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First genome size assessment in the genus *Peganum* and in the family Nitrariaceae: Iberian and North African data on *Peganum harmala*, including an intensive sampling in Tunisia

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Abstract: *Peganum harmala* is a halonitrophilous perennial herb that is relevant in the landscape of steppe, semidesert, and desert territories in southern Europe, northern Africa, and southwestern Asia. We present here data on the nuclear DNA amount assessment in one Iberian and 17 Tunisian populations of this species. The 2C values, belonging to the very small genome category, ranged from 0.61 to 0.67 pg. These data are the first on genome size for the species and for the whole genus and the whole family. In addition, the somatic chromosome number ($2n = 24$) has been counted in one population, confirming previous reports and constituting the first one in a Tunisian accession of the species. The results show a high degree of homogeneity of the character studied within the species. These new data, filling a gap in genome size knowledge at family level, together with the specific homogeneity in genome size in a large area, could be relevant for further large-reach analyses on genome size evolution.

Key words: C-value, flow cytometry, Iberian Peninsula, Nitrariaceae, nuclear DNA amount, *Peganum harmala*, somatic chromosome number, Tunisia

1. Introduction

The genus *Peganum* L. comprises 4–6 species of perennial herbs or shrubs distributed from the Mediterranean region to Central Asia and in southern North America (Mexico) (Güemes and Sánchez-Gómez, 2015). The genus had been classically included in the family Zygophyllaceae, but recent approaches currently classify it in the Nitrariaceae, a small family constituted by three genera and ca. 20 species (Stevens, 2001).

Peganum harmala L. is a halonitrophilous perennial herb living in dry and warm areas with anthropic influence. It is rather abundant in steppe, semidesert, and desert territories in southern Europe, northern Africa, and southwestern Asia (Güemes and Sánchez-Gómez, 2015). In Tunisia, *Peganum harmala* is present and abundant in an extensive dry area throughout most of the country, excluding the southernmost and northernmost parts, respectively with Saharan and wet climates (Pottier-Alapetite, 1979–1981). This species, rich in alkaloids, has numerous medicinal properties and is frequently reported in ethnobotany and phytotherapy (Koyuncu et al., 2009; Niroumand et al., 2015, and references therein). Among its uses, hallucinogenic properties have been reported (Gable, 2007).

Nuclear DNA content or genome size and chromosome number are two very relevant plant karyological and cytogenetic features (Bennett and Leitch, 2005; Stuessy, 2011). They are related to each other and may occur in relationship with many other plant traits, from morphological to ecological, including phylogenetic, systematic, and taxonomic ones (Bennett and Leitch, 2005, and references therein). Within the framework of a larger study devoted to diverse *Peganum harmala* characteristics in Tunisia (Hajji, in prep.), we realized that no information at all on genome size for this species existed and that its chromosome number had not been determined in Tunisian material. Consequently, the aims of the present paper were: (i) to perform an extensive prospection of *Peganum harmala* in Tunisia in order to assess its genome size and its variation across populations, (ii) to collect and analyze from the same point of view one European population of the taxon with the intention of comparing

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the results obtained on each side of the Mediterranean, and (iii) to count the chromosome number of the species in a Tunisian accession.

2. Materials and methods

2.1. Plant materials

Seventeen populations were collected in Tunisia, representing the area of distribution of *Peganum harmala* in this territory. In addition, and for the purpose of comparison, one population was collected in the Iberian Peninsula. Fresh leaves for flow cytometric measurements (kept on slightly humidified Kleenex paper), seeds (when present) for chromosome counts, and material for herbarium vouchers preparation were taken. Complete collection data are given in the Table, including the herbarium code of each voucher, deposited in the herbarium BCN (Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona).

2.2. Genome size assessments

Genome size was estimated by flow cytometry. Leaf tissue of the target plant and the internal standard was chopped together in a plastic petri dish with a razor blade in 1200 µL of LB01 isolation buffer (Doležel et al., 1989), and supplemented with 100 µg/mL ribonuclease A (RNase A, Boehringer). *Lycopersicum esculentum* L. 'Montfavet 63-5' (2C = 1.99 pg, Lepers-Andrzejewski et al., 2011) was used as internal standard. Five individuals per population were analyzed and two replicates per sample were processed. After filtering through a nylon mesh of 70 µm pores, samples were stained with 36 µL of propidium iodide to a final concentration of 60 µg/mL (Sigma-Aldrich, St. Louis, MO, USA), kept on ice until measurement, and processed in an Epics XL flow cytometer (Beckman Coulter, Brea, CA, USA) at the Centres Científics i Tecnològics, Universitat de Barcelona. Further technical details on the procedure and on the cytometer setting can be found in Pellicer et al. (2010).

