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**Karyological study of *Jatropha curcas* L. and  
*Argania spinosa* (L.) Skeels**

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{وَأَخِرُ دَعْوَاهُمْ أَنْ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ}

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And who taught me that success only comes with persistence. To whom I have made the precious, and from whom I have drawn my strength, and my pride in my precious

Mother : Kaltouma

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## **Karyological study of *Jatropha curcas* L. and *Argania spinosa* (L.) Skeels**

### **Abstract**

This study identified and analyzed the intraspecific diversity of chromosome numbers and karyotypes in *Jatropha curcas* L. and *Argania spinosa* (L.) Skeels using conventional cytogenetic techniques such as lacto-propionic orcein staining. *Jatropha curcas* has a consistent chromosome number of  $2n=2x=22$ , with chromosomes measuring between 0.14 and 1.42  $\mu\text{m}$ , and a karyotype characterized by metacentric and submetacentric chromosomes. *Argania spinosa* predominantly shows a chromosome number of  $2n=20$ , although some cells display  $2n=24$ , indicating the existence of variation in the number of chromosomes within the species. The length of the chromosomes ranges from 0.52 to 1.24  $\mu\text{m}$ , with a karyotype dominated by metacentric chromosomes. These data on the genomes of the two species would allow for the valorization and conservation of these genetic resources.

**Keywords:** *Argania spinosa* L., Chromosome, *Jatropha curcas* L., Karyotype, Mitosis.

## Étude caryologique de *Jatropha curcas* L. et *Argania spinosa* (L.) Skeels.

### Résumé

Cette étude a identifié et analysé la diversité intraspécifique du nombre de chromosomes et du caryotype chez *Jatropha curcas* L. et *Argania spinosa* L. Skeels en utilisant des techniques cytogénétiques conventionnelles comme la coloration à l'orcéine lacto-propionique. *Jatropha curcas* présente un nombre de chromosomes constant de  $2n=2x=22$ , avec des chromosomes mesurant entre 0,14 et 1,42  $\mu\text{m}$ , et un caryotype caractérisé par des chromosomes métacentriques et submétacentriques. *Argania spinosa* affiche principalement un nombre de chromosomes de  $2n=20$ , bien que certaines cellules montrent  $2n=24$ , ce qui indique l'existence d'une variation du nombre chez l'espèce. La longueur des chromosomes varie de 0,52 à 1,24  $\mu\text{m}$ , avec un caryotype dominé par des chromosomes métacentriques. Ces données sur les génomes des deux espèces permettraient la valorisation et la conservation de ces ressources génétiques.

**Mots clés:** *Argania spinosa* L., chromosomes, *Jatropha curcas* L., Caryotype, Mitose.

## دراسة كاريولوجية لجنسي *Argania spinosa* (L.) Skeel و *Jatropha curcas* L.

### ملخص

حددت هذه الدراسة التنوع داخل النوع في عدد الكروموسومات والهيكل النووي لدى *Jatropha curcas* L. و *Argania spinosa* L. Skeels باستخدام تقنيات علم الوراثة الخلوية التقليدية مثل تلوين الأورسين اللاكتو-بروبيوني *Jatropha curcas* . لديه عدد ثابت من الكروموسومات يبلغ  $2n=2x=22$ ، مع كروموسومات تتراوح أطوالها بين 0.14 و 1.42 ميكرومتر، وهيكل نووي يتميز بكروموسومات مركزية وشبه مركزية *Argania spinosa* . يظهر بشكل شكل رئيسي عدد كروموسومات يبلغ  $2n=20$ ، على الرغم من أن بعض الخلايا تظهر  $2n=24$ ، مما يشير إلى وجود تباين في عدد الكروموسومات داخل النوع. تتراوح أطوال الكروموسومات من 0.52 إلى 1.24 ميكرومتر، مع هيكل نووي تهيمن عليه الكروموسومات المركزية. ستسمح هذه البيانات حول جينومات النوعين بتثمين وحفظ هذه الموارد الجينية.

الكلمات المفتاحية : *Argania spinosa* L. , *Jatropha curcas* L. , نمط نووي, كروموزوم , انقسام خيطي.

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# ***Introduction***

## Introduction

The progress of sustainable development in an environment that is in balance with nature depends on a comprehensive plan that considers the balanced use of resources such as plants, soil, and water. Forest-dwelling species, such *Jatropha curcas* L. et *Argania spinosa* L., are essential to preserving this ecological balance, so they must be properly recognized and protected.

*Jatropha curcas* L., (Euphorbiaceae), also known as « green gold», is an oil-bearing tree probably native to Central America, but now cultivated in Africa and Asia. This plant meets the growing interest in renewable energy sources. an agrofuel plant that is increasingly attracting the attention for its oil. *J. curcas* has the potential of becoming the most important biodiesel plant producer (Dahmer *et al.* 2009) . Its seeds contain 38% oil (Dias *et al.* 2008), The oil from *J. curcas* can be easily converted into liquid biofuel that meets American and European quality standards (Berchmans & Hirata, 2008). Moreover, this plant has therapeutic virtues, helps prevent soil erosion, and can be cultivated by low-income farmers.

The Argan tree, *Argania spinosa* (L.) Skeels, from the Sapotaceae family, is an endemic species in Algeria (Tindouf region) and southwest Morocco. The Argan tree serves as the final barrier against desertification. As a forest tree with ecological significance, it combats soil erosion, provides forage for goat herds, and yields fruit. It is a crucial resource for rural populations in semi-arid and arid regions of Algeria ( Ould Safi, 2014).

Besides its traditional uses in food, medicine, and cosmetics, the Argan tree is an economically valuable plant. Its oil extracted from the fruits is widely marketed for food and various cosmetic applications (Errouane *et al.* 2015).

Although much effort is devoted to the cultivation and conservation of two species, *Jatropha curcas* and *Amaranthus spinosa*, little information is available on the diversity of their genome and the possibilities of selection. Classical and modern cytogenetic analyses have become valuable sources of information in recent years for assessing genetic resources.

The main objective of our study is to determine the number and morphology of chromosomes of both species, *J. curcas* and *A. spinosa*, in order to establish their karyotype and contribute to the understanding of their genetic diversity. We will employ the classical cytogenetic technique developed by Dyer (1963) for this purpose.

Our study is divided into three main chapters. In the first chapter, we present an overview of the literature, focusing mainly on the species under study. The second chapter covers the study material and experimental methods applied. Results and discussion constitute the third chapter.

# *Chapter I*

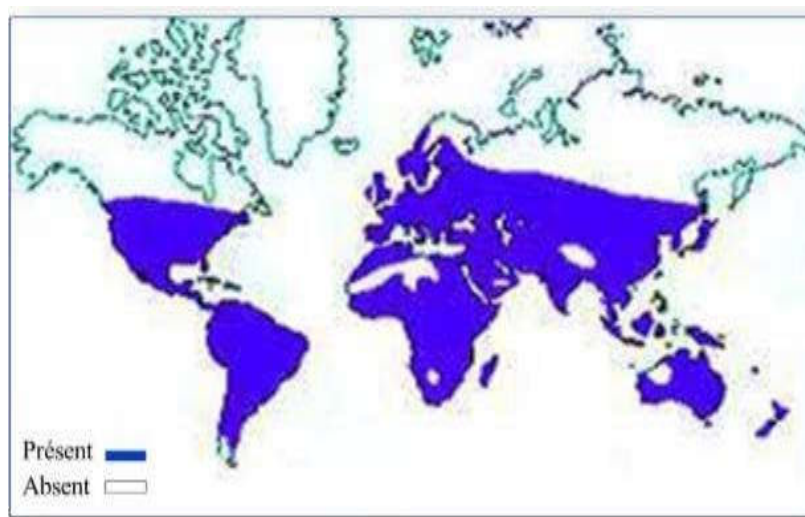
## *Bibliographic Synthesis*

## I.1 Euphorbiaceae Family and *Jatropha curcas* L.

### I.1.1 Euphorbiaceae family

The spurge family (Euphorbiaceae), commonly called euphorbias is of the most complex and largest families of angiosperms, after Orchidaceae, Asteraceae, Fabaceae, Poaceae and Rubiaceae. This family comprises 200-300 genera and over 6,000 species grouped into five subfamilies: Euphorbiaceae sensu stricto, Pandaceae, Phyllanthaceae, Picrodendraceae and Putranjivaceae (Tokuoka, 2007; APG III, 2009).

