Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2017) 41: 588-599
© TÜBİTAK
doi:10.3906/bot-1612-63

Comparative morphological and cytogenetic study of five *Asparagus* (Asparagaceae) species from Algeria including the endemic *A. altissimus* Munby

Kenza BOUBETRA[1,2], **Nabila AMIROUCHE**[1], **Rachid AMIROUCHE**[1,*]
[1]Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene, Algiers, Algeria
[2]National Institute of Forest Research, Cheraga, Algiers, Algeria

Received: 31.12.2016 • **Accepted/Published Online:** 23.07.2017 • **Final Version:** 22.11.2017

Abstract: Forty wild populations belonging to five *Asparagus* species including the endemic *A. altissimus* were sampled in an east-west bioclimatic gradient of North Algeria. More than 250 individuals were taxonomically examined and karyologically investigated. Meiotic behavior was analyzed for each species. New chromosome numbers were consistent with the base number x = 10 for the genus *Asparagus*, and two cytotypes, diploid and hexaploid, were observed. Four species were found to have 2n = 20 chromosomes, highlighting the prominence of diploidy in North Africa. One polyploid with 2n = 60 is reported here as the first karyological information on the endemic *A. altissimus*. Karyotype parameters were established and compared for diploid species. New data provided for *A. acutifolius* and *A. albus* indicate differences in the number of submetacentric chromosomes and genome size estimated by total chromosome length. *A. acutifolius* is distinguished by the largest genome, the smallest being that of *A. horridus*. The hexaploid cytotype consists of very small metacentric chromosomes. All diploid species displayed regular meiotic behavior with mostly bivalent pairing. Abnormalities, including univalents, multivalents, laggards, and bridges, were sometimes observed in *A. horridus* and *A. officinalis*. The new cytogenetic information provided in this study is discussed in the biogeographic context of the North African flora.

Key words: *Asparagus*, North Africa, karyotype, polyploidy, meiotic behavior, endemism

1. Introduction

The genus *Asparagus* L. (Asparagaceae) contains more than 210 species distributed throughout the world in temperate and tropical regions, with Africa and especially southern Africa as the main center of diversification (Kanno and Yokoyama, 2011). Species of *Asparagus* have economic value, particularly for their nutritional components. The most significant is undoubtedly *A. officinalis* L., the only one cultivated on a global scale. Several species have long been used in traditional pharmacopoeia (*A. racemosus* Willd., *A. verticillatus* L., *A. adscendens* Kunth), while others are ornamental (*A. plumosus* Baker, *A. densiflorus* Kunth, *A. virgatus* Baker) (Kumar et al., 2015).

Plants of this genus are characterized by various forms, with herbaceous perennials, woody shrubs and vines, photosynthetic stems (cladodes), leaves reduced to scales, and black or red berries (Clifford and Conran, 1987). The genus *Asparagus* is remarkable with high variability in reproductive behavior involving monoecious, dioecious, hermaphroditic, andromonoecious, and, in some cases, supermale plants (Kanno and Yokoyama, 2011).

From a systematic point of view, species of *Asparagus* are currently grouped within the subfamily Asparagoideae sensu APG IV (2016), including also species of *Hemiphylacus* S.Watson, a former small genus endemic to Mexico (Rudall et al., 1998). Three subgenera are currently recognized within the genus (Clifford and Conran, 1987): the subgenus *Asparagus* sensu stricto includes all the dioecious taxa with Eurasian distribution; the two others subgenera, *Protasparagus* and *Myrsiphyllum*, contain hermaphroditic taxa occurring mostly in Africa. However, this infrageneric subdivision was sometimes rejected (Fellingham and Meyer, 1995). Recent phylogenetic studies on the genus *Asparagus* (Kubota et al., 2012; Norup et al., 2015) have confirmed the monophyly of this genus with sexual dimorphism and polyploidy as the main force of evolution (Castro et al., 2013). All these phylogenetic studies have revealed conflicts between the different classifications, highlighting recurrent questions about delimitation of the currently recognized species.

