic chromosomes was 2*n*=48 in *B. alba* and *B. subalternans* and 2*n*=72 in *B. pilosa* (Figure 1). The chromosomal numbers were the same in all populations of each species. In addition, *B. alba* and *B. subalternans* had the same chromosomal numbers but differed morphologically. There was no difference in the chromosomal numbers of the two *B. pilosa* morphs. Of the three species studied, *B. pilosa* had the highest chromosomal number (2*n*=72), although this species is not as "vigorous" as some autopolyploids (STEBBINS 1971).

Bidens alba, *B. pilosa* and *B. subalternans* have very similar vegetative parts. *Bidens alba* is perennial, short-lived, and restricted to the seacoast, whereas *B. pilosa* and *B. subalternans* are syntopic and widely distributed in agricultural areas and along roadsides. The three species have green or winny erect, square stems and are 0.3-1.2 m tall. The disc-floret is yellow, tubular and ca. 5 mm long, while the cypcelas are dark brown, costate and 6.5-11 mm long.

Bidens pilosa has two morphs, one radiated (with ray-florets) and the other discoid (without ray-florets). When present, the ray florets vary from white to salmon, are ca. 5 mm long and have cypselas chiefly with 2-3 awns. *Bidens alba* has radiated flower heads with white reflexed ray-florets 6-16 mm long; most cypselas have two awns. *Bidens subalternans* has radiated flower heads with yellow-cream ray-florets 5-6 mm long; most cypselas have four awns (Figure 1).

The chromosoaml counts agreed with other data for the genus *Bidens*. Of the 57 *Bidens* species for which the chromosomal numbers are known, 50 have x=12 or multiples of this number, thus confirming *n*=12 as the basic number for this

Fig. 1 — A. *B. alba* flower head; B. Mitotic metaphase with 2*n*=48 chromosomes; C 1. *B. pilosa* radiate flower head and C 2. Discoid flower head; D. Mitotic metaphase with 2*n*=72 chromosomes; E. *B. subalternans* flower head; F. Mitotic metaphase with 2*n*=48 chromosomes. Bar=10 µm.

genus (SOLBRIG *et al.* 1972; POWELL *et al.* 1974; TURNER *et al.* 1979; KEIL *et al.* 1988). Based on our results and on literature reports for the other species of *Bidens*, we suggest that the mechanism responsible for the chromosomal variations in the genus could be polyploidy.

The chromosomal number of 2*n*=48 for *B. alba* was the same as that previously reported by BALLARD (1986) and KEIL *et al.* (1988) for this species. Our data support the identification of Brazilian species reported by MORAES (1997) and MAGENTA (1998).

There were some discrepancies between our results and those reported in the literature. The chromosomal number for *B. subalternans* (2*n*=48) agreed with that reported by COVAS and SCHNACK (1946) (*apud* MOORE 1977), but differed from the *n*=35+B reported by JANSEN *et al.* (1984) and the *n*=34 observed by Powell & King (1969) (*apud* MOORE 1973).

The reviews by MOORE (1972; 1973; 1977), GOLDBLAT (1981; 1984; 1985; 1988) and GOLDBLAT and JOHNSON (1990; 1994; 1996) showed

$2n$=72 (or n=36) for *B. pilosa*. However, n=12, 23, 24 and $2n$=36, 46, 48, 76 have been observed in other studies. BALLARD (1986) reported $2n$=72, as also found here.

MARIANO and MARIN-MORALES (1999) analyzed the chromosomal numbers of nine Brazilian populations of *B. pilosa* and reported values of $2n$=48, 70 and 72. This variation was considered to represent different levels of ploidy and cytotypes as part of a geographical gradient. However, these authors provided no clear evidence of a cline, no details of the morphological characters were given, and we were unable to locate voucher specimens to confirm the identification. Confusion among different species of this complex should be considered as a possible explanation for this variation.

According to MAGENTA (1998), several specimens of *B. alba* and *B. subalternans* have been erroneously identified as *B. pilosa,* giving rise to problems in the interpretation of data. *Bidens* species are widely distributed throughout tropical and subtropical regions and have many characters that are highly variable. The taxonomic analyses by SHERFF (1937) were based only on herbarium specimens and did not take into consideration the morphological variations within populations (BALLARD 1986). In addition, the lack of vouchers deposited in herbaria precludes any critical analysis of data previously reported by others.

Despite the fact that the three *Bidens* species have a broad geographical distribution, the same chromosomal number has been found in plants of the same species from distinct regions: $2n$=48 for *B. subalternans* from South America (COVAS and SCHNACK 1946 *in* GOLDBLAT 1971) and Brazil, $2n$=48 for *B. alba* from Brazil, Central America (BALLARD 1986) and North America (KEIL *et al.* 1988) and $2n$=72 for *B. pilosa* from Brazil, Central America (SOLBRIG *et al.* 1972) and North America (BALLARD 1986). Thus, there is no variation in the chromosomal number despite the wide geographical distribution of each species. The high degree of morphological variation within populations makes it difficult to establish clear boundaries that could be helpful in identifying the material used by other authors in previous studies. The cytological results reported here provide a useful tool for the taxonomy of *Bidens* at the interspecific level. The extent to which this may be applied to other *Bidens* species remains to be determined.

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REFERENCES

BALLARD R., 1986 — *Bidens pilosa complex (Asteraceae) in North and Central America*. American Journal of Botany, 73: 1452-1465.