Species of the Euphorbiaceae include annual or perennial herbaceous plants, shrubs climbers, trees succulents and cactiforms, found in tropical regions tropical Indo-Malaysia, America and Africa, occurring worldwide except in cold alpine and arctic regions (Koekemoer *et al.* 2014; Christenhusz & Byng, 2016) ( Fig.1).

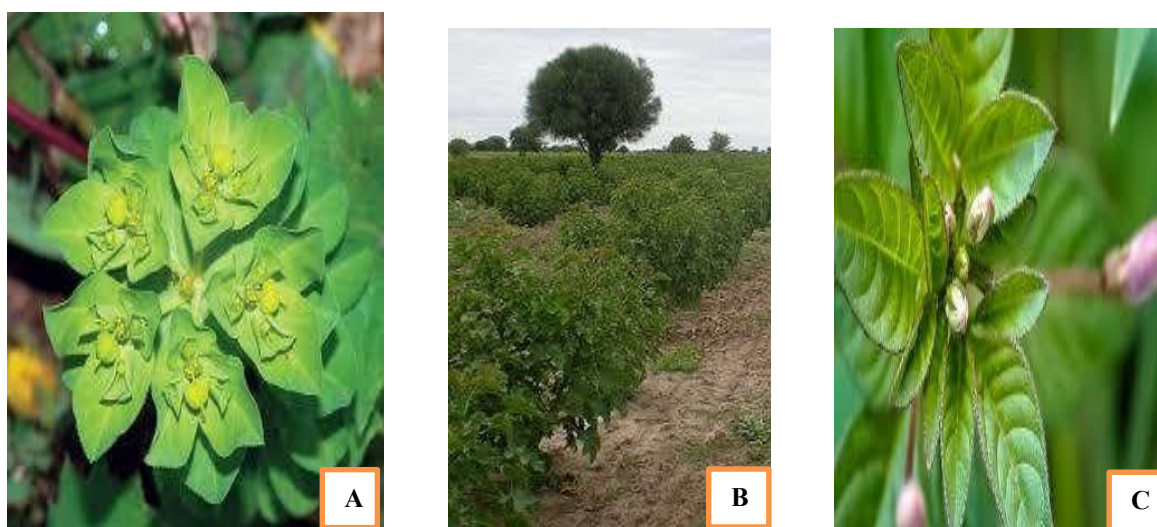


**Figure. 1 .** Geographical distribution of Euphorbiaceae (*In* Elorfi & Saadallah, 2022).

The Euphorbiaceae family is highly heterogeneous, with plants varying both in their vegetative structures and in the structure of their flowers:

- Leaves are generally alternate or simple, often much reduced in succulent species. Some genera and species have opposite, palmate leaves
- Inflorescences are very varied: clusters, spikes, panicles and cymes, but the *Euphorbia* genus forms a very special type of inflorescence ;

- Flowers are unisexual, with or without perianth. Male flowers have fused or branched stamens, while female flowers have three uniovulate or two biovulate carpels.
- The fruit is a tricoque capsule with loculicidal or septicidal dehiscence, or an explosively dehiscent schizocarp.
- Seeds are wattled and styles are bifid or feathery ( Quezel & Santa, 1963).



**Figure 2.** Some species of the Euphorbiaceae family (Site 1.2.3v).  
A, *Euphorbia serrata* L. ; B, *Tree of Jatropha curcas* ; C, *Euphorbia heterophylla* L.

### I.1.2 Importance and uses of Euphorbiaceae

Cassava, or *Manihot esculenta* (Crantz), is a food plant with fleshy roots used to produce tapioca. The plants of *Phyllanthus emblica* L., commonly known as Indian gooseberry, produce edible fruits (Ma et al. 1997).

Some species in this family are grown as ornamental plants: *Euphorbia pulcherrima* Willd. ex Klotzsch, *Euphorbia milii* Des Moul., *Codiaeum variegatum* L., *Dalechampia scandens*

Many species of Euphorbiaceae are known for their use in traditional medicine to cure various illnesses such as diabetes, diarrhoea, heart disease, haemorrhage, hepatitis, malaria, ophthalmic diseases, rheumatism and scabies. The diversity of the use of the Euphorbiaceae species in ethnomedicine is due to the presence of a wide range of unusual

secondary metabolites, including terpenoids, flavonoids, tannins, alkaloids, and steroids (Seigler, 1994).

- Latex from the fresh leaves of *Euphorbia nerifolia* Linn., is rubbed into the skin to heal wounds and pimples (Tripathi *et al.* 2010).
- The juice from the leaves of *Euphorbia dracunculoides* Lamk., is used by certain tribes in Asia to kill lice in humans and animals (Tripathi *et al.* 2010).  
The seeds of *Chrozophora rottleri* (Geis.), are used for their emetic and corrosive properties (Kirtikar *et al.* 1975).
- The latex of *Euphorbia macroclada* Boiss, is used as an antipyretic against malaria, warts and snake and scorpion bites and as an analgesic to relieve toothache and treat wound healing, eczema and fungal infections (Altundag *et al.* 2011).

The seeds of *Ricinus communis* L. produce a versatile oil that has long been used in industrial applications, particularly in the production of soaps, paints, and varnishes (Jeske *et al.* 1995).

The spurge family is home to many well-known biodiesel plants, including castor bean (*Ricinus communis* L.), cassava (*Manihot esculenta* Crantz), tung tree (*Vernicia fordii* (Hemsl.) Airy Shaw), rubber (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.), and caper spurge (*Euphorbia lathyris* L.). The biennial herb known as caper spurge, which is endemic to the Mediterranean region, has been identified as a prime candidate for biodiesel production due to its high seed yield, high oil content (48% on seed weight), and inexpensive inedible attribute (Wang *et al.* 2021).

### **I.1.3 *Jatropha curcas* L.**

The *Jatropha* genus is a member of the Jatrophaeae tribe under the Crotonoideae subfamily. This genus is morphologically diverse with more than 200 species composed mainly of diploids ( $2n=2x=22$ ), although triploid and tetraploid chromosome numbers have rarely been reported (Ganesh *et al.* 2008 ; Machua *et al.* 2011) . The majority of his species are found in temperate subtropical and coastal regions as well as the tropics during dry seasons (Domergue & Pirot, 2008). One of the most famous and economically important species of the *Jatropha* genus is *Jatropha curcas* L.

