

Genome size, chromosome number, and rDNA organisation in Algerian populations of *Artemisia herba-alba* (Asteraceae), a basic plant for animal feeding facing overgrazing erosion

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Abstract

Bougoutaia, Y., Garcia, S., Garnatje, T., Kaid-Harche, M. & Vallès, J. 2016. Genome size, chromosome number, and rDNA organisation in Algerian populations of *Artemisia herba-alba* (Asteraceae), a basic plant for animal feeding facing overgrazing erosion. *Anales Jard. Bot. Madrid* 73(2): e043.

Artemisia herba-alba is a largely-distributed and often landscape-dominating taxon in arid areas of the Mediterranean and Irano-Turanian regions. In Algeria, in 2010 its communities covered 10% of the steppe territory, but its populations have been subjected to overgrazing. A karyological study based on 22 populations together with a cytogenetic characterisation of this species has been performed for the first time in Algerian materials, through genome size and chromosome number determination. Fluorescence *in situ* hybridisation (FISH) was also used to assess the rDNA loci number and distribution in the two ploidy levels detected. The studied accessions are diploid ($2n = 2x = 18$ chromosomes, 6 populations) or tetraploid ($2n = 4x = 36$ chromosomes, 15 populations). One population, occupying a more or less central geographic position among the studied area, presented both cytotypes. Genome size reflects well the two ploidy levels, with no evidence of downsizing with polyploidy. The karyotypes are rather symmetric (2A Stebbins' class). FISH analyses detected four signals (2 loci) in diploid and eight signals (4 loci) in tetraploid cytotypes for both ribosomal DNA genes, which present an L-type (linked) organisation, i.e. with loci from both rDNA genes colocalised. The presence of two ploidy levels suggest a genomic dynamism and even a possible differentiation underlying the morphological uniformity and despite the dramatic decrease experienced by this plant in Algeria in terms of surface coverage.

Keywords: Anthemideae, *Artemisia herba-alba*, chromosome number, cytogenetics, fluorescent *in situ* hybridisation, genome organisation, genome size, karyology, polyploidy, rDNA loci.

Resumen

Bougoutaia, Y., Garcia, S., Garnatje, T., Kaid-Harche, M. & Vallès, J. 2016. Tamaño del genoma, número cromosómico y organización del rDNA en poblaciones argelinas de *Artemisia herba-alba* (Asteraceae), una planta básica para la alimentación animal en el contexto de la erosión por sobrepastoreo. *Anales Jard. Bot. Madrid* 73(2): e043.

Artemisia herba-alba es un taxon ampliamente distribuido y dominante en el paisaje en áreas áridas de las regiones mediterránea e irano-turania. En Argelia, sus comunidades cubrían en 2010 el 10% de su territorio de estepa, pero sus poblaciones se encuentran sometidas a sobrepastoreo. Se ha llevado a cabo un estudio cariológico basado en 22 poblaciones, se han caracterizado citogenéticamente por primera vez poblaciones argelinas de esta especie y se ha estimado el tamaño del genoma y el número cromosómico, además de la determinación por hibridación *in situ* fluorescente (FISH) del número y la distribución de los loci del ADN ribosómico en los dos niveles de ploidía. Las accesiones estudiadas son diploides ($2n = 2x = 18$, 6 poblaciones) o tetraploides ($2n = 4x = 36$, 15 poblaciones), y una, que ocupa una posición más o menos céntrica entre las consideradas, presenta ambos niveles de ploidía. El tamaño del genoma refleja los dos niveles de ploidía y no se observan indicios de disminución con la poliploidía. Los cariotipos son simétricos (clase 2A de Stebbins). Los análisis de FISH detectan cuatro señales (2 loci) en los citotipos diploides y ocho señales (4 loci) en los tetraploides para ambos genes del ADN ribosómico, que presenta una organización del tipo L (ligada), es decir, con los loci de los dos genes del ADNr colocalizados. La presencia de dos niveles de ploidía sugiere un dinamismo genético e incluso una posible diferenciación más allá de la uniformidad morfológica, con independencia del retroceso experimentado por esta planta en Argelia.

Palabras clave: Anthemideae, *Artemisia herba-alba*, cariológia, citogenética, hibridación *in situ* fluorescente, loci del ADN ribosómico, número cromosómico, organización del genoma, poliploidia, tamaño del genoma.

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INTRODUCTION

Artemisia is one of the biggest genera in the family Asteraceae, with around 500-600 taxa at specific and sub-specific levels and organised in five subgenera (Vallès & al., 2011). *Artemisia herba-alba* Asso belongs to *A.* subgenus *Seriphidium* (Besser ex Less.) Rouy (*A.* sect. *Seriphidium* Besser ex Less., basión.) and is basically distributed in the

Mediterranean region. It has been referred to as a species complex, with closely related taxa considered as independent species in the Irano-Turanian region, or as a single species (Vallès, 1987; Ouyahya & Viano, 1988; Podlech, 2013).

