Cytogenetics characterization of *Conyza bonariensis* (L.) Cronquist populations from Brazil

André Guareschi, Tamara Pastori,
Nelson Dihel Kruse, Sergio Luiz de Oliveira Machado,
Thais Scotti do Canto Dorow, Solange Bosio Tedesco

Departamento de Biologia – CCNE/UFSM; Santa Maria, RS
e-mail: tamarapastori@gmail.com

Resumo

A espécie *Conyza bonariensis* (L.) Cronquist, conhecida por buva, pertence à família Asteraceae e é originária da América do Sul. Apresenta ciclo de desenvolvimento anual e é prolífica, podendo produzir até 200.000 sementes por planta, estabelece-se em diversas condições climáticas, apresenta boa adaptabilidade, sendo, geralmente, considerada planta daninha, mas também usada como medicinal. Para auxiliar na identificação precisa dessa espécie, este trabalho teve como objetivo realizar a caracterização citogenética, partindo de sementes coletadas em populações ocorrentes na região do planalto médio do Rio Grande do Sul. Para determinação do número de cromossomos, as sementes foram colocadas para germinar em placas de Petri com papel filtro duplo no germinador a 20° C. As radículas com 2 a 5 mm de comprimento foram coletadas e submetidas ao pré-tratamento a frio (4° C) por 18h, fixação em etanol: ácido acético (3:1) por 24h a temperatura ambiente e conservação em álcool 70% sob refrigeração até o uso. Para o preparo das lâminas, as radículas foram hidrolisadas em HCl 2N por 5 min, lavadas em água destilada e coradas com orceína acética (2%) pela técnica de esmagamento. As lâminas foram analisadas em microscópio ótico com auxílio da objetiva de 40x e 100x. Os resultados obtidos para as populações oriundas

de diferentes locais do RS foram 2n=4x=36 cromossomos para as populações de Júlio de Castilhos, Tupanciretã, Cruz Alta, Victor Graeff; e 2n= 5x= 45 para a população de Não-Me-Toque, correspondendo morfologicamente a *Conyza bonariensis* var. *microcephala* (Cabrera) Cabrera, com exceção da população de Tupanciretã, a qual se trata de *Conyza bonariensis* var. *bonariensis*.
Palavras-chave: Asteraceae, buva, número de cromossomos, planta daninha.

Abstract

The species *Conyza bonariensis*, known as hairy fleabane, belongs to the Asteraceae family and has its origin in South America. It has an annual cycle of life, being prolific and able to produce up to 200,000 seeds per plant. It occurs in various climatic conditions, showing good adaptability, and is generally considered weed, but also used as medicinal. To help on the identification of this species, the study aimed to perform cytogenetic characterization, starting from seeds collected of populations occurring in the hig-medium plains region of Rio Grande do Sul. To determinate the number of chromosomes, the seeds were germinated and the root-tips with 2-5 mm in length were collected and subjected to cold pretreatment (4°C) for 18h, fixed in ethanol: acetic acid (3:1) for 24h and kept in 70% ethanol under refrigeration. For the preparation of slides, the root-tips were hydrolyzed in 2N HCl for 5 min and stained with acetic orcein (2%) by the squashing technique. The results were 2n = 36 chromosomes to populations of Julio de Castilhos, Tupanciretã, Cruz Alta, Victor Graeff; and 2n = 5x = 45 for the population of Nao-Me-Toque, morphologically corresponding to *Conyza bonariensis* var. *microcephala*, except the population of Tupanciretã, which it is *Conyza bonariensis* var. *bonariensis*.
Keywords: Asteraceae, buva (horseweed), chromosome number, plant, weed.

Introduction

Conyza bonariensis (L.) Cronquist is an annual weed species, herbaceous plant with seed dispersal and belongs to the Asteraceae (Compositae) family (Lorenzi, 2000). According Lazaroto et al., (2008) this species infests orchards, annual crops, pastures and uncropped areas. There is a wide morphological variability among species, varieties, biotypes and access of hairy fleabane (*Conyza*), which causes difficulties in taxonomic identification. In Brazil, it is often the occurrence of different species of *Conyza* in simple or

associated populations; and the correct identification is important to find out the resistance mechanism that is involved in each species and also for the appropriate selection of the best management strategy .