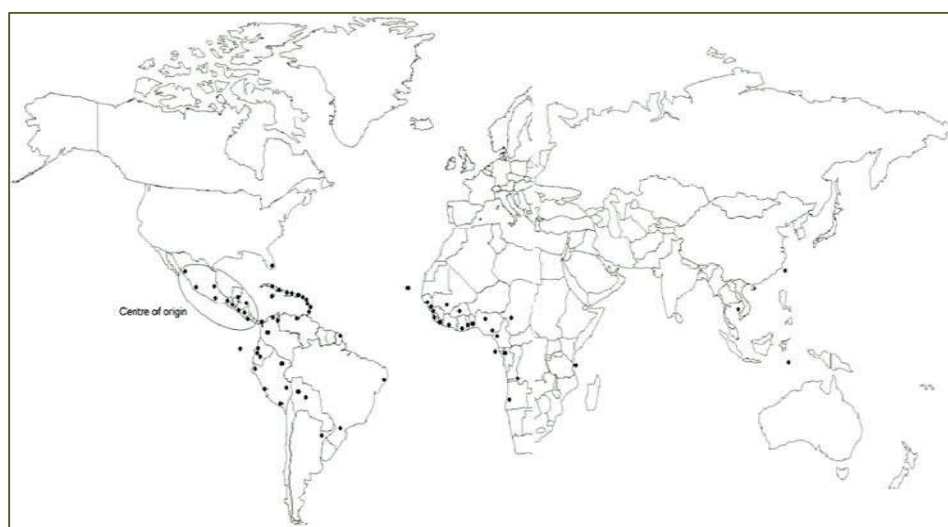
*Jatropha curcas* L., is a perennial small tree of 5 to 8 m height, native to tropical America, from where it has been distributed widely in tropical and subtropical regions of the world (Reddy *et al.* 2013 ).

The name “*Jatropha*” derives from the Greek *jatros* (doctor) and *trophe* (food), implying medicinal uses (Kumar and Sharma, 2008). *Jatropha* is also known as physic nut or purging nut in English, *pourghère* or *pignon d'Inde* in French, *purgeernoot* in German, *piñoncillo* in Mexican and *mundubi-assu* in Brazil (Münch, 1986 ; Schultze, 1986).

### I.1.3.1 Geographical origin

*Jatropha curcas* still has a disputed origin story. The earliest fossilized remains of the genus *Jatropha* were discovered in the early Tertiary age geological formations of Peru, South America, between 66 million and 3.0 million years ago. Their closest relatives were discovered in the late Miocene, between 23 million and 5.0 million years ago, in wet tropical regions of Central and northern South America (Sine *et al.* . 2020).

Originating in the Caribbean, the *jatropha* was most likely brought to other African countries as well as Asia by Portuguese navigators sailing from the Cup-Vert Islands and the Guinea-Bissau Islands (Cano-Asseleih,1986 ; Heller, 1996). Today, this plant is widely cultivated in most dry tropical and subtropical regions ( Garg *et al.* 2011). At an elevation rangin from 200 to 800 meters above sea level, *jatropha* can be found in pastures, by roadsides, wastelands, and open forests in tropical and subtropical climates. It is resistant to heat and drought (Zhang *et al.* 2008).



**Figure 3.** Distribution of *Jatropha curcas* L. (Heller, 1996).

### I. 1.3.2 Classification la classification of *Jatropha curcas* e

The classification of *Jatropha curcas* is as follows:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Malpighiales

Family: Euphorbiaceae

Genus: *Jatropha*

Species: *curcas* L.1753 (APG III, 2009).

### I.1.3.3 Botanical description

*Jatropha curcas* is a 2–5 m tall shrub with latex that is pungent, astringent, and yellowish/milky. It has tap root system with four lateral roots. The deciduous leaves are apically crowded, oval, acute to acuminate, and basally cordate, with dimensions ranging from 6 to 40 cm × 6 to 35 cm (Fig.3).

The plant is monoecious, yet it occasionally produces unisexual blooms at the terminal inflorescence. The flowers are in greenish cymes, bell-shaped, and yellowish. In general, male flowers have 10 stamens in two whorls, while female flowers have three carpels and two split stigmas (Wu *et al.* 2011).

The *jatropha* fruit is a tricoque capsule which opens by septicidal and then loculicidal dehiscence. The exocarp remains fleshy until the seeds are mature. There are usually three seeds per fruit. The seeds are black and average 18 cm (Singh, 1970).

The *J.curcas* reproduction is essentially via amphimixis, mainly by outcrossing (entomophylous pollination), even if the species is self-compatible and hence selfing is also possible (Ambrosi *et al.* 2009). Only a small proportion of seeds are set through apomixis. The plant can also be propagated clonally by anthropic management via cuttings of stem or branch (Konan *et al.* 2018).



**Figure 4.** Botanical characteristics of *Jatropha curcas* L. (Aponte & Deghan, 1994).  
 a : flowering branch, b : bark, c: leaf veins, d: female flower, e: male flower, f: section of green fruit, g: fruit, h: longitudinal section of fruit, i: seed.

#### I.1.3.4 Importance and uses of *Jatropha curcas*

*Jatropha curcas* is commonly cultivated because the plant has medicinal values, Each of the bark's extracted tannins and latex has astringent and antimicrobial ropha extracts are well-known for qualities. The seeds are commonly used to treat constipation, the sap is used to speed up the healing process after wounds, and the leaves are used as a therapy for malaria and high fever. The plant also has anti-tumor qualities (Verma *et al.* 2021).

Jatropha oil is known to be a powerful purgative, and is also used in the treatment of dermatological diseases and pain caused by rheumatism (Heller, 1996). Preparations of stems, fruits and roots are used to treat leishmaniasis (Odonne *et al.*, 2011). Leaf and root extracts are known for their anti-leukaemic activity (Duke,1983).

From an economic point of view, *Jatropha* is widely used in several areas, it fits easily into agricultural system in the form of hedges, windbreak, and erosion barrier or as a source of firewood (Figueroa *et al.* 2015). Fruit cake can be used as an organic fertiliser because of its high nitrogen content (3,2 à 3,8 %) (Heller, 1996).

Actually, *J. curcas* is a source of oil-rich seeds (46 to 58% of oil on kernel weight and 30 to 40% on seed weight) not edible as it contains toxic compounds and anti nutritional factors. It is considered to be an agrofuel plant that is attracting increasing attention for its oil and has the potential to become the most important biodiesel producer, with a quality that is compatible with that of diesel (Dahmer *et al.* 2009) . The *Jatropha* oil is considered as an environment friendly renewable source of energy (Ambrosi *et al.* 2009 ; Nguema *et al.* 2013).

#### I.1.3.5 Caryology

Information on chromosome numbers in this genus is limited. Chromosome data is available for only about 36 species and some interspecific hybrids. Almost all of the species are diploid, with  $2n=2x=22$  chromosomes, and the few reports on meiosis point to a regular meiotic behavior ( Dahmer *et al.* 2009).

Chromosome number variation exists within the genus *Jatropha*. While most species are diploid ( $2n=22$ ), *J. cuneata*, *J. dioica*, and certain *J. heterophylla* populations are tetraploid ( $2n=44$ ), *J. tirucalli* stands out with  $2n=20$  chromosomes (Fedorov, 1969; Soontornchainaksaeng and Jenjittikul, 2003; Carvalho *et al.* 2008). .

Most reported artificial interspecific *Jatropha* hybrids are diploid (22 chromosomes), two triploid hybrids ( $2n=3x=33$ ) were created from crosses between *J. curcas* and *J. cathartica* Teran & Berlan or *J. podagrica* Hook (Dehgan, 1984).