In Algeria, *A. herba-alba*, locally called *chih* in Arabic and *armoïse blanche* in French, occurs mostly in lime-sandy soils with 200-600 mm of mean annual precipitation, across the

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so-called steppe areas ranging from upper semiarid to lower perarid (or Saharian) in the bioclimatic range (Pouget, 1980; Aidoud, 1988).

According to the Algerian *Haut commissariat au développement de la steppe* (HCDS), *A. herba-alba* covers 2 million hectares in Algeria, i.e., ca. 10% of all steppe surface of the country, but this represents a 30% reduction as compared with the situation in 1970 (HCDS, 2010). Nedjimi & Guit (2012) estimated in 3 million hectares the extension of *A. herba-alba* in Algeria. In any case, it is the second most consumed species by domestic animals—mostly sheep—after *Stipa tenacissima* L. (with 4 million hectares) and plays a relevant role in rural areas economy and development (Houmani & al., 2004; HCDS, 2010; Nedjimi & Guit, 2012). Its regression is being replaced by scarcely-palatable taxa, such as *Noaea mucronata* (Forssk.) Asch. & Schweinf. and *Anabasis* spp. (HCDS, 2010). For these reasons, a protection strategy should be undertaken for this species, and a characterisation of its genetic structure is necessary as a basis for conservation policies.

Karyological and cytogenetic data are fundamental for characterising genome organisation, which is key for systematic, evolutionary, and conservation approaches to plant studies (Levin, 2002). Chromosome number is a basic, but also a relevant trait for plant systematic and evolutionary studies, since it allows detecting evolution-driving processes, such as polyploidy and dispolyploidy (Stebbins, 1971; Guerra, 2008; Stuessy, 2009, 2011; Garbari & al., 2012). Genome size is also a crucial cytological parameter because it is related to many others and sometimes also to systematic, ecological and other factors (Bennett & Leitch, 2005). Molecular cytogenetics, and particularly fluorescent *in situ* hybridisation (FISH), offer powerful tools for a deep karyotype analysis, by providing physical maps for some chromosomal regions and allowing to infer mechanisms of chromosomal evolution, such as different kinds or rearrangements (Siljak-Yakovlev & al., 2014). The genus *Artemisia* has been largely studied from this point of view (Vallès & al., 2011), but the *A. herba-alba* complex still needs attention in this subject: despite its large distribution area only a few works on chromosome counts are available (Vallès, 1987; Torrell & Vallès, 1995; Ferchichi, 1997; Bougoutaia & al., 2014) and even less on more in depth karyological or cytogenetic approaches (Vallès, 1987; Torrell & Vallès, 2001; Torrell & al., 2003).

This paper performs a karyological and cytogenetic investigation of a representative set of Algerian populations of *A. herba-alba*, with the aim to provide a comprehensive picture of its genome organisation, which could serve as the basis for further genetic studies and for the conservation and management of this species. Specifically, the objectives were to: 1) estimate the genome size, 2) determine chromosome number, 3) establish the karyotype, 4) physically map rRNA genes in chromosomes, and 5) assess any differences in the above characters in populations with different degrees of conservation.

MATERIAL AND METHODS

Plant material

Achenes and/or fresh leaves were collected from several individuals of twenty-two *A. herba-alba* populations, representing a large sorting of the species in Algeria

(Table 1, Fig. 1). Vouchers were prepared and deposited in the herbarium BCN, of the Centre de Documentació de Biodiversitat Vegetal (Universitat de Barcelona).

Genome size assessment

Nuclear DNA content of each population was estimated by flow cytometry at the Centres Científics i Tecnològics (Universitat de Barcelona). Fresh leaf material, directly collected in the field or obtained from seedlings from germinated achenes, was chopped with a razor blade together with leaf material of an internal standard in 1,200 μ l of LB01 buffer (Doležel & al., 1989), supplemented with 100 μ g/ml of ribonuclease A (RNase A, Boehringer). The resulting liquid was filtered and the nuclei suspension was stained with 36 μ l of propidium iodide (1 mg/ml, Sigma-Aldrich Química, Madrid, Spain) to a final concentration of 60 μ g/ml and kept on ice for 5-20 min. Measurements were carried out with an Epics XL (Coulter Corporation, Hialeah, Florida, USA), with the following configuration of the instrument: excitation of the sample was carried out using a standard 488 nm air-cooled argon-ion laser at 15mW power; forward scatter (FCS), side scatter (SSC) and red (620 nm) fluorescence (FL3) for propidium iodide were acquired. Cells were triggered using the FL3. Although FSC is conventionally used to trigger all, the use of FL3 channel as a triggering parameter allows to discard all particles that do not correspond to nuclei. In order to remove background from nuclei, a consecutive gating strategy of FCS vs FL3log and SSC vs FL3log was used. Finally, FL3 linear histogram was used to assess the DNA content of samples. One run was done per preparation, in which 8000 particles were measured, meaning a minimum of 2000-3000 of them per each fluorescence peak (*Artemisia* and standard). Two independent replicates of each individual and five individuals per population were analysed. The analyses for a given population were all performed on the same day. *Petunia hybrida* Vilm. 'PxPc6' and *Pisum sativum* L. 'Express long' (2C = 2.85 pg and 8.37 pg, respectively; Marie & Brown, 1993) were used as internal standards. Seeds of the standards were provided by the Institut des Sciences du Végétal (CNRS, Gif-sur-Yvette, France). Nuclear DNA contents (2C) were calculated by multiplying the known DNA content of the standard by the quotient between the peak positions (mode) of the target species and the standard in the histogram of fluorescence intensities, assuming a linear correlation between the fluorescent signals from the stained nuclei of the unknown specimen, the known internal standard, and the DNA amount (Doležel, 1991). From the holoploid 2C value estimated as described above, we calculated the monoploid 1Cx value by dividing the latter by the ploidy level (Greilhuber & al., 2005).

