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Short communication

# Karyotype characterization of *Argania spinosa* (L.) Skeel (Sapotaceae)

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**Abstract**

*Argania spinosa* (L.) Skeel is an endemic species from Southwest Morocco being the unique representative of the tropical Sapotaceae in this area. The cytology of this species is poorly known in spite of its great socio-economical and ecological interest in these arid and semi-arid zones. The objective of this work is to characterize the karyotype of *A. spinosa* species in somatic cells from root tips. Samples analyzed showed a karyotype constituted for ten pairs of chromosomes ($2n=2x=20$) and the putative karyotype proposed has been of four submetacentric and six metacentric pairs. The four submetacentric pairs were the longest with a mean total length between 1.14 and 1.69 μm and the total length of six metacentric pairs were between 0.59 and 1.03 μm.
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*Keywords:* Argan tree; *Argania spinosa*; Chromosome number; Karyotype; Sapotaceae; *Sideroxyleae*; Taxonomy

## 1. Introduction

Argan tree [*Argania spinosa* (L.) Skeels (synonyms *Argania sideroxylon* Roem. and Schult., *Sideroxylon spinosum* L.)] belonging to the *Sideroxyleae* is the only representative species of the tropical family Sapotaceae in Morocco. *Argania* is a monospecific genus with *A. spinosa* as the unique representative (Pennington, 1991). It is an endemic species from Southwest Morocco where it occupies an area of around 800,000 ha. Argan tree plays a great socio-economical and ecological role in these arid zones due to the prised oil from its kernels and its foliage to feed cheep and goat herds. Nineteen percent of the local population incomes depend of this tree (Benchakroun, 1990). However, a great zoo-anthropologic action led to the absence of natural regeneration of this species (Benchakroun and Buttoud, 1989). To protect argan groves from the overexploitation, it was classified in 1998 as a "Biosphere reserve" by UNESCO, and many efforts are being undertaken for its rehabilitation. The cytology of this species is poorly known in spite of its great socio-economical and ecological interest and data available are scarce and controversial. Johnson (1991) reported that the basic chromosome number of *Sideroxyleae* is suggested to be $x=11$, whereas the most common chromosome number in Sapotaceae is $x=13$ followed by 12 and 14. However, previous work on the cytology of *A. spinosa* failed to establish the chromosome number. In fact, Miège in 1954 reported a chromosome number of 20 ($x=10$) in somatic cells whereas Humphries et al. (1978) in meiotic cells reported 24 ($x=12$).

The objective of this work is to characterize the karyotype of *A. spinosa* species in somatic cells from root tips.

## 2. Materials and methods

### 2.1. Plant material

Plant material assayed included *A. spinosa* seeds collected on May 2006 at Essaouira city (Southwest of Morocco). Seedlings used for root sampling were planted and maintained in the experimental field of CEBAS-CSIC of Murcia (Spain).

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### 2.2. Karyotype analysis

*A. spinosa* seeds were sowed in Petri dishes filled with moisten vermiculite and incubated at 25 °C until radicules were about 5 cm length. Root tips were collected at mid-day placed in cold water for 4 h at 0 °C, and later treated with 0.1% colchicine for 3 h at room temperature. Samples were then fixed for 24 h at 4 °C in ethanol–glacial acetic acid (3:1), stored in 70% ethanol solution and kept until use at 4 °C. Sampled roots were then hydrolyzed in 5 N HCl at room temperature for 5 min. Longer periods produce an excessive breaking of cells. Finally, staining of samples was performed in acetic acid/orcein 45% for 2 h and later squashed on a slide. Several cells from more than one root tip were counted to ensure that a consistent count was achieved. The observations of samples were realized in a Leica DMRB DC 500 (Wetzlar, Germany) light microscope and the images were captured with IM 1000 version 1.20 software (Barcelona, Spain). The images were analyzed with MicroMeasure (version 3.3) software (Reeves, 2001) to determine chromosomes length. Finally, Adobe Photoshp® 7.0 software was used to prepare the karyotype.

## 3. Results and discussion

The diploid number determined in the somatic cells of the *A. spinosa* root tips analyzed is of $2n=2x=20$ (Fig. 1). The karyotype consists of four submetacentric and six metacentric pairs. The four submetacentric pairs were the longest with a mean total length between 1.14 and 1.69 μm whereas the lengths of the six metacentric pairs were between 0.59 and 1.03 μm (Table 1). Our result confirms the information reported previously by Miège (1954) but differs from that of Humphries et al. (1978). These differences can be due to a misidentification of the material and the small chromosome size. The chromosomes of *Argania* were very small, which is in agreement with previous works describing other Sapotaceae (Johnson, 1991) where the precise identification of some chromosome characteristics such as arms length or presence or absence of satellites is very difficult.

This study showed that *A. spinosa* has a different chromosome number with respect to the other genus in the *Sideroxyleae* (basic chromosome number of $x=11$), and in the Sapotaceae (basic chromosome number of $x=13$, $x=12$ or $x=14$) (Johnson, 1991). An ascending or descending dysploidy process could be the hypothesis explaining this situation. In the case of descending dysploidy (meroaneuploid reduction), the decrease of parts of normal parental chromosomes can occur in plants adapted to a more rigorous environments than the ancestral species and this could be the case of *Argania*, the only species of the tropical family Sapotaceae adapted to the arid environment of Southwest Morocco (Pennington, 1991). *Argania* species is an extremely drought resistant plant being one of the few nontropical species of the Sapotaceae family (Schnell, 1970, 1971). Such reduction in chromosome number by multiple translocations was reported in other species like *Eleocharis subarticulata* (DaSilva et al., 2005).