## I.2 *Argania spinosa* L. Skeels

*Argania spinosa* (L.) Skeels, 1911, اركان in Arabic, arganier in French, the argane tree in English and Argan in Amazigh. This Amazigh name refers to the hard wooden core of the tree's fruit (Boudy, 1952).

The argan (*Argania spinosa* (L.) Skeels, 1911) is an oleo-agro-sylvo-pastoral tree, the only representative of the Ebenales order and the tropical Sapotaceae family, which contains over 600 species and 40 genera, several of which are of great economic interest for food or industrial purposes (M'hrite, 1989).

The argan tree is endemic to Morocco and is also found scattered across south-west Algeria, particularly in the Tindouf region (Radi, 2003), it is the second largest forest species after the holm oak (*Quercus ilex* L.) and before the cedar (*Cedrus atlantica*), with a lifespan of 200 years or more (El-Bakouchi, 2023) .

### I.2.1 Origin and geographical area of distribution

In Algeria, its geographical range covers a relatively large area in the north-west of the wilaya of Tindouf, where this species is the second most important forest species after *Acacia radianna*. In this area (Hamada of Tindouf), it forms scattered populations, grouped together in a contracted pattern, along the banks of the wadis where it finds the necessary water compensation (Fig. 4).

The Tindouf argan tree probably originally formed a single ecological unit with that of Morocco, which covered vast territories. With the advent of the ice ages, the entire argan tree area that covered the Moroccan territory moved towards southern Morocco (Kechabar *et al.* 2013).

Glacial dynamics, particularly during the Würm period (the last ice age), considerably reduced its range, and the southward „crash“ caused it to spill over into what is now the Hamada de Tindouf, hence the existence of a veritable relic in Algeria today (Benkheira, 2009). In Algeria, the argan tree grows in the Tindouf region, north of Tindouf. It is distributed in three main areas: Touaref Bouam, Targuent and Merkela (According to the Wilaya Tindouf forest conservations, 2013).

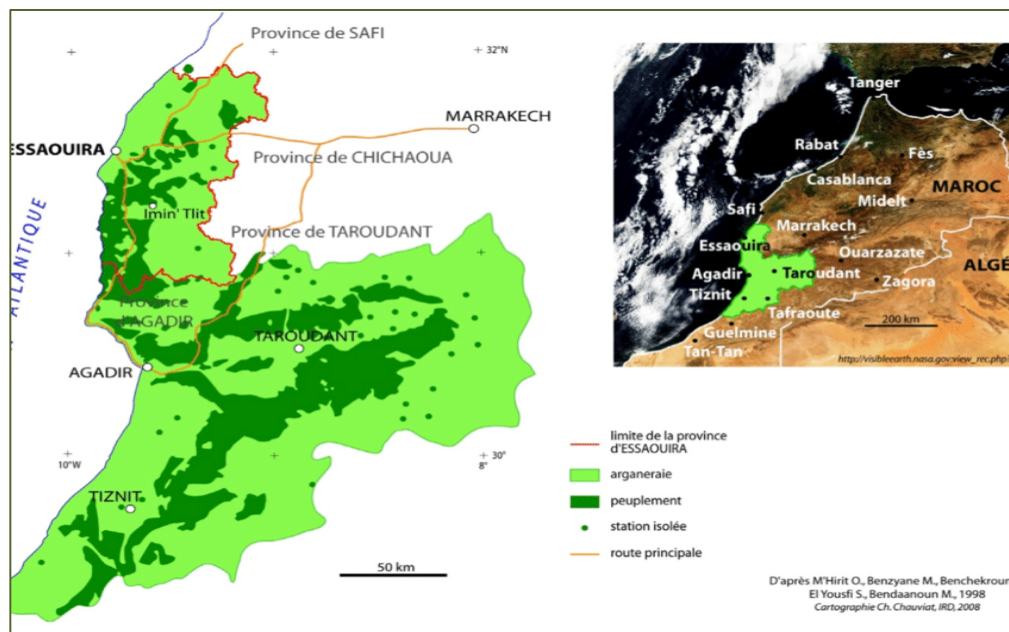


Figure 5. Geographical distribution of the argan tree forest ( Site 4).

## I 2.2 Classification

The classification of *Argania spinosa* is as follows:

Phylum: Spermatophytes

Subphylum: Angiosperms

Class: Dicotyledons

Subclass: Gamopetalae

Series: Superovariatae pentacyclie

Order: Ebenales

Family: Sapotaceae

Genus: *Argania*

Species: *spinosa* L. Skeels 1911.

Varieties: A. *Sideroxylon* Rom et Schl.

A. *Sideroxylon spinosium* L.Sp.

Common Name: Argan (Berber) (Quezel et Santa, 1963).

## I .2. 3 Botanical description

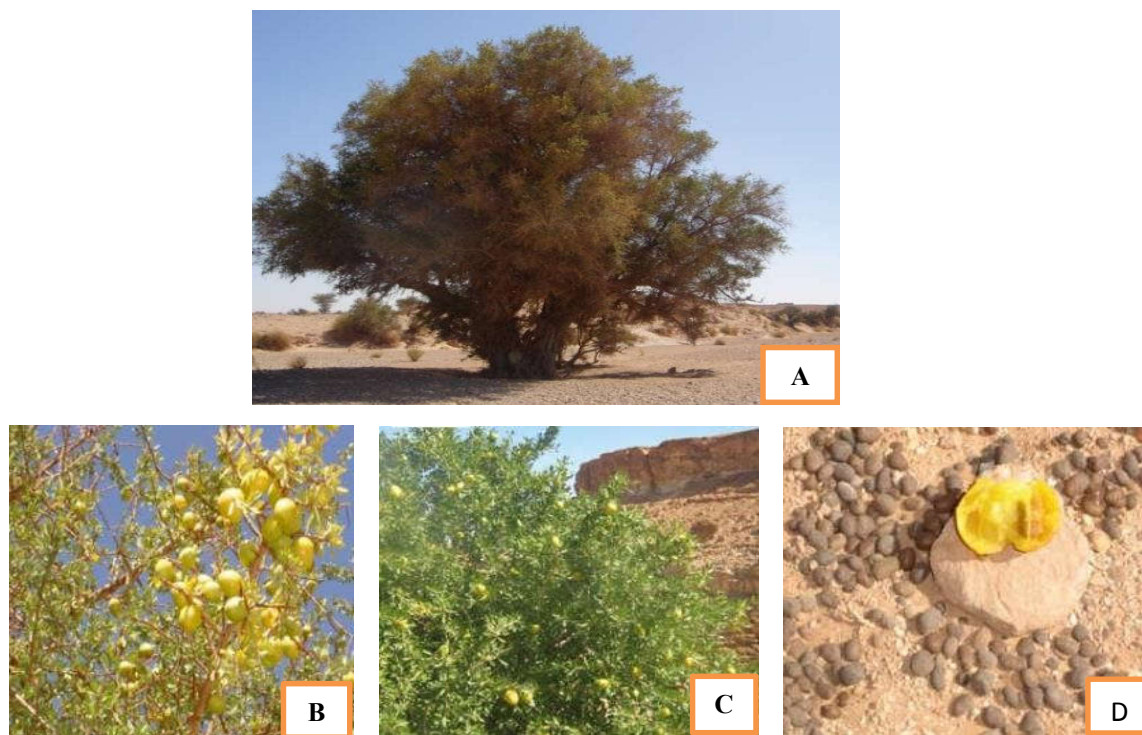
The tree, which resembles an olive tree in certain ways, can grow to a height of 8 to 10 meters, depending on the environmental circumstances. The Argan tree is very hardy and can live for 150 to 200 years. It has thorny branches, hence the name 'spinosa', and grows to a height of 8 to 10 meters, with leaves that taper into a short stalk (Emberger, 1938) .