Chromosome counting

Root tips were pre-treated with 0.05% aqueous colchicine for 2h 15min – 2h 45min at room temperature and fixed in absolute ethanol and glacial acetic acid (3:1) at room temperature for 1-2 h, then kept in the same fixative at 4°C. For chromosome counts, root tips meristems were excised, hydrolysed for 1.5-2 min on 1M HCl at 60°C, washed with

Table 1. Data on chromosome number and nuclear DNA amount of the populations studied

| Population number ¹ | Collection data (collector: Y. Bougoutaia) | Herbarium voucher | 2n ² | 2C ³ ±SD (pg) | 1C ⁴ (Mbp) | 1Cx ⁵ (pg) | HPCV ⁶ <i>Artemisia</i> | HPCV ⁶ standard |
|--------------------------------|---|----------------------|-----------------|-----------------------------|-----------------------|-----------------------|---------------------------------------|-------------------------------|
| 1 | Ain Oussera (W Djelfa), 35°20'52"N, 2°57'14"E. 780 m. 06.III.2014 (voucher 10.IV.2015) | BCN 121316 | 36 | 12.63±0.70 | 6177.93 | 3.16 | 2.89±0.35 | 2.68±1.38 |
| 2 | Oued Sedar (W Djelfa), 34°28'27"N, 3°16'43"E. 1270 m. 10.III.2014 | BCN 121317 | 36 | 13.07±0.37 | 6391.03 | 3.27 | 1.22±0.99 | 0.98±1.08 |
| 3 | Benhamed (W Djelfa), 34°20'19"N, 2°53'21"E. 1220 m. 10.III.2014 | BCN 121318 | 36 | 12.42±0.12 | 6071.62 | 3.10 | 4.04 ± 0.60 | 3.10±1.40 |
| 4 | El-Ach (W Bordj Bou Arreridj), 35°52'26"N, 4°34'31"E. 724 m. 7.III.2014 | BCN 121319 | 36 | 13.31±0.28 | 6509.05 | 3.33 | 1.26 ± 0.97 | 0.72±0.62 |
| 5 | El-Hamel (W M'sila), 35°05'15"N, 04°06'01"E. 960 m. 16.V.2014 | BCN 121320 | 18 | 5.54±0.13 | 2707.46 | 1.38 | 3.89±0.47 | 3.01±0.87 |
| 6 | Zerarka (W M'sila), 35°35'50"N, 04°09'37"E. 550 m. 07.III.2014 | BCN 121321 | 18 | 6.87±0.21 | 3359.48 | 1.72 | 2.16±1.08 | 5.67±1.89 |
| 7 | Mohamed Boudiaf (W M'sila), 34°52'56"N, 04°21'18"E. 883 m. 18.V.2014 (voucher 9.IV.2015) | BCN 121322 | 18 | 5.39±0.07 | 2636.78 | 1.35 | 2.80±0.29 | 2.71±0.37 |
| 8 | Ben-Srou Est (W M'sila), 35°01'59"N, 4°35'30"E. 700 m. 18.V.2014 (voucher 9.IV.2015) | BCN 121323 | 18 | 7.18±0.25 | 3509.93 | 1.79 | 3.32±1.00 | 4.20±2.92 |
| 9 | Ouled Slimane Sud (W M'sila), 34°51'50"N, 4°49'49"E. 600 m. 18.V.2014 (voucher 9.IV.2015) | BCN 121324 | 18 | 5.64±0.79 | 2755.62 | 1.41 | 3.32±1.00 | 4.20±2.92 |
| 10 | Guellet Sidi Saad (W Laghouat), 34°15'15"N, 1°58'54"E. 1210 m. 13.III.2014 | BCN 121325 | 36 | 11.62±0.26 | 5684.45 | 2.91 | 3.06±0.63 | 3.37±2.29 |
| 11 | Seboga (W Laghouat), 34°03'14"N, 1°53'46"E. 1380 m. 13. III.2014 | BCN 121326 | 36 | 13.84±0.85 | 6767.95 | 3.46 | 1.59±1.32 | 1.15±1.09 |
| 13 | Bouaiche (W Médéa), 35°34'33"N, 2°18'41"E. 870 m. 06.III.2014 | BCN 121327 | 36 | 13.10±0.24 | 6404.85 | 3.27 | 2.69±0.81 | 2.39±0.69 |
| 14 | Rechaiga (W Tiaret), 35°26'18"N, 2°03'54"E. 871 m. 13.III.2014 | BCN 121328 | 36 | 12.07±0.39 | 5903.78 | 3.02 | 3.28±0.53 | 1.43±0.99 |
| 15 | Ain Dheb (W Tiaret), 34°48'60"N. 1°34'17"E. 1100 m. 13.III.2014 | BCN 121329 | 36 | 13.29±0.47 | 6498.83 | 3.32 | 3.25±0.57 | 4.01±2.15 |
| 16 | Sidi Abderrahmane (W Tiaret), 34°39'07"N, 01°06'53"E. 1050 m. 13.III.2014 | BCN 121330 | 36 | 13.18±0.88 | 6445.06 | 3.30 | 1.24±1.03 | 1.24±1.25 |
| 17 | Baniane (W Biskra), 34°59'52"N. 6°02'01"E. 545 m. 18.III.2014 (voucher 9.IV.2015) | BCN 121331 | 18 | 6.84±0.09 | 3346.53 | 1.71 | 3.01±0.42 | 2.89±0.52 |
| 18 | Tifefal (W Batna), 35°07'25"N, 6°14'11" E. 845m. 18.V.2014 (voucher 9.IV.2015) | BCN 121332 | 18 | 7.01±0.24 | 3427.19 | 1.75 | 3.36±0.58 | 2.87±0.75 |
| 19 | Arris (W Batna), 35°09'57"N, 6°14'12"E. 970 m. 18.III.2014 (voucher 9.IV.2015) | BCN 121333 | 36 | 12.51±0.54 | 6116.22 | 3.13 | 2.30±0.98 | 1.59±1.03 |
| 20 | Zerzour (W Batna), 35°06'39"N, 5°01'28"E. 575 m. 18.V.2014 (voucher 9.IV.2015) | BCN 121334 | 36 | 13.35±0.26 | 6526.52 | 3.34 | 2.82±0.85 | 2.96±0.70 |
| 26 | Oum Ali (W Tébessa), 35°07'06"N, 8°16'39"E. 1017 m. 12.III.2014 | BCN 121335 | 36 | 11.70±0.31 | 5719.04 | 2.92 | 2.45±1.08 | 3.62±0.62 |
| 31 | Taoura (W Souk-Ahras), 36°02'20"N, 8°06'50"E. 626 m. 12.III.2014 | BCN 121336 | 36 | 12.54±0.56 | 6131.58 | 3.13 | 2.59±0.66 | 1.96±1.16 |
| 37 | Slim Est (W M'sila), 35°00'33"N, 3°53'36"E. 1020 m. 24.V.2014 (voucher 9.IV.2015) | BCN 121337 | 36 | 12.88±0.25 | 6300.29 | 3.22 | 1.97±1.09 | 3.45±0.82 |

