The chromosomes of the rare and endemic genus *Famatinanthus* (Famatinanthoideae, Asteraceae)

Los cromosomas del raro y endémico género *Famatinanthus* (Famatinanthoideae, Asteraceae)
Abstract

Classical staining and in situ fluorescent hybridization with probes for the 18–5.8–26S and 5S genes were performed in *Famatinanthus decussatus* (endemic from Argentina), in order to know its main chromosomal characteristics and to compare them to related genera. The species is a paleopolyploid with 2n = 54, likely originated from x=9, and its karyotype features are conservative: one pair of 5S signals and two of 18–5.8–26S per complement were found. All the data are congruent with the basal position of this species in the Asteraceae phylogenies.

**Keywords:** Chromosomes, endemism, *Famatinanthus decussatus*, polyploidy, rDNA.

**Resumen**

Los cromosomas del raro y endémico género *Famatinanthus*. Se aplicaron tinción convencional e hibridación in situ fluorescente con sondas para los genes de 18–5.8–26S y 5S en *Famatinanthus decussatus* (endémica de Argentina), con el fin de conocer su características cromosómicas principales y compararlas con géneros relacionados. La especie es un paleopoliploide con 2n = 54, probablemente originado a partir de x=9; sus caracteres cariotípicos son conservados: se encontraron un par de señales de 5S por complemento y dos de 18–5.8–26S. Los datos son congruentes con la posición basal que esta especie ocupa en las filogenias de Asteraceae.

**Palabras clave:** Cromosomas, endemismo, *Famatinanthus decussatus*, poliploidía, rDNA.

**Introducción**

*Famatinanthus* Ariza & S. E. Freire is a recently described genus of Asteraceae endemic to a restricted area in the province of La Rioja, Argentina (Freire et al., 2014). Its single species, *F. decussatus* (Hieron.) Ariza & S. E. Freire, was formerly placed within *Aphyllolcladus* Wedd. However, analyses of floral characters (i.e. corollas, anthers, style, achenes), stem anatomy, trichomes and pollen (Freire et al., 2014), indicate that the species has a combination of features that do not correspond with the circumscription of *Aphyllolcladus* or any other genus within the Mutisioideae tribe. Consequently, a new genus was proposed to accommodate it. The singularities of *F. decussatus* not only involve morpho-anatomical features; in fact, in a molecular phylogenetic study using 14 chloroplast DNA loci, Panero et al. (2014) found that the recently named genus has the two chloroplast inversions present in all Asteraceae except the nine genera of the subfamily Barnadesioideae and concluded that it is sister to the Mutisioideae–Asteroideae clade that represents more than 99% of Asteraceae. These characteristics made *Famatinanthus* to deserve a subfamily only for itself. Considering this background it is important to know the chromosomal characteristics of the species.

From the cytological point of view, chromosome counts provide a key tool in studies of systematics, phylogeny and evolution, and they are especially useful for understanding speciation and hybridization (Stebbins, 1971). Structural and quantitative characteristics of karyotypes have been significant in evolutionary and taxonomic studies in many angiosperm groups (Stebbins, 1971), being decisive in establishing linkage groups and natural classifications. (e.g. Weiss-Schneeweiss et al., 2003; Pellicer et al., 2010). The FISH procedure allows homologous chromosomes in a complement to be differentiated and permits the comparison among related species (Heslop-Harrison, 2000; Garcia et al., 2007; Leitch et al., 2008). The procedure also provides information...
stored at –20 °C. Some of these slides were used for classical staining with Giemsa. The remaining stored slides were used for determining the location and number of rDNA sites by FISH. One of the probes was the pTa71 containing the 18–5.8–26S rDNA (Gerlach & Bedbrook, 1979) labeled with biotin-14-dATP (BioNick; Invitrogen, Carlsbad, USA). For the 5S rDNA, a probe was obtained from the genome of Prionopsis ciliata by PCR (Moreno et al., 2012) labeled with digoxigenin-11-dUTP (Roche Diagnostics). The FISH procedure was in accordance with Schwarzacher & Heslop-Harrison (2000), with minor modifications. The preparations were incubated in 100 lg/ml RNase, post-fixed in 4 % (w/v) paraformaldehyde, dehydrated in a 70–100% graded ethanol series, and air-dried. On each slide 15 μl of hybridization mixture was added (3 ng/μl of probe, 100% formamide, 50% dextran sulfate, 20 x SSC and 10% SDS), previously denatured at 70°C for 10 min. Chromosome denaturation/hybridization was done at 90°C for 10 min, 48°C for 10 min, and 38°C for 5 min using a thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany), and slides were placed in a humid chamber at 37°C overnight. The probes were detected with avidin-FITC conjugate and antidigoxigenin-rodamine conjugate and counterstained and mounted with 25 μl antifade (Vectashield Vector Lab., Burlingame, USA), containing 2ng/μl of DAPI.