Conyza bonariensis, known as hairy fleabane or flax-leaved, is a native plant of South America, occurring abundantly in Argentina, Uruguay, Paraguay and Brazil, where the presence is more intense in the South, Southeast and Midwest (Kissmann & Groth, 1999). [*Conyza bonariensis*]. Differs from other species of *Conyza* by its branches of the upper canopy which rise and surpass the stem apex; leaf margins usually entire, but some varieties may have tiny teeth; and the composite heads in the maturation have more than 1 cm of diameter.

Thebaud & Abbott (1995) reported the occurrence of hybridization between *C. canadensis* and other species of the genus *Conyza*, especially *C. sumatrensis* (Retz.) E. Walker and *C. bonariensis*, because they often grow in populations associated. However, *C. canadensis* is diploid, with chromosome number 2n = 18, while the other species are polyploids. It must be considered the genus *Conyza* has polymorphic species, which makes harder the identification and classification of species based only on morphological characters (Urdampilleta et al., 2005).

Early studies of somatic chromosomal counting in *Conyza* (Solbrig, 1964; Bernardelli, 1986; Hunziker, 1990) refers to a set hexaploid with 2n = 6x = 54. Cytogenetic studies performed in *C. bonariensis* by Carr et al., (1999) report the presence of 18 chromosomes in bivalents associations, equivalent to a chromosome number of 2n = 4x = 36. In the same study, the authors reported other species such as *Conyza aegyptica* Dryand. (2n = 18), *C. apurensis* Kunth (2n = 54), *C. bonariensis* (2n = 36), *C. primulifolia* (Lam.) Cuatrec. & Lourteig (2n = 72), *C. sophiifolia* Kunth (2n = 18) and *C. uliginosa* Pers. (2n = 54). Other species of *Conyza* were studied by Turner et al., (1979), Wulff (1998) and Vilar (2006) and showed a divergent chromosome number: *C. bonariensis* collected in Argentina (2n = 4x = 36), *C. bonariensis* (2n = 2x = 18) and a population of *C. bonariensis* originated from Brazil (2n = 6x = 54). Besides the studies already mentioned, there are records of the chromosome for *Conyza bonariensis* IPCN (Index Plant Chromosome Number) showing that counts were carried out through the gametic number by Turner et al., (1979) (n = 27), Jansen et al., (1984) (n = 26), Razaq et al., (1988) (n = 27), Razaq et al., (1994) (n = 27) and counts through the somatic number, as Buttler (1984) (2n = 54) and Wulff (1998) (2n = 36).

Paula (2009) examined the most appropriate method for chromosomal counts in *Conyza* spp., determining the chromosomal number and dyers for testing pollen viability. The results showed hexaploid chromosomal complement: 2n = 6x = 54 in all the specimens examined, collected in Capão do Leão, RS. It was also established the Reactive Alexander dye as the most appropriate to estimate the pollen viability in C. bonariensis, due it is able to stain the viable and nonviable pollen with distinct colors.

It was considered the difficulties in defining the species of *Conyza* due to polymorphism, and because it is a genus with species arranged in a polyploid complex, this study aimed to determine the number of chromosomes in populations of *Conyza bonariensis* sampled at different locations in High(medium) Plains of Brazil of RS state.

Material and methods

For this study, seeds were collected from eight populations of horseweed naturally occurring in the RS state, Brazil, in Julio de Castillos, Tupanciretã, Cruz Alta, Victor Graeff and Não-Me-Toque cities.

Individuals were identified as *Conyza bonariensis* var. *microcephala* and *Conyza bonariensis* var. *bonariensis* by Dr. Thais Scotti Canto-Dorow, Universidade Federal de Santa Maria (UFSM) and are deposited in the Herbarium SMDB Department of Biology-UFSM under numbers SMDB 12505, SMDB 12506 (Julio de Castillos), SMDB 12507, SMDB 12508 (Tupanciretã), SMDB 12509, SMDB 125010, SMDB 125011 (Cruz Alta), SMDB 12513, SMDB 12514 (Victor Graeff) and SMDB 12516 (Não-Me-Toque) (Table 1).