*A. spinosa* develops under the hot arid Mediterranean floor and have very different distribution and ecological exigencies in comparison with the rest of *Sideroxylon* species (Emberger, 1939). Quezel and Barbero (1993) hypothesized that argan groove had taken place during the Mio–Pliocene area. In

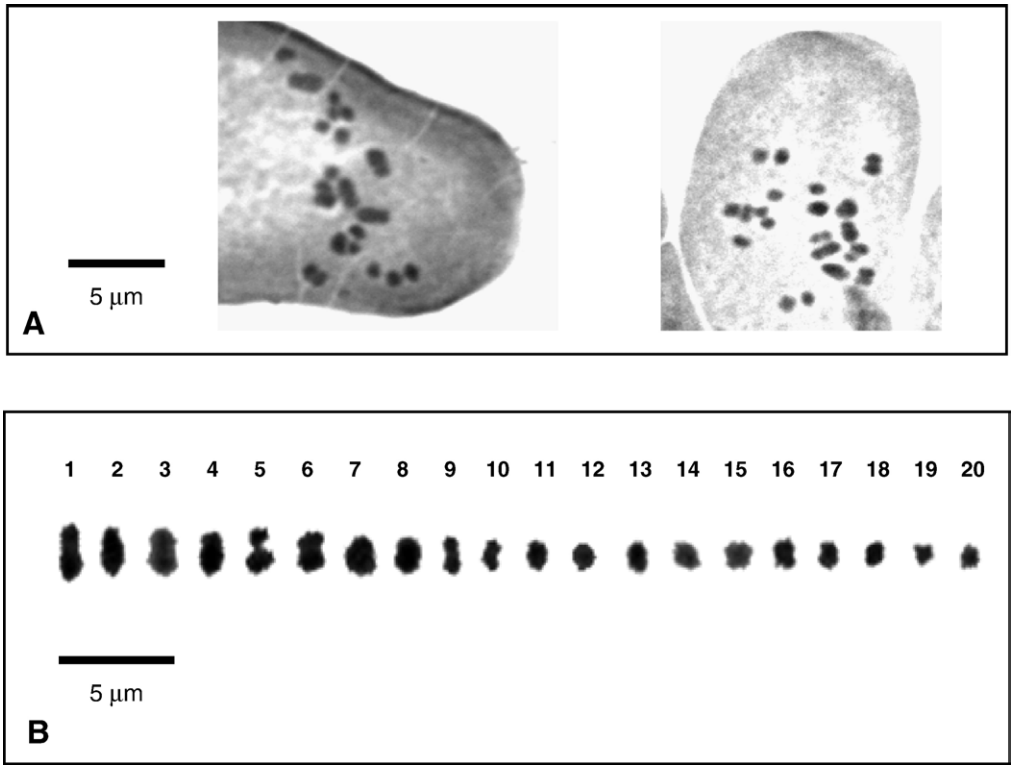


Fig. 1. Metaphasic plaques (A) and karyotype (B) of *Argania spinosa*.

Table 1
Average length, mean pair length and morphology of *Argania spinosa* chromosomes

| Chromosome | Average length ± S.D. (μm) | Mean pair length (μm) | Morphology |
|---|---|---|---|
| 1 | 1.78 ± 0.25 | 1.69 | Submetacentric |
| 2 | 1.60 ± 0.24 | | |
| 3 | 1.49 ± 0.27 | 1.46 | Submetacentric |
| 4 | 1.43 ± 0.27 | | |
| 5 | 1.39 ± 0.38 | 1.34 | Submetacentric |
| 6 | 1.30 ± 0.32 | | |
| 7 | 1.18 ± 0.32 | 1.14 | Submetacentric |
| 8 | 1.10 ± 0.34 | | |
| 9 | 1.06 ± 0.36 | 1.03 | Metacentric |
| 10 | 0.99 ± 0.36 | | |
| 11 | 0.91 ± 0.34 | 0.89 | Metacentric |
| 12 | 0.87 ± 0.35 | | |
| 13 | 0.82 ± 0.35 | 0.81 | Metacentric |
| 14 | 0.80 ± 0.35 | | |
| 15 | 0.77 ± 0.34 | 0.75 | Metacentric |
| 16 | 0.73 ± 0.30 | | |
| 17 | 0.71 ± 0.30 | 0.69 | Metacentric |
| 18 | 0.68 ± 0.27 | | |
| 19 | 0.60 ± 0.22 | 0.59 | Metacentric |
| 20 | 0.58 ± 0.19 | | |

addition, *Arganioxylon sardum*, a fossil plant encountered in Sardinia (Italy) (Biondi, 1981), was similar to *A. spinosa*. This evidence can be the element to demonstrate the old origin of *A. spinosa* and its old distribution out of the tropical zone.

In conclusion, the basic chromosome number in *Argania* ($n = 20$) is the lowest so far determined in the Sapotaceae. The derived number is consistent with the specialized ecology of the species.

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