2.3. Chromosome counts

Roots were obtained from seeds germinated on wet filter paper in petri dishes in the dark and at room temperature. Root tips were pretreated with 0.002 M aqueous 8-hydroxyquinoline (Sigma-Aldrich, St. Louis, MO, USA) solution for 3–4 h in a cold room and then they were fixed in a freshly prepared 3:1 mixture of absolute ethanol and glacial acetic acid. Root tips were hydrolyzed into a 1 M solution of HCl at 60 °C for 10 min and then they were stained with aqueous 1% aceto-orcein (Sigma-Aldrich, St. Louis, MO, USA) for a minimum of 30 min at room temperature. Finally, the apexes were squashed between slide and cover with a drop of a 9:1 solution of 45% acetic acid and glycerol. Metaphase plates were observed and photographed with a digital camera (Zeiss Axio Cam MRc 5) on a Zeiss Axioplan microscope (Carl Zeiss AG Corporate, Oberkochen, Germany).

3. Results and discussion

The results of flow cytometric genome size estimations in the 18 accessions studied are provided in the Table. The half-peak coefficients of variation for both the target plants and the internal standard, always lower than 5, account for the reliability of the data obtained. The Figure illustrates the somatic chromosome count performed in one of these populations.

To the best of our knowledge (http://data.kew.org/cvalues, accessed August 26, 2016), the present data constitute the first estimations of nuclear DNA amount in the studied species, the whole genus *Peganum* and the whole family Nitrariaceae. Genome size is extremely homogeneous in all the populations sampled, with 2C values ranging from 0.61 to 0.65 pg for the 17 Tunisian accessions, covering a large and diversified area of this territory, and being 0.67 pg in the Iberian population, given for comparison. Two varieties are quoted in some Tunisian floras (Le Floc'h et al., 2010), which are no longer considered (http://www.theplantlist.org, accessed August 26, 2016). Genome size data do not provide any evidence at all of infraspecific differentiation. In addition, this coincidence in North African and European populations may allow us to hypothesize a very short range of variation of nuclear DNA content over the complete distribution area of the genus.

The nuclear DNA amounts in *Peganum harmala* are placed among the lowest in angiosperms: they belong to the very small genome size category, constituted by those plants with 2C values lower than or equal to 2.8 pg (Leitch et al., 2005). Herbaceous condition and annual life cycle have classically been considered characters linked to small genome sizes (Bennett, 1972; Bennett and Leitch, 2005) in relationship with generation time, although this rule has proved not always to have been followed (Pellicer et al., 2014, and references therein). The studied species is herbaceous, though not annual, but, in any case, it shows this low nuclear amount trait. Arid climate and extreme conditions (such as salinity) have also been postulated as characteristics implying low genome size amounts for the plants adapted to them (Bennett and Leitch, 2005, and references therein), although examples of higher genome sizes in desert and other arid lands have been reported (Levin et al., 2002; Greilhuber and Leitch, 2013). This is in agreement with the large genome constraint hypothesis, suggesting that plants with large genomes are underrepresented in extreme environments, among which arid and saline ones may be included (Knight et al., 2005). This could be one satisfactory explanation for the very small genomes in *Peganum harmala*, which meets the ecological characters of drought and salt tolerance. Similarly, *Reaumuria soongarica* (Pall.) Maxim (Tamaricaceae), adapted to extreme drought and salinity,

Table. Provenance, herbarium voucher, and nuclear DNA content of the *Peganum harmala* populations studied.