Results

Seeds resulted inviable in most collected capitula. Only accession G.B. 4271 showed a good germination rate, thus the analyzed cells were all from this sample and they presented 2n = 54 (Fig. 1). Chromosomes are small compared to related genera of Asteraceae with 2n = 54, being the average length c = 1.75 ± 0.21 μm and the total...
haploid length of LT = 94.05 ± 2.95 μm (Table 1). The chromosomes are homogeneous in size, with a ratio between the largest and the shortest of the complement ca. 1.87. Notable secondary constrictions were detected in two chromosome pairs in most of the metaphases (Fig. 1A). Centromeres were difficult to visualize, and the high number of chromosomes hindered identifying the homologue pairs.

FISH technique with 18-5.8-26S probe evidenced four loci in terminal position, coinciding with the secondary constrictions visualized with the conventional staining, while the 5S probe showed two terminal signals per cell (Fig. 1B), located in asynteny respect to the chromosomes bearing 18-5.8-26S.

Discussion

Chromosome number. As well as most basal Asteraceae, *Famatinanthus* resulted to be a high polyploid with a hypothetical basic number x = 9. Polyploids with chromosome numbers derived from x = 9 have been reported for Barnadesioideae (Wulff, 1990), Mutisioideae (Ward, 1983; Waisman *et al*., 1984; Grau, 1987) and Stiffioideae (Gibbs & Ingram, 1982) (Table 1). In fact, this number has been pointed out as basic for the entire family, which is congruent with the basal position of *Famatinanthus* within Asteraceae (Panero *et al*., 2014). Semple & Watanabe (2009) hypothesized that multiple downward dysploid events from polyploids based on x = 9 account for nearly all the base numbers reported for Mutisieae, the numbers x = 8 to 32 would be the result of a long dysploid series from x2 = 27. In this sense, *Famatinanthus* is remarkable by conserving the paleopolyploid number.

Recurrent polyploidy. Polyploidy is important for many aspects described in several review articles (e.g. Soltis *et al*., 2003; Hegarty & Hiscock, 2008; Leitch & Leitch, 2008; Van de Peer *et al*., 2009). Autopolyploidy is a common phenomenon and it is regarded as frequent in angiosperms (Ramsey & Schemske, 1998). Within Asteraceae, Barker *et al*. (2008) revealed at least three ancient whole-genome duplications: a first one (shared by Mutisioideae, Carduoideae, Cichorioideae and Helianthoideae) placed near the origin of the family just prior to the rapid radiation of its tribes, and two independent genome duplications near the base of the tribes Mutisieae and Heliantheae. We can speculate that the genome duplication near the base of Mutisieae also affected *Famatinanthus* since they are sister clades. Thus, *Famatinanthus* would be an ancient polyploid.

Chromosome size. There is a phenomenon called genome downsizing whereby polyploids do not have a genome size exactly equal to the multiple of their diploid progenitors but which is somewhat smaller, as a result of a removal of redundant DNA (Leitch & Bennett, 2004). *Famatinanthus* has small chromosomes compared to hexaploid members of Barnadesioideae and Mutisioideae (Table 1). Only species of Doniophyton have c and LT values minor than *F. decussatus*. Genome size is related to the size of single chromosomes (e.g. Garnatje *et al*., 2004), and therefore the relatively small size of the chromosomes of *F. decussatus* would be due to the longer time that the species have had from its polyploidization to rearrange its genome and eliminate redundant elements.