Notes: ¹Population numbers are the same as in Figure 1. ²Somatic chromosome number (ploidy level). ³Holoploid nuclear DNA content (2C value ± standard deviation). ⁴Nuclear DNA content; 1pg = 978 Mbp (Doležel & al., 2003). ⁵Monoploid genome size. ⁶Half-peak coefficient of variation for flow cytometric assessments, performed with *Pisum* as standard (see Materials and methods for details).

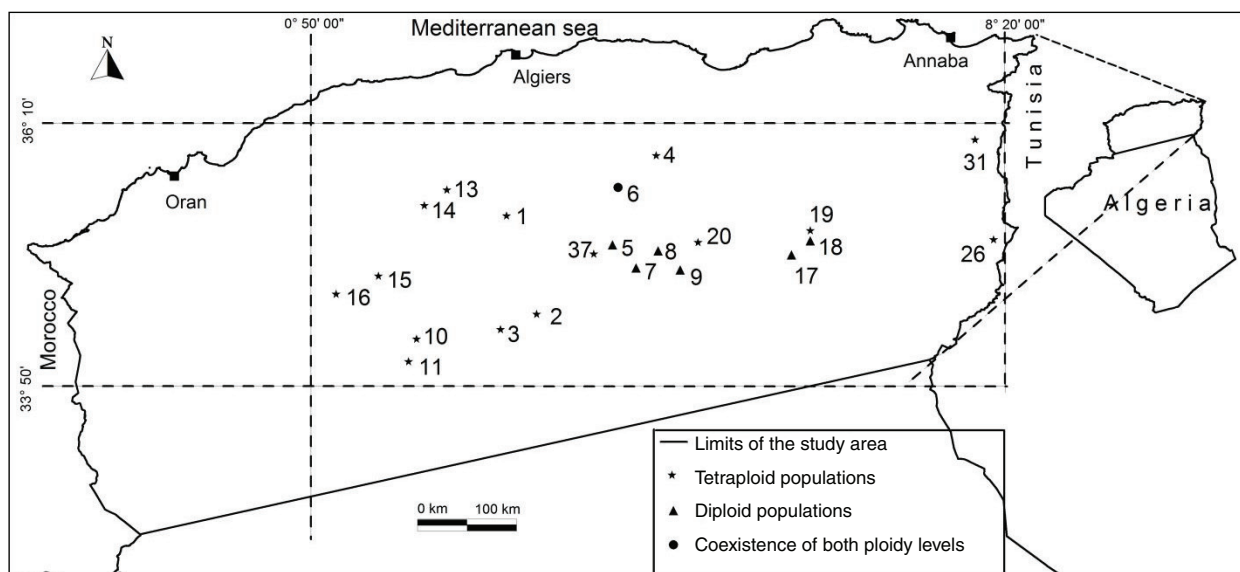


Fig. 1. Geographical location of the populations studied.

distilled water, stained on 2% aceto-orcein and squashed on a drop of 9:1 45% acetic acid:glycerol. Slides were observed with a Zeiss Axioplan microscope and the best metaphase plates were photographed with an AcioCam HRm camera.

Fluorescent in situ hybridisation (FISH)

FISH for the detection of 35S (45S; 18S-5.8S-26S) and 5S rDNA loci was performed in one population for every detected ploidy level. To obtain protoplasts, root tips pre-treated and fixed as mentioned above were washed in 0.01 M citric acid-sodium citrate buffer (pH 4.6) for 10 min and digested in an enzyme mixture [3% cellulase “Onozuka” R-10 (Yakult Honsha Co. Tokyo, Japan) and 30% pectinase (Sigma) or 4% cellulase “Onozuka” R-10 (Yakult Honsha Co. Tokyo, Japan), 1% pectolyase Y-23 (Seishin Co. Tokyo, Japan), and 4% hemicellulase (Sigma)] diluted at 50% in citrate buffer, for 30 min at 37 °C. Then, they were rinsed with distilled water and squashed on a drop of freshly prepared 45% acetic acid; the coverslips were removed after freezing and air dried. The probe used for 35S rDNA localisation was a plasmid carrying a 2.5 kb insert of 26S rRNA gene from *Solanum lycopersicum* L. labelled with Cy3 (Jena Biosciences) using the Nick Translation Mix (Roche). The 5S rDNA probe was an approximately 0.7 kb-long trimer of 5S rRNA genes from *A. tridentata* Nutt., labelled with Green dUTP using the Nick Translation Mix (Abbott Molecular). This probe contained three units of the 5S rRNA gene (120 bp) and the non-coding intergenic spacers (about 290 bp). FISH was carried out according to Gouja & al. (2015). Samples were counterstained with Vectashield (Vector Laboratories, Inc., Burlingame, CA, USA), a mounting medium containing 500 ng/μl of 4',6-diamidino-2-phenylindole (DAPI). The fluorescence signals were analysed and photographed using a digital camera (AxioCam HRm, Zeiss) coupled to a Zeiss Axioplan microscope; images were analysed with Axiovision HR Rev3, version 4.8 (Zeiss) and processed for colour balance, contrast and brightness uniformity in Adobe Photoshop. The data were submitted to