Population	2C in pg (standard deviation)	Half-peak coefficient of variation for *Peganum*	Half-peak coefficient of variation for the standard	1C in Mbp[2]
Tunisia: Kasserine, Hassi Frid, 30-VI-2014, Ali Hajji, Ridha Hajji (BCN 113783)	0.63 (0.01)	4.62	0.33	308
Tunisia: Gafsa, Gsar Gafsa, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113781)	0.61 (0.02)	4.79	0.15	298
Tunisia: Gafsa, Belkhir, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113780)	0.63 (0.01)	4.02	0.44	308
Tunisia: Gabès, Manzel Habib, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113784)	0.62 (0.02)	3.44	0.90	303
Tunisia: Gabès, Chnini, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113782)	0.64 (0.01)	4.63	0.60	313
Tunisia: Mednine, Koutine, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113788)	0.63 (0.01)	2.71	0.70	308
Tunisia: Mednine, north of Mednine city, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113787)	0.64 (0.01)	3.87	0.62	313
Tunisia: Sfax, Mahres, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113786)	0.63 (0.02)	3.44	0.90	308
Tunisia: Sfax, south of Sfax city, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113785)	0.61 (0.01)	4.02	0.44	298
Tunisia: Mahdia, Eljam, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113792)	0.62 (0.02)	3.17	0.42	303
Tunisia: Mahdia, Karkar, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113791)	0.65 (0.02)	3.52	0.59	318
Tunisia: Sousse, Hay Zouhour, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113789)[1]	0.64 (0.01)	3.90	0.90	313
Tunisia: Sousse, Sidi Bouali, 1-VII-2014, Ridha Hajji, Jaklouti Mohamed Salah (BCN 113790)	0.65 (0.02)	3.43	0.43	318
Tunisia: Seliana, Rouhia, 10-VII-2014, Ridha Hajji, Mohamed Hajji (BCN 113776)	0.63 (0.01)	4.19	2.94	308
Tunisia: Seliana, north of Seliana city, 10-VII-2014, Ridha Hajji, Mohamed Hajji (BCN 113777)	0.63 (0.01)	4.65	2.70	308
Tunisia: Kef, Gsour, 10-VII-2014, Ridha Hajji, Mohamed Hajji (BCN 113779)	0.62 (0.02)	4.61	0.90	303
Tunisia: Kef, Dehmeni, 10-VII-2014, Ridha Hajji, Mohamed Hajji (BCN 113778)	0.61 (0.01)	3.45	2.47	298
Spain, Catalonia, Lleida, Segrià: Tossal de Moradilla, 7-VIII-2014, Abdelhamid Hajji, Joan Vallès, Josep Vigo (BCN 113775)	0.67 (0.02)	4.60	1.01	328

[1] Population in which somatic chromosome number ($2n = 24$) has been assessed (see text).

[2] 1 pg = 978 Mbp (Doležel et al., 2003).

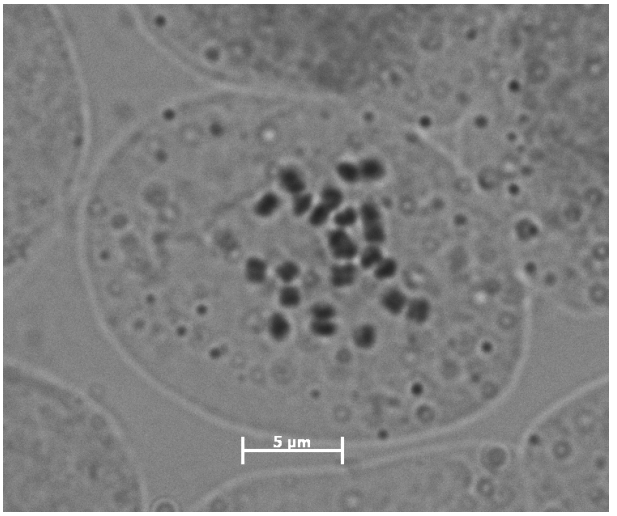

Figure. Mitotic metaphase ($2n = 24$) of *Peganum harmala* from Sousse (see the Table for locality details).

and with a chromosome number ($2n = 22$) close to that of *Peganum* (see next paragraph), has a nuclear DNA content of 2C = 1.61 pg (Wang et al., 2011), much higher than that of *Peganum harmala*, but included in the very small genome category too.

The somatic chromosome number counted in one of the Tunisian populations (Sousse, see the Table for details) is $2n = 24$ (Figure). The degree of condensation of the chromosomes does not permit karyotype elaboration (which was not our purpose), but confirms this karyological character in the species. The same number (either in meiotic or in mitotic preparations) had been reported in material from Spain (Lorenzo-Andreu, 1951; Díaz Lifante, 1991), Iraq (Hilu, 1979), the Caucasus (Magulaev, 1979), China (Ma et al., 1990), and Morocco (Ruíz de Clavijo, 1991). Our present count is the first one on Tunisian and the second one on North African material of the species. The only discrepant count for this taxon is $2n = 22$ (Ma et al., 1984). The fact that the same first author later reports $2n = 24$ for the species in the same territory (Xinjiang; Ma et al., 1990) makes us consider the possibility of a misinterpretation in the first count. In any case, the constancy in the species' chromosome number, in agreement with the above-commented matter of nuclear DNA content, is deduced from the present result and the previous ones, covering the taxon's whole distribution area.

The present results contribute the first data on genome size for *Peganum harmala* and its whole genus and family, and confirm its chromosome number, the low nuclear DNA content being in agreement with some ecological characters. They show a very high degree of homogeneity in these karyological and cytogenetic traits, which cannot be associated with differentiation among populations. Other characters (morphological, genetic, chemical…) could throw more light on possible variation within this species. The new data here contributed, filling a gap in genome size knowledge at family level, together with the specific homogeneity in genome size in a large area, could be relevant for further large-reach analyses on genome size evolution.

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