Chromosome Numbers in Brazilian and Argentine Populations of *Pfaffia glomerata* (Amaranthaceae)

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Received February 12, 2003; accepted February 26, 2003

**Summary**  Somatic chromosome numbers were determined in 10 populations of *Pfaffia glomerata*, also known as “Brazilian ginseng”, collected from different regions of Brazil and Argentina. Nine populations showed 2n=34 and one 2n=32, 33. Although chromosomes were very small, a pair of satellited chromosomes per karyotype was reported in almost all the populations. Chromosomes were predominantly metacentric and submetacentric. Meristematic interphase nuclei showed areticulate chromatin structure. Prophase chromosomes had deeply stained proximal blocks of condensed chromatin. This is the first cytological report including chromosome counting for the genus *Pfaffia*, a genus of the Amaranthaceae family with several species of economic importance due to their high content of ecdysteroid glycosides. The latter is a substance similar to that found in the genus *Panax*, “the Korean ginseng”, widely used as medicine.

**Key words**  *Pfaffia glomerata*, Brazilian ginseng, Chromosome number, Chromatin.


The use of these plants went beyond the regional indigenous populations (Arenas and Azorero 1997, Oliveira 1986) since these transferred their knowledge to the local rural people. Owing to the plant’s properties being concentrated in its roots, it has been commonly employed “for all diseases”. Recently, due to some chemical compounds found in plants of the genus *Pfaffia*, mainly ecdysteroid glycosides (Nishimoto *et al.* 1987, 1988), very similar to those in the genus *Panax* (Korean ginseng), certain species have been popularly called “Brazilian ginseng”.

In the folk medicine, the Brazilian ginseng has been used for the treatment of rheumatic diseases, for antidiabetic purposes and inflammatory processes (Taniguchi *et al.* 1997, Nicoloso *et al.* 1999). It has also been widely used as a ginseng-like tonic herb against fatigue and stress (Teràn 1990, Nicoloso *et al.* 1999) and as an aphrodisiac tonic (Teràn 1990, Taniguchi *et al.* 1997).

In the southern region of Brazil, mainly in the area of the floodplain of the Paraná river basin, *Pfaffia glomerata* is very abundant. However, extensive extraction activities are compromising the wild resources (Agostinho and Zalewski 1996). In spite of their economic and medicinal interest, few studies have been developed on some *Pfaffia* species. Physicochemical properties (Nishimoto *et al.* 1984, 1987, 1988, Shiobara *et al.* 1992, 1993), vegetative propagation methods (Nicoloso *et al.* 1999), leaf ultrastructure characteristics (Estelita-Teixeira and Handro 1984), leaf anatomy (Handro, 1964), and medicinal properties (Taniguchi *et al.* 1997) have been reported. Moreover, there is some confusion in genus *Pfaffia* systematics. To date, *P. iresinoides* is being recognized as *P. glom-
erata (Smith and Downs 1972). Cytological studies, including chromosome number counting and evaluation of the meiotic behavior have never been undertaken. Thus, for the first time, we are reporting the chromosome numbers for 10 populations of *P. glomerata* collected in different regions of Brazil and Argentina as an initial contribution to the chromosomal knowledge of the genus *Pfaffia*.

Materials and methods

Plants of 10 populations of *P. glomerata* were collected in different sites of Brazil and Argentina where they occur wide (Fig. 1). Origin of populations is shown in Table 1. Voucher specimens are deposited in the Herbarium of the Pontíficia Universidade Católica do Rio de Janeiro, Brazil. Fig. 2 illustrates some morphological aspects of *P. glomerata*, including its characteristic leg-like roots. Plants were kindly identified by Dr Josafá Carlos de Siqueira from the Pontíficia Universidade Católica do Rio de Janeiro.

Plants were maintained at the Medicinal Herb Garden of the State University of Maringá. Shoots from the basis and middle positions were put in water for rooting. Root tips, 1 or 2 cm in length, were pretreated for 3 h in a solution of 8-hidroxiquinoleine 0.002 M at 9°C, fixed in Carnoy’s solution (3 : 1, ethanol: glacial acetic acid) and stored in 70% alcohol. Hydrolysis was done in 5 N HCl, for 25 min, at room temperature. Standard chromosome preparations were done according to Feulgen technique (Schiff’s reagent). Photomicrographs were taken with Kodak Imagelink-HQ, ISO 25, black and white film.

Results and discussion

Among the 10 populations analyzed, 9 presented \(2n=34\) chromosomes (Table 1). All
chromosome counting in the population of Ilha São Francisco (Guaíra municipality) had \(2n=32\) and 33 chromosomes, with a predominance for the latter number. On the other island (Ilha São Francisco), with similar environmental conditions, no changes have been observed in the chromosome number predominantly found in the other populations.