the Plant rDNA database, a database compiling information on rDNA signal number, position and organisation (Garcia & al., 2012).

Karyological analyses

Pictures of 3-6 metaphase plates with a similar degree of chromosome condensation were used to calculate karyotype data and elaborate idiograms in three populations, representing both ploidy levels. Morphometric karyotypic parameters were calculated with MicroMeasure 3.3 (Colorado State University). Graphics of the haploid idiograms were performed with PowerPoint (Microsoft Office 2010).

Statistical analyses

A one-way ANOVA was used to analyse interpopulation variation of genome size within both ploidy levels. Bonferroni tests for mean comparisons were also carried out. Statistical analyses were performed with XLSTAT-Pro, v.7.5.2 (Addinsoft).

RESULTS AND DISCUSSION

Chromosome number and nuclear DNA amount data of the 22 accessions studied are presented in Table 1. Table 2 contains karyotype morphometric data and rDNA FISH results obtained in selected populations, comprising both ploidy levels found. Fluorescence histograms for each ploidy level are provided in Fig. 2.

Ploidy level variation and its relationships with distribution and conservation status

The studied accessions are diploid $-2n = 2x = 18$ chromosomes, 6 populations— or tetraploid $-2n = 4x = 36$ chromosomes, 15 populations—, one of them —number 6— presenting both ploidy levels; additionally, a few counts

Table 2. Summary of karyological results. The superscripts indicate: ¹chromosomal formula according to Levan & al. (1964); ²total karyotype length; ³mean chromosome length; ⁴chromosome length range; ⁵standard deviation; ⁶length ratio of long and short chromosome arms (Levan & al., 1964); ⁷centromeric index [I or index in Levan & al. (1964)]; ⁸intrachromosomal asymmetry index (Romero, 1986); ⁹interchromosomal asymmetry index (Romero, 1986); ¹⁰symmetry class according to Stebbins (1971); ¹¹FISH results (number of colocalised 35S and 5S loci). Karyological results correspond to populations 06 (2x and 4x), 17 and 26, and FISH results correspond to populations 17 (2x) and 26 (4x).