The tree features a dense, round, and powerful crown. Its trunk is gnarled, twisted, and relatively short, often composed of several intertwined sections, giving the Argan tree a distinctive appearance (fig .6) .

The root system of the argan tree is a taproot type, capable of descending to great depths, up to 30 meters (Nouaim *et al.*, 1991).

The tree is formed by several intertwined stems. Its leaves are leathery, subaltern or fasciculate, generally 2 to 3 cm long, dark green on the upper surface and lighter on the underside, with a very distinct central vein and highly branched secondary veins. The flower is pentamerous and hermaphroditic, consisting of 15 or more white to greenish-yellow flowers in glomerules located between the nodes ( Boudy, 1952).

The fruit is a yellow or red drupe. It comes in six different shapes: oval, rounded, globular, drop-shaped, apiculate and spindle-shaped. They vary in size from 1 to 5 cm. It consists of a fleshy pericarp (or pulp) which covers a very hard stone (or nut) in the centre, representing a quarter of the fruit's weight. The nut consists of one to three oily, albuminous kernels (Adlouni, 2010).



**Figure 6.** Aspect of the argan tree (A), Argan tree in fructification (B), Fruits of the Argan tree (C), Detail of a fruit (D) ( Benkhaira, 2009).

### I.2.4 Uses of *Argania spinosa*

- **Food**

The argan tree truly serves several purposes; every part of it is useful and provides food or income for the people who depend on it for survival (Charrouf & Guillaume, 2002).

The oil supplies up to 25% of the dweller's daily lipid diet and has several other intriguing nutritional qualities (Collier & Lemaire, 1974). Its exceptional quality makes it a highly sought-after product, considerably more costly than olive oil due to its restricted availability (Yaghmur *et al.* 2001).

There is ample proof that argan oil is a significant dietary component. It is made from roasted argan kernels, which can be pressed using both conventional and contemporary methods (Mohammed *et al.* 2013; Zeghlouli *et al.* 2021).

The resulting argan oil is golden in both instances and tastes distinctively of hazelnuts (Charrouf & Guillaume, 2014). One of the world's most nutrient-dense oils, food argan oil has a nutty flavor and is sweet. This explains both the growing demand from consumers and the use of argan oil to a wide range of other foods.

Bread is typically consumed with argan oil when it is consumed as a meal. It also works well as a finishing touch when drizzled over seafood, couscous, tajin, and grilled salads. Additionally, edible argan oil serves as the primary component (Guillaume and Charrouf, 2013).

For animal feed, the leaves of the argan tree are eaten by camels and goats. The fruit pulp is also a source of animal feed. It seems that this fodder is the most important part of the animals' diet, especially the goats. The oil cake, residue of oil extraction, is used as an energy supplement for fattening cattle (Kechabar, 2016).

- **Medical and cosmetics**

Argan oil is widely used in traditional medicine because of its many therapeutic qualities. It's a priceless natural remedy. It is used in the treatment of athérosclérose, hypercholestérolémie, and rhumatismes (Charrouf, 2002).

Bnouham *et al.*, (2008), studied the antidiabetic effect of Argane oil in healthy and induced diabetic rats, and showed that Argane oil reduces significantly the glycemia and improve the body mass.

Argan oil is gaining interest for its potential cancer-fighting abilities. Since its composition resembles olive oil, which is known for its chemoprotective effects, scientists believe Argan oil might offer similar benefits. However, Argan oil's higher levels of gamma-tocopherol and squalene suggest it could be even more effective in protecting against cancer than olive oil (Monfalouti *et al.* 2010).

By preventing the appearance of wrinkles, argan oil has anti-ageing properties. It quickly regenerates and soothes even the driest, flakiest skin. Applying argan oil as a hydrating oil to the face, hands, and feet is common. It continuously feeds the hair and keeps it from falling (Moukal, 2004).

- **Environmental conservation**

The argan tree is a typically multi-use tree, due to the ecological and socio-economic character it occupies, it was declared a “protected species” by the United Nations Educational, Scientific and Cultural Organization (UNESCO) in 2007 (Venegas *et al.* 2011).

The argan tree is a thermophilous and xerophilous species, and plays an ecological role in protecting the soil against water and wind erosion. This role is attributed to its very powerful root system, which burrows deep into the soil to capture water (Mokhtari, 2002). Roots are the last line of defence against the expansion of deserts and are the main stabilising element in these arid and semi-arid ecosystems (Morton & Voss, 1987).

Trees such as the Argan are also essential for stabilising the course of wadis and regulating their flow. On the other hand, the argan forest ensures soil protection against wind erosion and runoff, thus promoting the infiltration of rainwater, which replenishes groundwater aquifers (Benhammou, 2007).

### I -2-5 Caryology

The chromosome numbers of 95 species belonging to the Sapotaceae family showed that all the specimens had a haploid chromosome number ( $n$ ) of 10, 11, 12, 13 or 14 ( Johnson, 1991, Bougoutaia *et al.* 2023).

The tribes Chrysophylleae and Omphalocarpeae stood out with a higher prevalence of individuals having  $n = 13$  or  $14$  chromosomes. In contrast, the tribes Mimosopeae, Isonandreae, and Sideroxyleae primarily had chromosome numbers closer to  $n = 10$  or  $11$  (Johnson, 1991).

For *Argania spinosa*, several studies have highlighted the variation in chromosome number within this species. Miège (1954) reported a diploid ( $2n$ ) number of 20 chromosomes, while Humphries *et al.* (1978) indicated a haploid ( $n$ ) number of 12 chromosomes. Majourhat *et al.* (2007) confirmed the  $2n = 20$  number and performed a morphometric and karyotypic analysis. More recently, El Boukhari *et al.* (2023) observed  $2n$  numbers of 20, 22, or 24 in different accessions of the species.

***Chapter II***

***Material and***

***Methods***

## II.1 Plant material

The study was undertaken on two species *Jatropha curcas* L. (2n=22) and *Argania spinosa* (L.) Skeels (2n=20). Seeds of *J. curcas* were purchased on social networks.

For *A. spinosa*, the ripe seeds were collected from trees at ouad Maa in the Tindouf region in Southwestern Algeria.

The region of Tindouf is delimited by the following coordinates: 25°30' - 29°40' latitude North and 03°00' - 08°60' longitude West. It is bordered to the north by Morocco, to the northeast by the wilaya of Béchar, to the west by the territory of Western Sahara, to the east by the wilaya of Adrar, and to the south by Mauritania.

According to Benkheira (2009), the Tindouf Argan Forest is located in the infra-Mediterranean zone, also known as the "oceanic type of the lower thermo-Mediterranean zone" (Fig.7).

