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 2n=72 and *B. subalternans* and *B. alba* has 2n=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. *pilosa*, 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 morphological 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 Fernández 2000). Some phenotypic characteristics of polyploids, such as the greater size and vigor of seeds, may enable them to tolerate a wider
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 ($2n=48$), B. odorata ($2n=24$) and B. pilosa ($2n=36$). With few exceptions, cytogenetic studies of the genus Bidens have been restricted to chromosomal counts (Solerig 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 cypsela were used for the chromosome 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 $2n=48$ in B. alba and B. subalternans and $2n=72$ 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 ($2n=72$), 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 sympatric 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 cypselas 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 cypsela with 2-3 awns. Bidens alba has radiated flower heads with white reflexed ray-florets 6-16 mm long; most cypsela have two awns. Bidens subalternans has radiated flower heads with yellow-cream ray-florets 5-6 mm long; most cypsela have four awns (Figure 1).

The chromosomal counts agreed with other data for the genus Bidens. Of the 57 Bidens species for which the chromosomal numbers are known, 50 have $n=12$ as the basic number for this
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 $2n=48$ 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* ($2n=48$) 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...
2\(n=72\) (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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