Ecologically, *Asparagus* species are tolerant to drought and high temperatures growing under forest cover as well as in open habitats including predesertic steppes.

* Correspondence: ramirouche@usthb.dz

Together with species of other genera such as *Smilax* and *Ruscus*, they constitute a lianascent layer characterizing the Mediterranean forests (Schnitzler and Arnold, 2010). These lianas play a key role in the ecology and dynamic of forests and may be helpful as indicators for the monitoring and management of forest ecosystems (Naidu et al., 2014).

Unfortunately, in Algeria, efforts devoted to the monitoring of forests and protected areas are currently limited by an obvious lack of data on taxonomic units and their cytogenetic traits, which are essential for understanding the genetic diversity and evolutionary process of a species (Amirouche and Misset, 2009). In fact, the genus *Asparagus* is poorly known in our country. According to the main ancient floras of Algeria (Munby, 1855; Maire, 1958; Quézel and Santa, 1962), it comprises five species, namely *A. acutifolius* L., *A. albus* L., *A. horridus* L., *A. officinalis* L., and the endemic *A. altissimus* Munby, which is narrowly located in the northwest of Algeria.

This work is based on several natural populations sampled in contrasting bioclimatic conditions along the east-west biogeographic gradient of Algeria. It aims to establish by morphological, chorological, and karyological criteria a distinction between the Algerian species. New chromosomal counts, ploidy levels, and meiotic behavior should highlight the interspecific diversity and help complete our knowledge on the biogeographical distribution of polyploidy on the global scale.

2. Materials and methods

2.1. Plant materials

Forty wild populations were sampled in several localities from various bioclimatic areas in northern Algeria (Table 1). At each site, 5–7 plants per taxon were collected. Taxonomic determinations were conducted on the basis of diagnostic criteria of the main Algerian floras (Munby, 1855; Maire, 1958; Quézel and Santa, 1962). Herbarium specimens of the National Agronomic School of Algiers (ENSA) were also examined.

2.2. Mitotic and meiotic preparations

Karyological observations were performed using the Feulgen staining technique. Chromosome counting was carried out on metaphase plates of mitotic root tip cells from seed germinations. Root tips were pretreated with 0.02 mol 8-hydroxyquinoline for 2–4 h at 4 °C and then washed before being fixed in ethanol-acetic acid (3:1) for 48 h and hydrolyzed in 1 N HCl at 60 °C for 8–10 min. Staining was done in Schiff's reagent for 2–3 h in the dark at room temperature. Root meristems were squashed in a drop of 2% acetocarmine.

Meiotic behavior was analyzed on pollen mother cells (PMCs) of young floral buds previously fixed in a fresh ethanol-chloroform-acetic acid solution (6:3:1) for 48 h at room temperature. Flower buds were washed and conserved in 70% ethanol at 4 °C before hydrolyzation and staining by Feulgen's method. Mitotic metaphase plates and PMCs were observed and photographed with a Zeiss Axiostar-Plus microscope equipped with a Canon digital camera.

2.3. Karyotype reconstruction

Karyotypic formulas and idiograms were based on measurements of mitotic metaphase plates. The chromosome morphology was determined by the centromeric index according to the nomenclature of Levan et al. (1964): metacentric (m), submetacentric (sm), and subtelocentric (st). Idiograms were constructed from at least five good metaphase plates. Karyotype asymmetry indexes were estimated by the (A1) and (A2) indexes of Romero Zarco (1986), which are defined as the intrachromosomal asymmetry index (A1) = $1 - (\Sigma b/B)/N$ and interchromosomal asymmetry index (A2) = SE/L, where b and B are the mean lengths of short and long arms, respectively; N is the number of homologs; SE is the standard error; and L is the mean chromosome length.