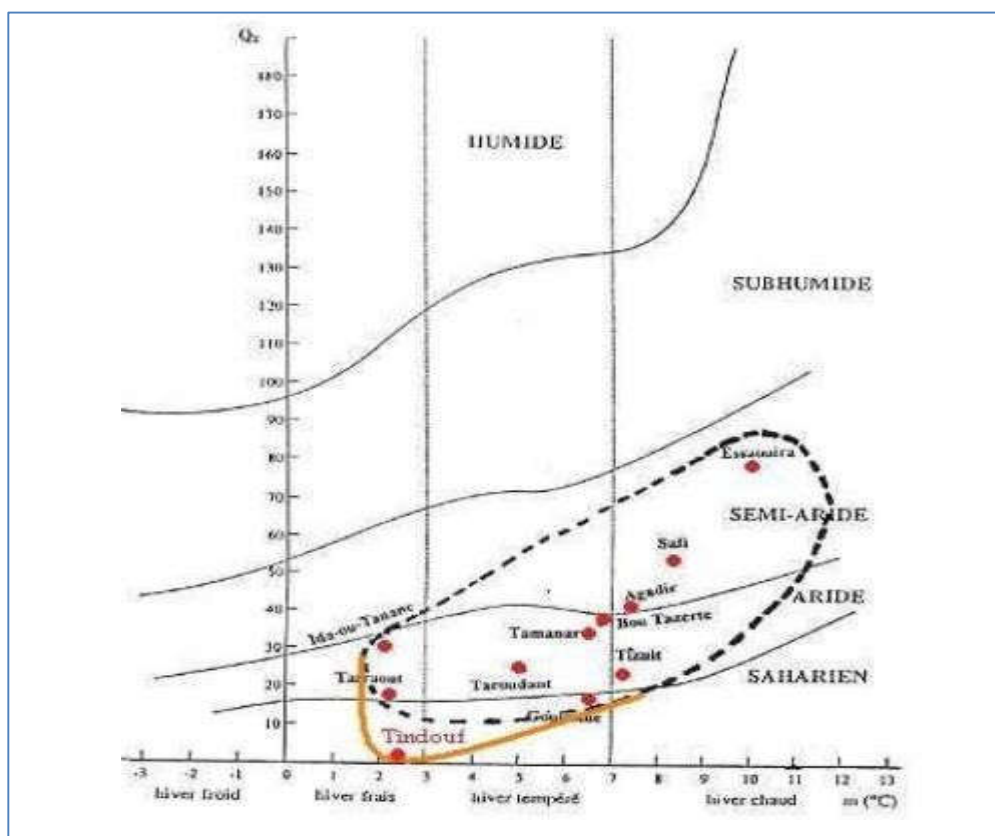


Figure 7. Bioclimatic area of the argan tree in Algeria (Tindouf region) (In Kechebar, 2016).



**Figure 8.** The seeds of the studied species  
a: *Jatropa curcas* L., b: *Argania spinosa* L.

## II.2 Methods

### II. 2.1 Germination

It is carried out in 3 steps:

- **Scarification**

The seeds of each studied species (*J.curcas* and *A.spinosa*), were scarified by rubbing with sandpaper to remove tegumental hardness, a necessary step in xerophilic plants.

- **Sterilization**

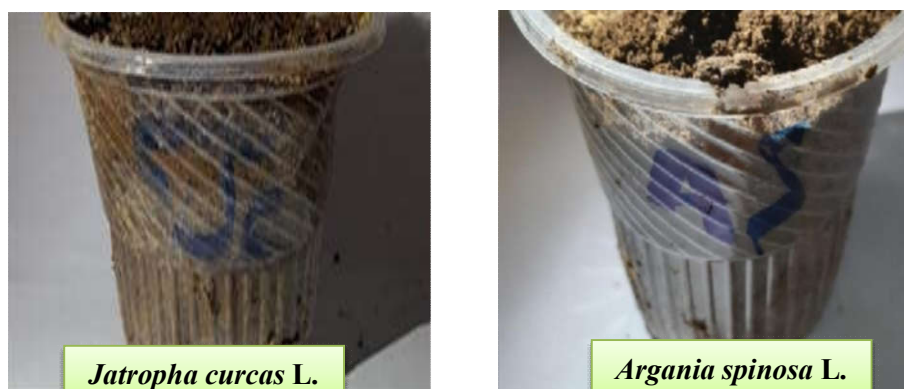
The seeds belonging to the two species (*J.curcas* and *A.spinosa*) were disinfected using Sodium Hypochlorite (5%), rinsed with sterile water, and then submerged in hot water (about 50 %) for five minutes.



**Figure 9.** Sterilization of seeds (personal)  
1: *Jatropa curcas* L. ; 2 : *Argania spinosa* L.

- **Soil sowing**

The seeds were sown 2 centimeters deep in the soil in a well-ventilated area with a temperature not exceeding 15 degrees Celsius and not falling below 28 degrees Celsius.



**Figure 10.** Plantation in soil (Personal)

### II.2.2 Mitotic study

Different methods are described for the study of chromosomes, they involve the application of chemical agents for the pre-treatment, fixation and coloring of cells in divisions. These methods are widely described in the "Plant Cytogenetics Techniques" specification. (Jahier ,1992).

The use of an appropriate technique must allow for proper separation of chromosomes with morphological details that allow for the correct determination of the karyotype.

To carry out the chromosome count for the two species, (*J. curcas* and *A.spinosa*), the protocol of Majourhat *et al.* (2007) was adopted with minor modifications:

➤ **Sampling and pretraitement**

Firstly, the root tip meristems, approximately 1 to 1.5 cm long , were excised in the morning at 8.00 am (period of cell division), and tips were pretreated with  $\alpha$ -bromonaphtalene (1%) for 1 h 20 min at room temperature.

This step aims to increase the number of metaphase mitotic cells, allows the chromosomes to be well spread in the cell, straightens the chromatids, and contracts the

chromosomes, which makes primary and secondary constrictions very noticeable (Singh 2018).

➤ **Fixation**

After rinsing with distilled water, the root tips were fixed in Carnoy II solution (In Jahier et al., 1992), composed of Ethanol - Chlorophorm - Acetic Acid (6v :3v:1v) for at least 24 hours at 4°C. This step blocks all cell life and preserves the structural integrity of the chromosomes (Jahier *et al.* 1992).

Fixed roots can be stored for several months in a 70% ethanol solution at 4°C (Jahier *et al.* 1992).

➤ **Hydrolysis**

For better chromosome analysis and optimal spreading, the root tips were hydrolysed in a hydrochloric acid solution (5N HCl) for 3 minutes.

Hydrolysis dissolves the pectic salts of the middle lamella and allows the clarification of the cytoplasm. In addition, hydrochloric acid releases aldehyde clusters onto DNA sugar molecules by destroying the bonds between the puric bases and deoxyribose (Jahier *et al.* 1992).

➤ **Coloration**

In order to easily visualise the metaphase plates, the hydrolysed root tips of two species were stained in orcein – lacto propionic solution ( Dyer, 1963) for 24 h at room temperature.

➤ **Microscopic observation**

The meristematic area is crushed in A drop of orcein lacto- propinique and crush between slide and coverslip , then the mitotic cells were first identified under the light microscope using a low magnification objective (X10), then observed in detail with a higher magnification objective (X40) for better visualisation of the cellular structures.

Chromosomes were counted on several metaphasis plates using a high magnification objective (X100) and photographed using a Zeiss microscope with a digital camera.

For each of studied species, chromosome counts and karyotype analyses were obtained on the basis of the three best metaphase plates.

### II.2.3 Karyotype analysis

#### ❖ Karyogram

In order to establish an accurate and representative karyotype for each of the two species studied, three metaphase plates from distinct individuals were carefully selected.

To determine chromosome type and centromere position, two parameters were calculated. These parameters were used to identify pairs of homologous chromosomes and to determine the position of each pair in the karyogram.

✓ Arm ratio,  $r = L/S$  ;

✓ Centromeric index  $CI\% = S/LT \times 100$

**L** : long arm, **S** : short arm.

The results obtained were compared with the standard chromosome nomenclature established by Levan *et al.* (1964) (Table 1).