Chromosome rearrangements, *i.e.*, inversions, translocations and other events, such as segment transpositions, duplications, and deletions, constitute the raw material for karyotype repatterning during speciation, which finally leads to a homozygous restructured karyotype in the new reproductive community. According to Greilhuber and Ehrendorfer (1988), chromosome number changes in karyotype with monocentric chromosomes, as reported in the *Pfaffia glomerata* populations (Fig. 3), require changes in centromere number without significant loss, and with only tolerable duplications in coding chromosome parts. This kind of chromosomal rearrangement, known as dysploidy, may occur by centromeric fission or fusion (Robertsonian translocations). In centromeric fission, a 2-armed chromosome breaks in the centromere to give 2 telocentric chromosomes, while in centromeric fusion, a telocentric or acrocentric chromosome is translocated into another chromosome of this type, so that a 2-armed chromosome results. Although karyograms were not undertaken for the present populations of *P. glomerata*, owing to the quality of karyotypes obtained, reports from many cells of all populations reveal that chromosomes are metacentric and submetacentric. Telocentric or acrocentric chromosomes that facilitate centromeric fusion were never found (Fig. 3).

The karyotype \(2n=32\) and 33 found in the Ilha do Alemão population (Fig. 3e) clearly shows metacentric and submetacentric chromosomes. Robertsonian systems, frequent in animals, have been described in a few plant groups, mainly in Commelinaceae (Jones 1977). The cause of chromosome variation in the Ilha do Alemão population of *P. glomerata* remains unknown, and more studies are necessary to elucidate it.

Despite its economic interest, the Amaranthaceae family is cytologically not well characterized, while genus *Amaranthus* is the best studied (Federov 1969, Goldblatt 1981). The predominant base number in the family, including the genus *Amaranthus*, is \(x=16\) and 17, as reported in *P. glomerata*. Pal *et al.* (1982) suggested that the base number \(x=17\) originated from \(x=16\) through primary trisomy. Based on the cytogenetic analysis of interspecific hybrids, Greizerstein and Poggio (1992) support this hypothesis and suggest that the species with \(2n=32\) are polyploid (base number \(x=8\)) and that \(x_1=16\) is a derived base number. The base number \(x_2=17\) would have appeared later by primary trisomy. In *Amaranthus hypochondriacus*, \(x=16\) and \(x=17\) have been reported for the same species (Greizerstein and Poggio 1994), and this cytological feature could predict segregational sterility observed in some intra- and inter-specific hybrids.

One pair of chromosomes with a satellite was found in the karyotype of all populations (Fig. 3). Since chromosomes were very small, it was not easy to identify the chromosome with the sec-
Secondary constriction in all cells studied. One nucleolus was observed in microsporocytes in prophase I. These data are in agreement with those reported by Greizerstein and Poggio (1994) for some *Amaranthus* species with $2n=32$ and 34.

Fig. 3. C metaphases of *P. glomerata* populations. a) Baitaporã, $2n=34$, b) Itaquiraí, $2n=34$, c) Eldorado, $2n=34$, d) Ilha São Francisco (Guaíra), $2n=34$, e) Ilha do Alemão (Guaíra), $2n=33$, f) Porto Rico, $2n=34$; g) Maringá, $2n=34$, h) San Ignácio, $2n=34$, i) Posadas, $2n=34$, j) Governador Virasoro, $2n=34$. Arrows point out satellites. Bars=0.5 µm.

Fig. 4. Meristematic nuclei of *P. glomerata*. a) Interphase nucleus showing areticulate chromatin structure. b) Prophase nucleus showing deeply stained proximal blocks of condensed chromatin (arrows).

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In meristematic interphase nuclei the chromatin structure was areticulate in all analyzed populations of *P. glomerata* (Fig. 4a). The chromatin reticulum was very small and slightly stained. Areticulate nuclei are related mainly to karyotypes with small chromosomes and low DNA content (Barlow 1977), as in the plants under analysis, and are commonly associated with phylogenetically more advanced (Guerra 1987) or more specialized taxa (Barlow 1977).

Prophase chromosomes in all populations showed deeply stained proximal blocks of condensed chromatin, allowing an estimate of the chromosome number (Fig. 4b). The presence of high amounts of condensed chromatin as proximal blocks at prophase could be an important feature in the evolution of the genus.

Cytogenetic studies on plants have been employed, among other things, to establish genomic relationships in biosystematic research and as an aid in applied crop improvement programs. This is the first report of chromosome counting in the genus *Pfaffia*, which up to the present has been only poorly studied. Because of its medicinal properties *Pfaffia glomerata* is highly explored in our region by several commercial groups that extract its active substances for world wide commercialization. Certain populations close to the Paraná river basin are disappearing and are now under governmental care. More studies are necessary to better characterize these populations.

Acknowledgements

The authors wish to thank Dr Josafá Carlos Siqueira, Pontifícia Universidade Católica do Rio de Janeiro, Brazil, for taxonomical determinations; to Dr. Maude Nancy Joslin Motta, Manager of the Parque Nacional de Ilha Grande (IBAMA) for permitting plant collection in the reserve areas; to Dr. Paulo Renato Taschetto for his collaboration in collection of material and its maintenance.

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