rDNA sites. Regarding Asteraceae species related to *Famatinanthus*, FISH experiments with 18-5.8-26S probe were conducted only in the Mutisioideae species.
**Chaptalia** nutans, 2n = 50, 100 (Fregonezi *et al.*, 2004) with two and four pairs of signals per complement, respectively, and also in two species of *Chaetanthera* (Baeza *et al.*, 2005a), where 1 and 3 pairs of signals in diploids with 2n = 22 were found. Regarding the 5S, data in species more or less related to *Famatinanthus* are known only for *Chaetanthera* (Baeza *et al.*, 2005a) in which 3 and 4 pairs of 5S signal in diploid species with 2n = 22 have been found. This situation evidenced the different pathways that the rDNA loci took since the two lineages split from each other. The great variability in amount and position of rDNA loci in related species is a fact already known (Hasterok *et al.*, 2006; Heslop-Harrison & Schwarzacher, 2011; Chacón *et al.*, 2012; Morales *et al.*, 2012). For these rapid changes of copy number and chromosomal location of rDNA, different types of transposable elements have been postulated as responsible (Raskina *et al.*, 2004a, b; Belyayev *et al.*, 2005; Altinkut *et al.*, 2006; Datson & Murray, 2006).

In higher eukaryotes, the 18-5.8-26S rDNA and 5S rDNA loci are transcribed by different RNA polymerases and

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**Fig. 1.** Metaphasic chromosomes of *Famatinanthus decussatus* with (A) Giemsa staining and (B) FISH technique. Arrowheads point to NORs, arrows point to 18-5.8-26S loci (green signals) and asterisks indicate 5S loci (red signals). Bars represent 3 μm.
usually located in different positions of chromosomes (Srivastava & Schlessinger, 1991). Colocalization of 18S-5.8-26S and 5S rDNA loci has commonly been reported in animals (Dobigny et al., 2003) but it is less frequent in plants (e.g. García et al., 2007, 2009b; Abd El-Twab & Kondo, 2006; Chang et al., 2009). A dominant linked rDNA genotype was found within three large groups of Asteraceae (García et al., 2010): Anthemideae, Gnaphalieae and in the “Heliantheae alliance” (Asteroideae). The remaining five tribes of the Asteroideae displayed canonical non linked arrangement of rDNA, and also the remaining 12 subfamilies, with separate organisation. *Famatinanthus* fits perfectly into this scheme, with the 5S and 18S-5.8-26S loci in separate chromosomes. The results of García et al. (2010) indicate that nearly 25% of Asteraceae species may have developed unusual linked arrangement of rRNA genes, and the 5S gene integration within the 35S unit might have repeatedly occurred during plant evolution, and probably once in Asteraceae.

Genome downsizing, a phenomenon referred before, also affects rDNA genes. During the polyploidization process, gene reordering and gene silencing may occur (Stebbins, 1985; Soltis et al., 2003; Leitch & Bennett, 2004; Pires et al., 2004). In Solanaceae, for example, an analysis of the 18S-5.8-26S rDNA of Nicotiana indicated that parental loci were initially maintained in newly formed polyploids, although the sequences within a locus might be subject to concerted evolution, and over periods greater than 1 myr, individual loci would disappear (Kovařík et al., 2008). In Asteraceae, a similar situation was found in Tragopogon (Buggs et al., 2009; Chester et al., 2012), in which the number of 18S-5.8–26S sites seemed to evolve faster than the 5S sites, as they do not increase with the successive genome additions. By constrast, genome sizes of some polyploid taxa present additivity, although data pointing to genome upsizing in older polyploids are limited (García et al., 2009; Leitch et al., 2008).

FISH studies with rDNA probes would be desirable in genera more closely related to *Famatinanthus* (e.g. Barnadesia, Duseniella, Dasyphyllum) in order to achieve a more complete picture of chromosomal evolution in basal Asteraceae.

Acknowledgements

The authors thank Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina), SECyT-UNC, and SECyT-UNRC for financial support. Thanks are also tendered to D. Olivera for his unvaluable assistance in Famatina’s field work (La Rioja).
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Fig. 2. *Famatinanthus decussatus*. A. Hábitat; B. Planta mostrando las raíces; C. Tallo; D. Capítulo; E. Capitulecencia; F. Cipselas. (Fotografías G. Barboza, J. Cantero; R. Deanna & S. Leiva 4270, CORD, HAO).
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