The values for the parameters measured: short arm length ( **S** ), long arm length ( **L** ) and total length of the chromosomes **TL** ( $LT = L + S$ ), were converted to micrometres using the 'Micro Measure' cytogenetic software package developed by Reeve (2001).

Conversion to  $\mu\text{m}$  is given by the following formula:

$$\text{Length in } \mu\text{m} = \text{Length in pixel} * 10000 / \text{Res} * \text{Mag}$$

Res : Image resolution

Mag: Magnification

Other parameters of the caryotype were calculated :

- **R** : ratio of the longest pair and the shortest pair of the chromosomal set.

**Table 1.** Chromosomal nomenclature of Levan *et al.* (1964).

Centromere Position	d	r	CI %	Chromosomal Type
Median point	0.00	1.0	50.00	Metacentric senso stricto (M)
Median Region	0.00-2.5	1.0-1.7	50.0-37.5	Metacentric (m)
Submedian Region	2.5-5	1.7-3	37.5-25	Submetacentric (Sm)
Subterminal region	5-7.5	3-7	25-12.5	Subtelocentric (St)
Terminal region	7.5-10	7.0-∞+	12.5-0.00	Acrocentric (t)
Terminal point	10.0---	∞	0.00	Telocentric (T)

***Chapter III***  
***RESULTS***  
***And***  
***Discussion***

### III. Results and discussion

#### III.1 Chromosomal count and Morpho-metry

The karyotype is a systematised representation of the chromosomes of a mitotic (or meiotic) cell, taking into account the number, shape, size and all the other morphological characteristics of the chromosomes which may be representative of the genomes of a cell type, an individual or a species (Gorenflot and Raicu, 1980).

Other characters are also used to establish the karyotype of a species: the total length (TL), the relative size of the chromosomes (RTL), the symmetry of the karyotype determined by the asymmetry index (las %), the ratio of the longest pair to the shortest pair (R), which gives an idea of the general shape of the karyotype.

##### *III.1.1 Jatropha curcas*

Chromosome counts in the various metaphasic plates obtained showed that the *jatropha curcas* species has a stable chromosome number of 22. This number is frequently observed in all the individuals studied, indicating that its karyotype is diploid  $2n=2x= 22$  chromosomes ( Fig.9).

The diploid number ( $2n =22$  ) find in the present study was also was recorded by several authors within the same species (*Jatropha curcas*) : Fedorov (1969) ; Soontornchainaksaeng *et al.* (2003) ; Carvalho *et al.* (2008) and Sasikala & Paramathma (2010) ; Dahmer *et al.* (2009); Gong *et al.* (2013) ; Triadiati *et al.* (2019).

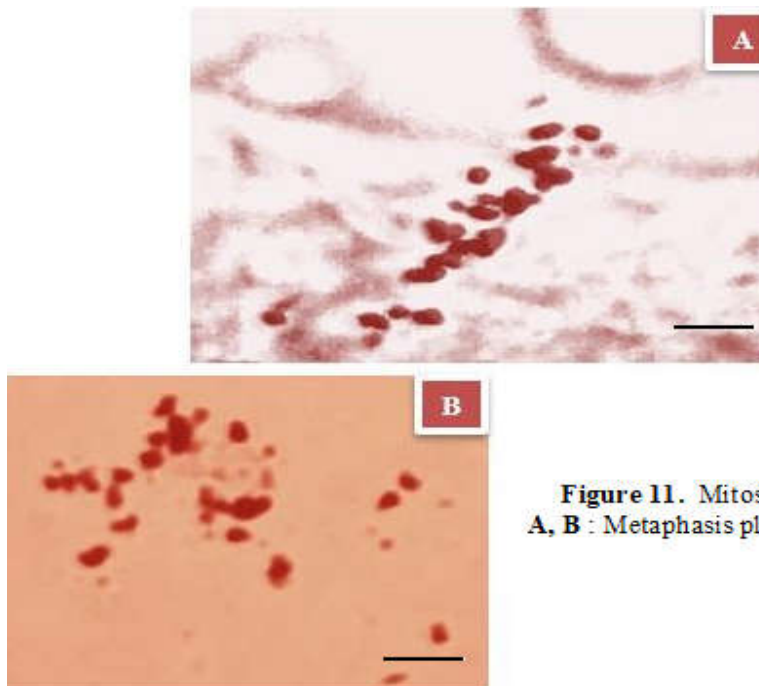
Concerning the size of the chromosomes, because of the small size of the chromosomes of *J. curcas* reported in several studies and for other problems encountered during the experimentation to establish the homology of which we cite:

- The high level of condensation due to the effect of pre-treatment and the the insufficient number of roots to establish the ideal time to obtain an acceptable level of condensation to facilitate chromosome measurements.
- The very low germination rate of the seeds of this species and therefore the limited of replicates.

Given these constraints, we limited our measurements to the longest and shortest chromosome pairs in order to get a general idea of the genome.

The present study showed a variation in the chromosomes total length, ranged from 0.14  $\mu\text{m}$  to 1.42  $\mu\text{m}$  (Table 2). These lengths seem to be inferior to those reported in several studies : Soontornchainaksaeng *et al.* (2003) (1 to 3,7  $\mu\text{m}$ ) ; Reddy *et al.* (2003) (1 to 3.67  $\mu\text{m}$ ) ; Dahmer *et al.* (2009) (1.24 to 1.71  $\mu\text{m}$ ) ; Gong *et al.* (2013) (1.27 to 2.09  $\mu\text{m}$ ) ; Triadiati *et al.* (2019) (1.19 to 2.69  $\mu\text{m}$ ).

For chromosomes morphology, according to figures 9A and 9B, and morphology of the pair N1 et N11, the karyotype de *J. curcas* consists of two chromosomes types : metacentric (m) and submetacentric (sm) chromosomes. This seems consistent with the results of Carvalho *et al.* (2008), Dahmer *et al.* (2009), and Wong *et al.* (2013), who indicated that the karyotype of the species *Jatropha curcas* presents a chromosomal formula composed of metacentric and submetacentric chromosomes.



**Figure 11.** Mitosis observed in *Jatropha curcas* L.  
A, B : Metaphasis plates presenting  $2n=22$  . Scale : 1  $\mu\text{m}$

**Table 2 :** Morphometric data within the species *Jatropha curcas* L., ( $2n=22$ ).

Chromosome pair n°	TL ( $\mu\text{m}$ )
Longest pair n°1	$1.42 \pm 0.06$
Shortest pair n° 11	$0.14 \pm 0.18$

### III.1.2 *Argania spinosa* L.

Chromosome counts in the various metaphasic plates obtained showed that *Argania spinosa* species has  $2n=20$  chromosomes (Fig 10 a,b). Our results are in accordance with those presented by Miege (1954), Majourhat *et al.* (2007), Boughetaia *et al.* (2023).

The results of our chromosomal counts revealed that some *Argania spinosa* individuals, in addition to the typical number for the species ( $2n = 20$ ), also possess a second chromosomal number ( $2n = 24$ ) (Fig. 10 C, D). This finding aligns with the observations of Humphries *et al.* (1978), who previously reported discovering 24 chromosomes in *Argania spinosa* pollen mother cells.