3. Results

Individuals belonging to the five Algerian *Asparagus* species were examined (Figures 1a–1p). Karyological data revealed that *A. acutifolius*, *A. albus*, *A. horridus*, and *A. officinalis* are diploids (2n = 20) with base number x = 10. The endemic *A. altissimus* is distinguished by higher chromosome number 2n = 60, expressing a hexaploid level.

In general, meiotic behavior shows the regularity and stability of the genome, expressed by predominant bivalent chromosomal pairing at diakinesis and metaphase I. Irregularities have been observed in anaphase I and telophase I.

We will describe below, for each taxon, the taxonomical, chorological, and cytogenetic results.

3.1. *Asparagus acutifolius* L., Sp. Pl. 1: 314 (1753)

The plant is dioecious, green with a short rhizome. The species is characterized by edible young turions sweet or slightly bitter, greenish and elongated. The stem is climbing, 0.5–2 m long, flexuous, glabrous, and striate, sometimes woody and highly branched. The leaves are reduced to small scales, triangular and brownish. The cladodes are cylindrical, 1.7–7.3 mm long, linear, spiny, persistent, subequal, and grouped in fascicles of 7–22. Flowers are yellowish white to greenish white and mixed with the cladodes 1–3. The pedicels are short, 2.3–3.9 mm, and slightly longer than flowers. The perianth is campanulate, 2.4–3.8 mm in length. In male flowers, the stamens are subequal, shorter than the perianth, with yellowish anthers and rudimentary ovary. In female flowers, the ovary is whitish and subglobose, 3-locular with 2 ovules per locule, stigmas are bifid. The berries

Table 1. Origin and geographical information of sampled sites in North Algeria.

Site	Biogeo. sect.	Bioclim.	Alt.	Coordinates		Populations/species collected
				Lat.	Long.	
Bainem	A1	SH	248	36°48′N	02°58′E	*A. acutifolius*
Tessala El Merdja	A1	SH	26	36°37′N	02°54′E	*A. officinalis*
Souidania	A1	SH	169	36°41′N	02°54′E	*A. acutifolius*, *A. albus*
Bouchaoui	A1	SH	23	36°44′N	02°51′E	*A. acutifolius*
Keddara	A1	SH	106	36°38′N	03°23′E	*A. acutifolius*, *A. albus*
Ahmer El Ain	A1	SH	97	36°28′N	02°33′E	*A. acutifolius*, *A. albus*
Zemmouri	A1	SH	5	36°48′N	03°35′E	*A. acutifolius*
El Affroun	A1	SH	123	36°27′N	02°36′E	*A. acutifolius*
Tablat	A2	SH	382	36°24′N	03°20′E	*A. acutifolius*, *A. albus*
Chiffa	A2	SH	154	36°25′N	02°45′E	*A. acutifolius*, *A. albus*
Redjredj	A2	H	1075	36°05′N	02°57′E	*A. acutifolius*
Ain El Hadjar	C	SA	565	36°20′N	03°47′E	*A. acutifolius*, *A. albus*
Ain Smara	C	SA	614	36°18′N	06°33′E	*A. acutifolius*
Ténès	O1	SH	139	36°14′N	01°14′E	*A. acutifolius*
Misserghin	O1	SA	211	35°35′N	00°54′W	*A. acutifolius*, *A. albus*, *A. altissimus*
El Ançor	O1	SA	114	35°40′N	00°53′W	*A. acutifolius*, *A. albus*, *A. horridus*
Mansourah	O2	SA	1030	34°51′N	01°18′W	*A. acutifolius*
Ksar Chellala	O3	SA	774	35°16′N	02°17′E	*A. horridus*
Hassi Fedoul	O3	SA	792	35°25′N	02°13′E	*A. horridus*
Emir Khaled	O3	SA	346	36°08′N	02°12′E	*A. horridus*
Bougaa	Hd	SA	758	36°20′N	05°04′E	*A. acutifolius*
Boussaâda	Hd	SA	510	35°13′N	04°10′E	*A. horridus*
Boughezoul	AS	SA	627	35°46′N	02°47′E	*A. horridus*
Yakouren	K1	H	762	36°44′N	04°26′E	*A. acutifolius*
El Aouana	K2	H	74	36°46′N	05°36′E	*A. acutifolius*
Guerbes	K2	H	25	36°55′N	07°10′E	*A. acutifolius*
Gouraya	K2	H	536	36°46′N	05°05′E	*A. acutifolius*
El Kala	K3	H	9	36°53′N	08°28′E	*A. acutifolius*
Senalba	AS	SA	1296	34°38′N	03°10′E	*A. acutifolius*
Tipaza	A1	SH	12	36°35′N	02°27′E	*A. albus*