CABRERA A.L., 1974 — *Flora ilustrada de Entre-Rios (Argentina).* Colección Científica del INTA, Buenos Aires, 6: 106-554.

DEMATTEIS M., 1998 — *Chromosome studies of some Vernonia species (Asteraceae).* Genetics and Molecular Biology, 21: 381-385.

DEMATTEIS M. and FERNÁNDEZ A., 2000 — *Chomosome studies on nine South American species of Vernonia (Vernonieae, Asteraceae*). Caryologia, 53: 55-61.

GUERRA M., 1983 — *O uso do Giemsa na citogenética vegetal – comparação simples e o bandamento.* Ciência e Cultura, 35: 190-193.

GOLDBLAT P., 1981 — *Index to plant chromosome numbers for 1975-78*. Monographs in Systematic Botany from the Missouri Botanical Garden, 5.

GOLDBLAT P., 1984 — *Index to plant chromosome numbers for 1975-78*. Monographs in Systematic Botany from the Missouri Botanical Garden, 8.

GOLDBLAT P., 1985 — *Index to plant chromosome numbers for 1982-83*. Monographs in Systematic Botany from the Missouri Botanical Garden, 13.

GOLDBLAT P., 1988 — *Index to plant chromosome numbers for 1984-85.* Monographs in Systematic Botany from the Missouri Botanical Garden, 23.

GOLDBLAT P. and JOHNSON D.E., 1990 — *Index to plant chromosome numbers for 1986-87*. Monographs in Systematic Botany from the Missouri Botanical Garden 30.

GOLDBLAT P. and JOHNSON D.E. 1991 — *Index to plant chromosome numbers for 1988-89*. Monographs in Systematic Botany from the Missouri Botanical Garden, 40.

GOLDBLAT P. and JOHNSON D.E., 1994 — *Index to plant chromosome numbers for 1990-91*. Monographs in Systematic Botany from the Missouri Botanical Garden, 51.

GOLDBLAT P. and JOHNSON D.E., 1996 — *Index to plant chromosome numbers for 1992-93.* Monographs in Systematic Botany from the Missouri Botanical Garden, 58.

GROMBONE-GUARATINI M.T, SOLFERINI, V. N. and SEMIR J. 2004 — *Reproductive biology in three Bidens L. species (Asteraceae).* Scientia Agricola, 61 (2): 185-189.

GUERRA M., 2000 — *Chromosome number variation and evolution in Monocots*. In: I. Wilson K.L. and

Morrison D.A. (Eds) "Monocots: Systematic and Evolution", pp 127-136. CSIRO, Melbourne.

JANSEN R.K.T., STUESSY, F., DIAZ-PIEDRAHITA S. and FUNK V.A. 1984 — *Recuentes cromosómicos en Compositae de Colombia*. Caldasia, 14: 7-20.

KEIL D.J., LUCKOW M.A. and PINKAVA D.J., 1988 — *Chromosome studies in Asteraceae from the United States, Mexico, the West Indies, and South America*. American Journal of Botany, 75: 652-668.

MAGENTA M.A.G., 1998 — *As subtribos Ambrosiinae, Galinsoginae e Coreopsidinae (Heliantheae – Asteraceae) no Estado de São Paulo*. Tese de Mestrado, Universidade de São Paulo, São Paulo.

MARIANO A.C. and MARIN-MORALES N.A., 1999 — *Chromosome polymorphism and cytotype establishment in Bidens pilosa (Asteraceae).* Cytobios, 97: 45-60.

MOORE R.J., 1972 — *Index to plant chromosome numbers for 1970*. Regnum Vegetabile, 84.

MOORE R.J., 1973 — *Index to plant chromosome numbers for 1967-71*. Regnum Vegetabile, 90.

MOORE D.M., 1977 — *Index to plant chromosome numbers for 1973-74*. Regnum Vegetabile, 96.

MORAES M.D., 1997 — *A família Asteraceae na planície litorânea de Picinguaba, Município de Ubatuba – São Paulo*. Tese de Mestrado, Universidade Estadual de Campinas, Campinas.

POWELL A.M., KYHOS W. and RAVEN P.H., 1974 — *Chromosome numbers in Compositae. X.* American Journal of Botany, 61: 909-913.

SHERFF E.E., 1937 — *The genus Bidens.* Field Museun of Natural History Botanica Serica, 16: 16-484.

SOLBRIG O.T., KYHOS D.W., POWELL M. and RAVEN P.H., 1972 — *Chromosome numbers in Compositae VIII: Heliantheae*. American Journal of Botany, 59:869-878.

STEBBINS G.L., 1971 — *Chromosomal evolution in higher plants*. Edward Arnold (Publishers) Ltd., London.

STUESSY T.F., 1977 — *Heliantheae - systematic review.* In: Heywood V.H., Harborne J.B. and Turner B.L. (Eds.)."The biology and chemistry of the Compositae", pp 621-671, Academic Press, London.

SUNDERBERG S., COWAN C.P. and TURNER B.L., 1986 — *Chromosome counts of Latin American Compositae.* American Journal of Botany, 73: 33-38.

TURNER B.L., BACON J., URBATSCH L and SIMPSON B., 1979 — *Chromosome numbers in South American Compositae*. American Journal of Botany, 66: 173-178.

VALLÈS J., TORRELL M., GARCIA-JACAS N. and KAPUSTINA L.A., 2001 — *New or rare chromosome counts in the genera Artemisia L. and Mausolea Bunge (Asteraceae, Anthemideae) from Uzbekistan.* Botanical Journal of Linnean Society, 135: 391-400.

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