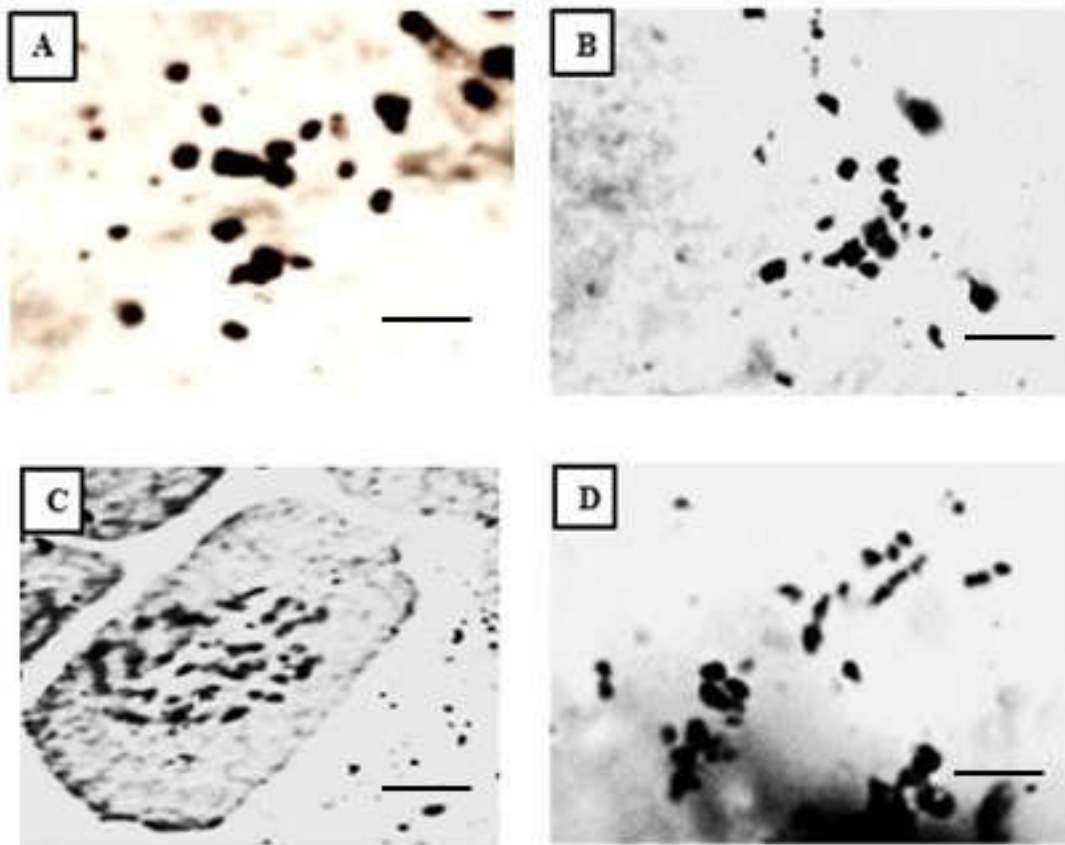
Furthermore, in their study on five genotypes of argan trees selected based on various morphological criteria in Morocco, El Boukhari *et al.* (2023) observed diversity in the chromosome count. They reported the presence of  $2n = 20$ ,  $2n = 22$ , and  $2n = 24$ , with a stable ploidy level ( $2n = 2x$ ), confirmed by flow cytometry. According to these authors, the highest diversity observed on argan tree forest could give an explanation of this variability of chromosome numbers.

The variability in chromosome number in the family *Sapotaceae* was already remarked by Arends (1976), reporting  $2n = 22$ , 24 or 28 in eight genera of African representatives, not including *Argania*. Majourhat *et al.* (2007) as well as Boguataia *et al.* (2023) have hypothesized a descending dysploidy as an evolutionary pathway within the family, given that Siljak-Yakovlev (1996) has shown this phenomenon to be quite common among plants. This could be the case of *Argania*, the only species of the tropical family *Sapotaceae* adapted to an arid environment du sud-ouest du Maroc (Majourhat *et al.* 2007). Descending dysploidy and polyploidy played crucial roles in chromosome number evolution in angiosperm and may be the origin of the variation (Carta *et al.* 2020).

The present study showed a variation in the chromosomes total length of Algerian argan, ranged from 0.52 to 1.24  $\mu\text{m}$  (Table 3). Majourhat *et al.* (2007) reported that the total

chromosome length of the Moroccan argan tree (0.59 to 1.69  $\mu\text{m}$ ) also confirms the small size of the chromosomes.

For chromosomes morphology, according to figures 10 A and 10 B, the karyotype of *A.spinosa* consists of the dominance of metacentric chromosomes (m) and for lack of better plates than those found, we were unable to determine the different chromosome types of the species. Bougoutaia *et al.*(2023) in their study of populations of Moroccan origin found a karyotype with a dominance of metacentric chromosomes and announced the chromosome formula of the argan tree:  $2n=20$  with respectively, st, st, m, m, m, m, m, sm, m, and sm.



**Figure 12.** Metaphasis plates within *Argania spinosa* L.  
**A and B** : somatic cells with  $2n=20$  ; **C and D** : cells presenting  $2n=24$ . Scale 1  $\mu\text{m}$ .

**Table 3** : Morphometric data within the species *Argania spinosa* (L.) (2n=20).

N° pair	TL (µm)
1	1.24±0.24
2	0.97±0.05
3	0.90±0.09
4	0.85±0.09
5	0.81±0.10
6	0.76±0.09
7	0.71±0.11
8	0.64±0.10
9	0.57±0.07
10	0.52±0.12
<b>R= 2.39</b>	

# *Conclusion*

## Conclusion

This study permitted to identify and analyse the intraspecific diversity of the chromosome numbers and karyotypes within two species *Jatropha curcas* L., and *Argania spinosa* L., by using conventional cytogenetic techniques such as lacto-propionic orcein staining (Dyer, 1963), which facilitates species chromosome counting.

At chromosomal level and thanks to staining with orcein, we were able to identify the following points:

- The chromosome numbers found in the somatic cells of the *Jatropha curcas* species studied are twenty-two ( $2n=2x=22$ ). This number is widely reported by previous authors.
- The chromosomes of *J. curcas* are small, varying in length from 0.14 to 1.42  $\mu\text{m}$ . The karyotype is characterized by metacentric and submetacentric chromosomes.
- Concerning the species *Argania spinosa*, most of the metaphase plates analyzed showed a frequent chromosome number of  $2n=20$ . However, some cells showed a chromosome number of  $2n=24$ , suggesting greater genetic variability within this species.
- The chromosomes of *Argania spinosa* are small, varying in length from 0.52 to 1.24  $\mu\text{m}$ . The karyotype of *A. spinosa* consists of the dominance of metacentric chromosomes (m).

This work has provided foundational data on the genomes of *Jatropha curcas* and *Argania spinosa*. However, the study is still incomplete. To enhance the findings, it would be beneficial to conduct further studies on mitosis and meiosis. These studies would provide more detailed insights into the genomes of these species and help confirm the initial results. Such research would support the valorization and conservation of these plant genetic resources.

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# *Annex*

## Composition of the solutions

### 1. 1% $\alpha$ -Bromonaphthalene

Dilute 1 ml of  $\alpha$ -Bromonaphthalene in 100 ml of distilled water.

### 2. Staining solution "Lacto-propionic Orcein"

#### -Stock solution of Orcein

Mix 2 g of orcein in 100 ml of lactic acid and 50 ml of propionic acid.

Subsequently, this solution is filtered and stored away from light.

#### -Dilution

At the time of use, the stock solution is diluted as follows:

- 45% of the stock solution;
- 55% distilled water;

### 3. Colchicine

- 1/20 in volume of HCl.

Mix 0.05 g of powdered colchicine in 100 ml of water.