Biogeo. sect.- Biogeographical sectors, subsectors, and bioclimates are from Quézel and Santa (1962): A1, A2: Algiers; C: Constantine; O1, O2, O3: Oran; Hd: Hodna; AS: Saharian Atlas; K1, K2, K3: Kabylie. Bioclimate: H: humid; SH: subhumid; SA: semiarid. Alt.: Altitude in meters, Lat: latitude, Long.: longitude.

are globose, 4.4–7 mm in diameter, green and then black when mature with 1 or 2 seeds (Table 2). The turions grow from March to April, flowering from June to September, fruiting from November to December. Seeds have a strong dormancy and do not germinate easily.

3.1.1. Karyology and meiotic behavior

The somatic chromosome number of all populations of *A. acutifolius* is 2n = 2x = 20 (Figures 2a and 2b). The karyotype is symmetric with 8 metacentric and 2 submetacentric pairs. The length of chromosomes varies between 4.21 and 6.61 µm with an intrachromosomal asymmetry index A1 = 0.92 and interchromosomal A2 = 0.20 (Table 3). The karyotype formula is 16 m + 4 sm. No satellites were observed. The majority of PMCs show regular meiotic behavior in all studied samples. Bivalents are predominant and may be ring-shaped (R) or rod-

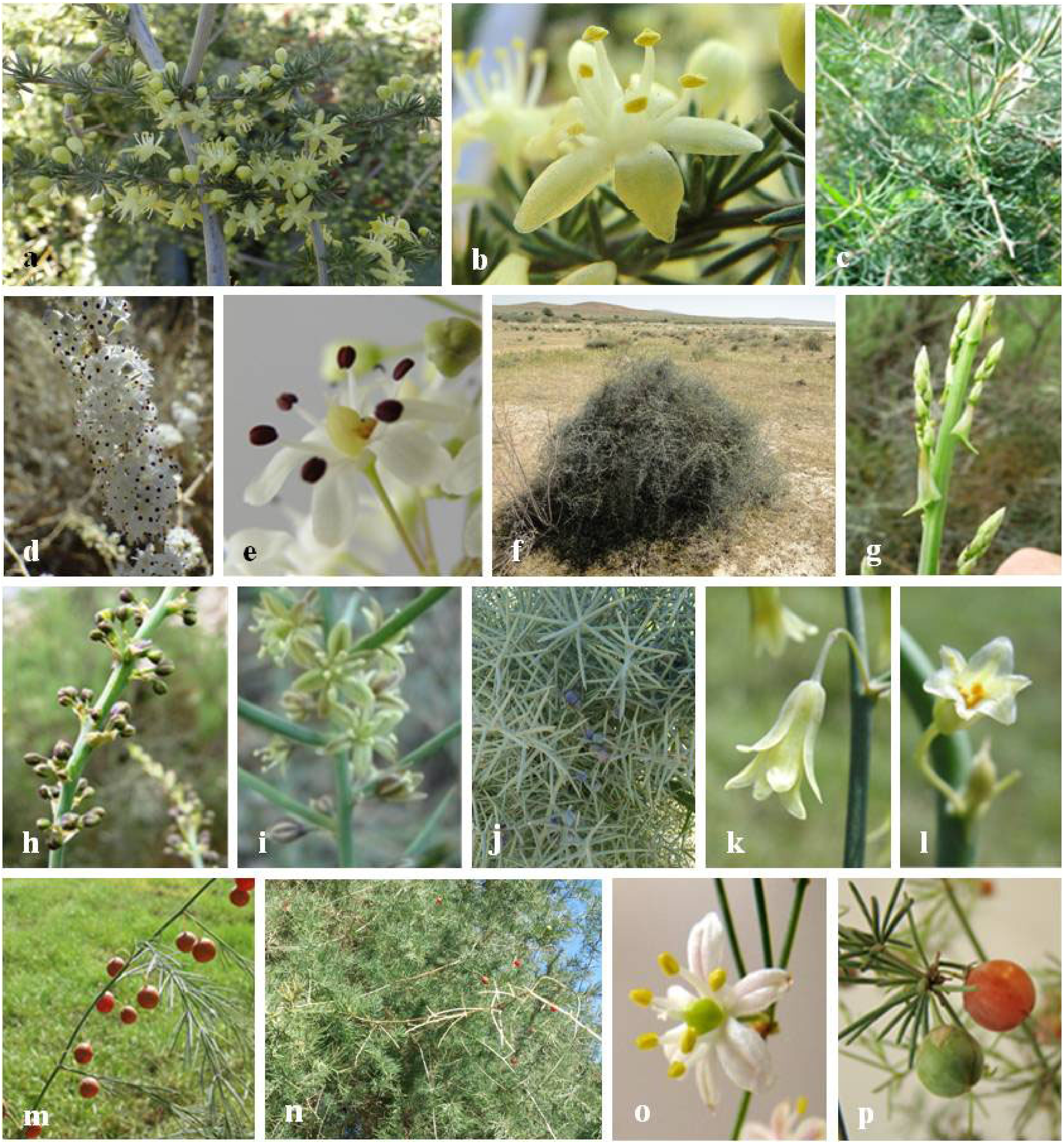

Figure 1. Habits, flowers, buds and berries of Algerian *Asparagus* species: *A. acutifolius* (a, b), *A. albus* (c–e), *A. horridus* (f–j), *A. officinalis* (k–m), *A. altissimus* (n–p). Photos by R Amirouche.