| Population number | 2n | Ploidy level | Chromosomal formula ¹ | TKL ² | MCL ³ | CLR ⁴ | SD ⁵ | R ⁶ | CI ⁷ | A1 ⁸ | A2 ⁹ | Stebbins class ¹⁰ | rDNA ¹¹ |
|-------------------|----|--------------|----------------------------------|------------------|------------------|------------------|-----------------|----------------|-----------------|-----------------|-----------------|------------------------------|--------------------|
| 6 | 18 | 2x | 12m+6sm | 40.69 | 4.52 | 3.29-4.59 | 0.14 | 1.54 | 0.40 | 0.29 | 0.03 | 2A | - |
| 6 | 36 | 4x | 30m+6sm | 76.08 | 4.23 | 3.74-4.68 | 0.27 | 1.43 | 0.42 | 0.25 | 0.06 | 2A | - |
| 17 | 18 | 2x | 16m+2sm | 34.85 | 3.87 | 3.90-4.28 | 0.27 | 1.37 | 0.43 | 0.24 | 0.07 | 2A | 2 |
| 26 | 36 | 4x | 30m+6sm | 51.56 | 2.86 | 2.44-3.21 | 0.21 | 1.40 | 0.42 | 0.25 | 0.07 | 2A | 4 |

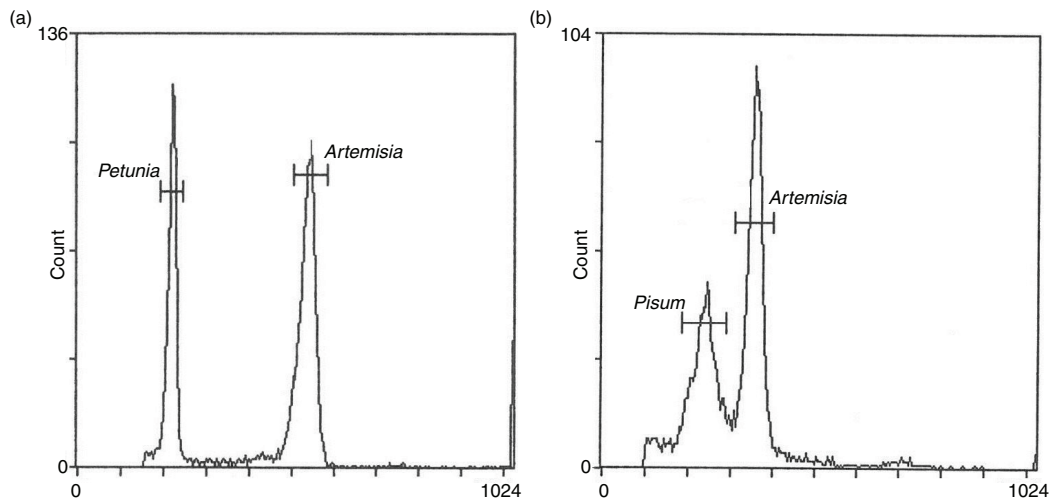


Fig. 2. Fluorescence histograms of genome size assessments by flow cytometry using propidium iodide: **a**, *Artemisia herba-alba* (diploid individuals of population n.º 6; 2C = 6.87 pg) with *Petunia* (2C = 2.85 pg) as internal standard; **b**, *A. herba-alba* (tetraploid population n.º 15; 2C = 13.29 pg) with *Pisum* (2C = 8.37 pg) as internal standard.

could also suggest the existence of polyploid individuals in population number 5 although genome size assessments indicate only diploids (Bougoutaia, unpubl. data). The results are coincidental with previous counts on this taxon, which already revealed the existence of two ploidy levels (Vallès & al., 2011). According to Watanabe's Index to Chromosome Numbers in Asteraceae (Watanabe, 2015), our study contributes the first diploid chromosome number for *A. herba-alba* from Algeria. From this country, only tetraploid cytotypes were previously recorded, after cytogenetic analysis of 10 populations (Betina & al., 2007; Bougoutaia & al., 2014). In Morocco, only the diploid level has been reported (Kawatani & Ohno, 1964; Ouyahya & Viano, 1988), whereas in Tunisian populations either diploid (Vallès & Torrell, 1995) or diploid and tetraploid (Ferchichi, 1997) cytotypes have been reported. A similar situation has been described for the northern part of the Mediterranean basin, in the Iberian Peninsula, where both cytotypes also coexist (Vallès, 1987). The karyotypes (Table 2, Fig. 3) are rather symmetric (2A asymmetry class; Stebbins, 1971), in agreement with the most common pattern in the genus (Vallès & al., 2011).