The seeds were germinated in Petri dishes with filter paper soaked with distilled water and kept in a growth chamber (temperature: ± 20° C and alternating periods of light/dark, 12h) at the Cytogenetics and Plant Genotoxicity Laboratory (LABCITOGEN) of UFSM. Radicles with 1.0 to 1.5 cm length, were collected and submitted to a cold pretreatment (4° C) for 18h. After, the radicles were fixed in Carnoy 3:1 (ethanol / acetic acid) for 24h at room temperature and stored in alcohol (70%) under refrigeration. For the slides preparation, the root-tips were washed in distilled water, hydrolyzed in HCl (2N) for 5 min, and washed again. For mounting the blade, the meristematic region was removed, crushing it with acetic orcein (2%). Cells with good chromosomes spreading were photographed and the chromosomes counted and measured using an eyepiece micrometer.

Results

The analysis was performed in two varieties of *Conyza bonariensis*, the var. *bonariensis* and the var. *microcephala*, this one with small flower heads (capitula), 4-5 mm involucres, shortly peciolated and arranged in large panicles, which makes it different from the typical variety, which has flower heads larger, 6 mm involucres, long pedicels, arranged in more contracted panicles.

The somatic cells examinations from root-tips of *C. bonariensis* populations showed variation in chromosomal number from tetraploid to pentaploid level (Table 1, Figure 1), which were found nine plants with 2n = 4x = 36 and one plant with 2n = 5x = 45 chromosomes.

Table 1. Populations and chromosome number of *C. bonariensis* collected in the High Plains region of RS state, Brazil, 2007/08 growing season.

Sities (RS)	Geographic coordinates	Populations (nº)	Chromosomes (nº)	Specimen (nº herbary SMDB)
Júlio de Castilhos	29°10'20.99" S 53°40'01.75" O	2	2n= 4x= 36	*C. bonariensis var. microcephala* (SMDB 12505, SMDB 12506)
Tupanciretã	29°03'19.47" S 53°46'35.99" O	2	2n= 4x= 36	*C. bonariensis var. bonariensis* (SMDB 12507, SMDB 12508)
Cruz Alta	28°36'11.68" S 53°40'29.55" O	3	2n= 4x= 36	*C. bonariensis var. microcephala* (SMDB 12509, SMDB 12510, SMDB 12511)
Victor Graeff	28°36'41.98" S 52°41'53.53" O	2	2n= 4x= 36	*C. bonariensis var. microcephala* (SMDB 12513, SMDB 12514)
Não-Me-Toque	28°22'45.78" S 52°47'13.78" O	1	2n= 5x= 45	*C. bonariensis var. microcephala* (SMDB 12516)