shaped (Rd); univalents (I) are rare. The meiotic formula is 0.8 I + 7.8 IIR + 1.8 IIRd. The mean number of chiasmata per cell is 17.4. No abnormalities were observed at all meiotic stages.

3.1.2. Habitat and distribution

A. acutifolius is widely distributed in Algeria. This taxon is frequent in woodlands and shrublands, growing in various moist and shady biotopes of humid, subhumid and semiarid

Table 2. Morphological comparisons between the five Algerian *Asparagus* species.

	A. acutifolius	*A. albus*	*A. horridus*	*A. officinalis*	*A. altissimus*
Plant eight (m)	0.5–2	0.95	0.25–1	0.50–2	1–5
Cladode length (mm)	1.7–7.3	11–27.20	19.90–86.2	12.9–26.70	14.89–19.7
Number of cladodes in a fascicle	7–22	7–32	1–2	4–6	7–10
Cladode appearance	Spiny	Smooth	Thick and strongly spiny	Smooth	Thin
Flower sexuality	Dioecious	Hermaphrodite	Dioecious	Dioecious	Hermaphrodite
Flower color	Yellowish to greenish white	White	Yellow-purple	Yellow	White
Shape of flower	Campanulate	Campanulate	±Stellate	Tubular	Campanulate
Flower mixed/cladodes	1–3	6–12	2–6	1–2	1
Length of the perianth (mm)	2.4–3.80	2.8–3.9	2.5–3.5	6.1–6.5	3.87–4.1
Stigmas	Bifid	Trifid	Trifid	Trifid	Trifid
Pedicel length (mm)	2.3–3.90	3.68–5.1	2.1–3	3.91– 5.6	3.29–3.91
Number of seeds per fruit	1– 2	1–2	1– 2	1– 3	1– 2
Shape of berry	Globose	Globose	Subglobose	Subglobose	Globose
Diameter of berry	4.4–7	4–6	5–7	4.5–5.2	5–6
Color of berry	Black	Red	Black then purple	Red	Red