Polyploidy is one of the main evolutionary drivers in plants (Cui & al., 2006; Soltis & al., 2009) and is very common in *Artemisia*, where ploidy levels up to 16x have been reported (Pellicer & al., 2010). The polyploid North African populations of *A. herba-alba* reflect a process of genome

differentiation. The same situation in Iberian accessions has led to the consideration of two taxa, separated either at specific or at subspecific levels [2x: *A. valentina* Lam., *A. herba-alba* subsp. *valentina* (Lam.) Mascl.; 4x: *A. aragonensis* Lam., *A. herba-alba* subsp. *herba-alba*], although their morphological distinction is very difficult (Vallès, 1987). The coexistence of diploid and tetraploid individuals in one population and the possibility of this being the case for another one is not rare in *Artemisia*, since some populations with mixed ploidy levels have been detected in other species (Kreitschitz & Vallès, 2003; Garcia & al., 2009a), and could be interpreted as a sign of genome dynamism that might be associated to some degree of differentiation.

Out of the 22 populations studied, 12 —2-4, 6, 10, 11, 13-16, 26, and 31— are in a rather good state of conservation, since they are located in areas protected by the HCDS, whereas the remaining 10 —1, 5, 7-9, 17-20, and 37— are in a more or less advanced degradation situation outside these protected areas. All populations in the HCDS areas are tetraploid —excepting population 6, which presents both diploid and tetraploid individuals—, while those populations in the degraded areas are mostly diploid —60%—. There is no significant difference in genome size among tetraploids from the degraded populations and the preserved ones ($p = 0.760$). Interestingly, all diploid populations are only found in arid environments while the tetraploid ones

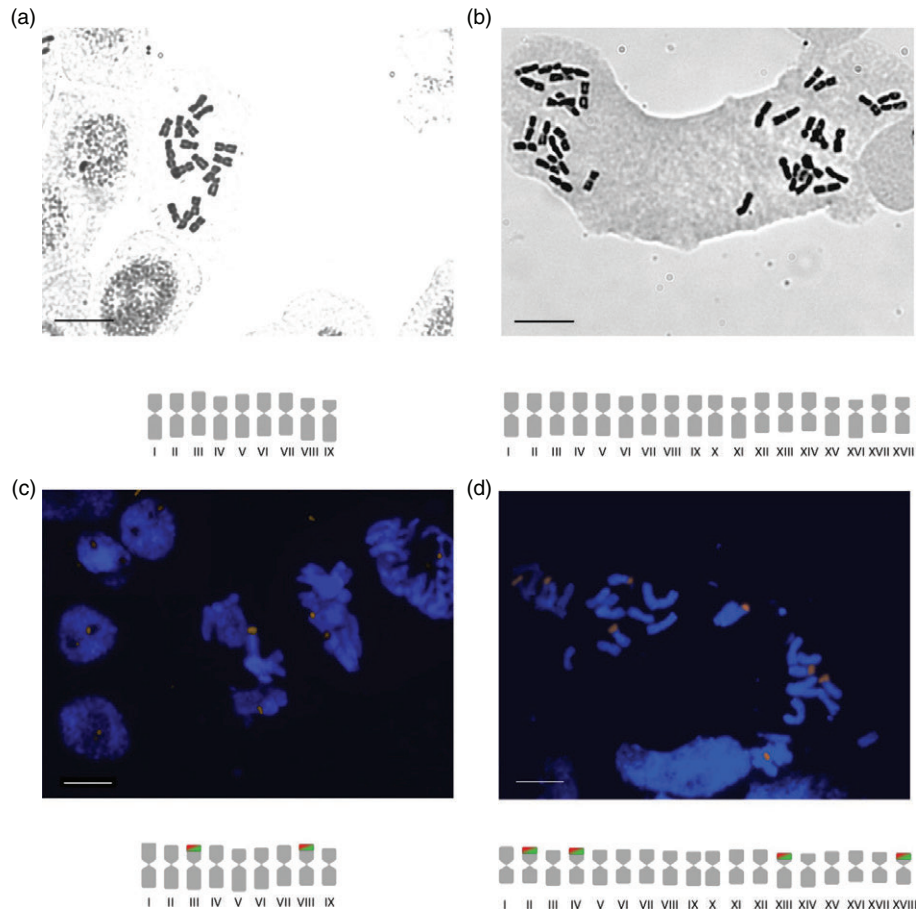


Fig. 3. Orcein-stained somatic metaphases and haploid idiograms of a diploid (a) and a tetraploid (b) individual (both from population 6), and somatic metaphases submitted to fluorescence *in situ* hybridisation with haploid idiograms showing the rDNA location in one diploid (c) and one tetraploid (d) individual (from populations 17 and 26 respectively). Scale bars, 10 μ m.

are found in both arid and semi-arid environments. In fact, all diploid populations grow in an arid corridor, whereas no polyploid population does; even polyploid populations growing relatively close to diploid ones, such as 20 and 37, are not under strictly arid conditions. Diploids may thus be specifically adapted to arid conditions while the bioclimatic range of tetraploids is wider. This could explain why diploid populations occupy only the central part of the studied area, whereas polyploid ones are distributed both in this central part and in other zones. Polyploidy confers evolutionary advantages derived from an increased genetic endowment and hence potential for variability. This can generate individuals capable of exploiting new niches (Leitch & Leitch, 2008). Lumaret & al. (1987) found that diploid populations of *Dactylis glomerata* L. were confined to habitats of ancient origin, while tetraploids were widespread. Polyploids may be more successful colonizers after glacial periods than their closely related diploids (Brochmann & Brysting, 2004) because polyploidy would contribute to their environmental stress tolerance and to their larger expansion.