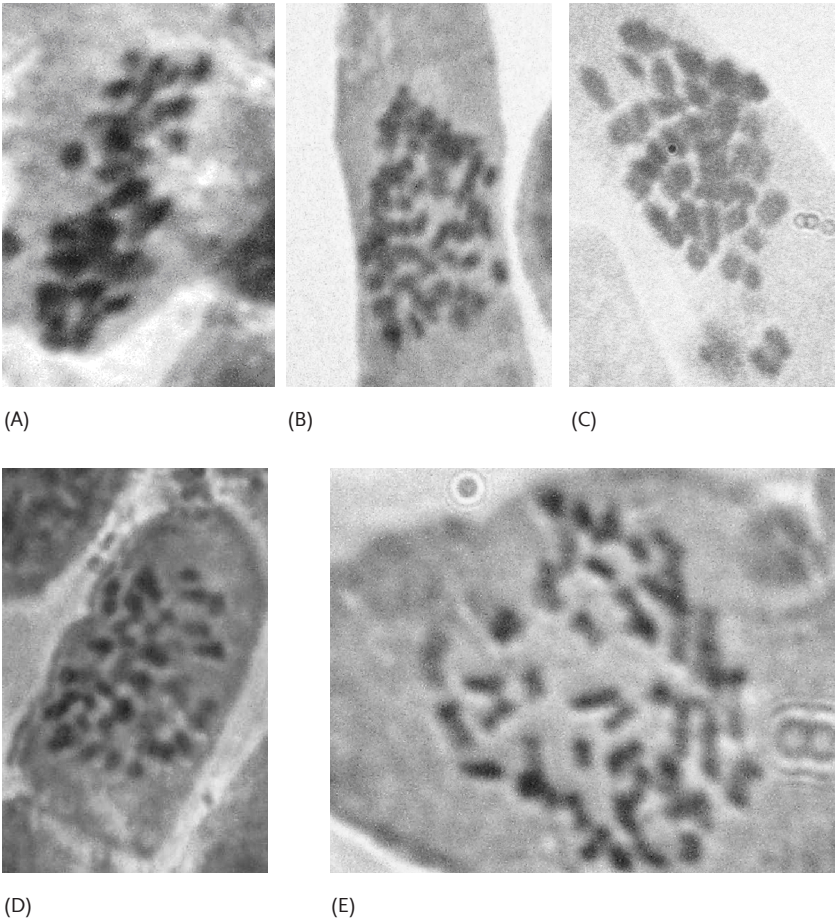

Figure 1. Somatic cells chromosomes from root-tips of *C. bonariensis* (L.) Cronquist from natural populations of the High Plains region of Rio Grande do Sul, Brazil. A- Populationof Julio de Castillos, 2n = 36 chromosomes, B- Tupanciretã population, 2n = 36 chromosomes, C- Cruz Alta population, 2n = 36 chromosomes; D- Victor Graeff population, 2n = 36 chromosomes, E-Não-Me-Toque population, 2n = 45 chromosomes. Bar = 10µ.

Discussion

For this species, others authors found plants with n = 27 chromosomes (Turner et al., 1979; Jansen et al., 1984) and hexaploid populations with 2n = 6x = 54 (Buttle, 1984; Paula, 2009).

Cytogenetic studies with another genus of the Asteraceae family, as *Bidens*, made by Barroso et al., (1991), found the basic number x = 12

chromosomes. Mariano & Marin-Morales, (1998) analyzing Brazilian *Bidens* populations found individuals with 2n = 48 and 2n = 72 chromosomes, pointing out polyploidy as an important evolutionary process for the genus *Bidens*. These authors also found plants with 2n = 68 and 2n = 70 chromosomes considered as diploid cytotypes, suggesting a secondary evolution by aneuploidy, besides populations with basic number of x = 12. In southern region of Brazil, Fachinetto et al., (2008) also found chromosomal variation of 2n = 38 to 2n = 56 for *Bidens*. The literature also has data with numerical variation of chromosomes for *Bidens* of 2n = 24 (Gill, Omoigui, 1989; Husaini, Iwo, 1990), 2n = 96 (Gill, Omoigui, 1989) and 2n = 72 (Mariano & Marin- Morales 1998). Some species of *Mikania* (Asteraceae) also shows variations in chromosome number 2n = 36 and 2n = 42 chromosomes for diploid cytotypes, and 2n = 72 for the tetraploid species *M. micrantha* Kunth (Maffei et al., 1999). These variations between species and populations with the same chromosome number are important for generating new forms of recombination, which have influence in the variability of natural populations of the adaptive process (Rees & Dale, 1974).

Cytogenetic in Brazil is still very focused on plants grown with economic interests. However, there is a growing interest in other plant species, both of medicinal value as an ornamental (Guerra, 1990; Lovatto & Battistin 1997; Pedrosa et al., 1999; Pagliarini, 2000).

The literature emphasizes the great variability in the chromosomes number in the genus *Conyza*. However, this study found tetraploid and pentaploid populations (Table 1). One can assume that the tetraploid plants found are originated from the non-reduced gametes of two diploid individuals of *C. bonariensis,* which should present 2n = 18, and then formed individuals 2n = 4x = 36. It is also possible to suggest the basic number of x = 9 for *Conyza bonariensis*. Pentaploid individuals may have originated from the natural crossing of a tetraploid individual who has formed unreduced gametes (4n) with a diploid individual of normal gametes (n) resulting in individuals 5n. In this study the first counting of the chromosomal number in *C. bonariensis* pentaploid plants, which are native from Rio Grande do Sul State, was performed. Additional studies are needed to elucidate these hypotheses developed for explain C. *bonariensis* populations.

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