Genome size

Nuclear DNA amounts (Table 1), evaluated here for the first time in North African representatives of the taxon, ranged from 5.39 to 7.18 pg for diploid accessions and from

11.62 to 13.84 pg for tetraploids. These results are similar to those obtained in Iberian populations (Torrell & Vallès, 2001), where 2C values of 6.57 and 12.48 pg were obtained for diploid and tetraploid accessions, respectively. Genome size in both Iberian and Algerian populations reflects well the two ploidy levels, with almost no downsizing with polyploidy. Although a relative decrease in nuclear DNA content is frequent in polyploids (Leitch & Bennett, 2004), Pellicer & al. (2010) found no genome downsizing in the South American endemic *A. mendozana* DC., and attributed this phenomenon to a recent polyploidy event, which could be the case as well here. However, in the North American *A.* subgenus *Tridentatae* (Rydb.) McArthur (*A.* sect. *Tridentatae* Rydb., basion.), Garcia & al. (2008) reported both decrease and conservation of genome size in whole genome duplicated genomes.

The expected nuclear DNA amount differences between diploids and polyploids have been addressed in the ploidy level variation section. In addition, significant differences in genome size were observed within both diploid and polyploid populations. The ANOVA test shows significant differences in genome size within diploid level — $F = 26.224$, $p < 0.0001$ — with two groups clearly differentiated in Bonferroni test, the first one comprising populations 6, 8, 17 and 18 and the second one including populations 5, 7 and 9. In the tetraploid level the observed differences are also

significant — $F = 8.106$, $p < 0.0001$ —. The mean comparison test indicates that the DNA amount from population 11 differs significantly from populations 10 and 26.

These intra-ploidy level differences do not seem to be directly associated to environmental factors such as altitude or climate, nor to geography and thus are not easy to explain. For example, the highest genome size in tetraploids corresponds to population 11, growing at 1386 m a.s.l. and the lowest one to population 10, growing at 1210, the two highest altitudes for all the studied populations. Both populations are geographically close to each other and at similar altitudes; population 11 grows in a mountain habitat with very cold winters, whereas population 10 is located in a high plain, where winters could be slightly milder. Also, the population with the second lowest genome size among the tetraploids —10, with a very similar value to 6— is located near Tunisia, at 1070 m and in the opposite side of the study area. Similarly, climatic conditions do not seem to explain genome size differences in the rest of our data. Duchoslav & al. (2013) also showed that environmental variables were not relevant in modulating genome size in *Allium oleraceum* L.

Such significant differences in GS within each ploidy level could be due to a few chromosome losses, gains, rearrangements, or to changes in chromosome dimensions. At a first glance, aneuploidy/dysploidy is not the most likely of those possible causes, since all the numerous chromosome counts in *A. herba-alba* reported $2n = 18$ or $2n = 36$ chromosomes (Watanabe, 2015) and since the subgenus *Seriphidium*, to which this species belongs, has been largely studied and is, in fact, one of the few groups in *Artemisia* lacking dysploidy (Vallès & al., 2011). To shed light on this matter, further studies will be undertaken focusing on a deep analysis of karyotypic features.

rDNA physical mapping

FISH analyses (Table 2, Fig. 3), performed for the first time in North African materials of the taxon, detected 4 signals (2 loci) in diploid and 8 signals (4 loci) in tetraploid cytotypes for both 35S and 5S rDNA, which are colocalised, presenting an L-type (linked) organisation. This agrees with the only previous FISH data in the species, made on Iberian populations: 4 signals were detected in the diploid accession studied for both 35S and 5S rDNA and 8 signals were reported in the tetraploid population (for 35S only). Colocalisation of 35S and 5S rDNA genes found in our study is in agreement with the first report within *Artemisia* (Torrell & al., 2001) as well as in *A. herba-alba* itself (Torrell & al., 2003) and in other species of this genus (García & al., 2007, 2009b).

Concluding remarks

This first karyological and cytogenetic study on Algerian *A. herba-alba*, based on an ample number of populations, shows the relevance of polyploidy within this taxon. The presence of two ploidy levels suggests a genomic dynamism and even a possible differentiation underlying the morphological uniformity and despite of the dramatic decrease experienced by this plant in Algeria in terms of surface coverage.

Further studies enlarging the sampling area in order to better assess the distribution limits of the polyploidy cytotypes are needed. Also, population genetic studies focusing on the possible effect of the decrease in surface covered by *A. herba-alba* on the persistence and conservation not only of this species but of the arid zone landscape, which constitutes an important economic resource.

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