bioclimates. It is common in the undergrowth of coastal pine forests with *Myrtus communis*, *Quercus coccifera*, and *Pistacia lentiscus* beside *Ruscus hypophyllum* and *Clematis cirrhosa*. It is well represented along the western littoral of Algiers (Bainem, Bouchaoui, Gouraya, Ténès) as well as the east littoral (Zemmouri, Guerbés, El Kala). This species also occurs in disturbed and more open pine forests of the southern slopes of the Tellian Atlas among *Chamaerops humilis* and *Ampelodesmos mauritanicus* (El Affroun, Redjredj, Ain Smara, Mansourah, Bougaa). In humid oak forest (*Quercus ilex*, *Q. suber*, *Q. faginea*), it is accompanied by *Tamus communis*, *Smilax aspera*, and *Ruscus aculeatus* (Yakouren, El Aouana). On the Saharan border (Senalba Mountains), it constitutes low bushes, often accompanied by *Jasminum fruticans* and *Rosmarinus tournefortii*.

3.2. *Asparagus albus* L., Sp. Pl. 1: 313 (1753)

The plant is hermaphroditic with a short rhizome. The turions are large, slightly bitter. The stem is erect up to 0.95 m, flexuous, smooth to very slightly striate, white, strongly spiny, woody and very branched. The leaves are reduced to small scales, triangular and brownish. The cladodes are subcylindrical, 11–27.2 mm long, subequal, smooth, and grouped in fascicles of 7–32. Flowers are grouped 6–12 at leaf axils, white and fragrant. The pedicels are longer than the flowers, 3.68–5.1 mm, with campanulate perianth from 2.8 to 3.9 mm in length. The stamens are subequal and shorter than the perianth, with purple anthers. The ovary is subglobose, 3-locular with 4 ovules per locule. Stigmas are trifid. The berries are globose, 4–6 mm in diameter, red becoming black with 1 or 2 seeds (Table 2). Turions grow in the beginning spring, differentiate and lignificate very quickly. Flowering in August and fruiting from November to December.

3.2.1. Karyology and meiotic behavior

All examined cytotypes share 2n = 2x = 20 chromosomes (Figures 2c and 2d). The karyotype consists of nine metacentric pairs and only one submetacentric with karyotype formula 18 m + 2 sm. The length of chromosomes is 3.19 to 6.25 µm. Intrachromosomal and interchromosomal asymmetry indexes are A1 = 0.91 and A2 = 0.25, respectively (Table 3). Meiosis in *A. albus* is regular with predominant bivalent pairing with similar behavior to that of *A. acutifolius*: 0.4 I + 7.6 IIR + 2.2 IIRd. The mean number of chiasmata per cell is 17.4. No irregularities were observed.

3.2.2. Habitat and distribution

This taxon is less common than *A. acutifolius*; it is linked to dry lands, rocky soils, and cliffs of subhumid and semiarid bioclimates. It is associated with *Ruscus hypophyllum*, *Calycotome spinosa*, and *Opuntia ficus-indica* (Keddara, Souidania, Ahmer El Ain, Tipaza). Sometimes it occurs with *A. acutifolius* and *A. horridus* in xerophytic shrubs of littoral formations (El Ançor, Misserghin near Oran) or on hills of Tellian Atlas (Tablat, Chiffa, Ain El Hadjar).

3.3. *Asparagus horridus* L., Sp. Pl. 1: 203 (1781)

Syn.: *A. stipularis* Forssk.; *A. stipularis* Forssk var. *horridus* (L.) Maire & Weiller; *A. stipularis* Forssk var. *brachyclados* Boiss. in Maire, Fl. Afr. Nord, 5: 220 (1958).

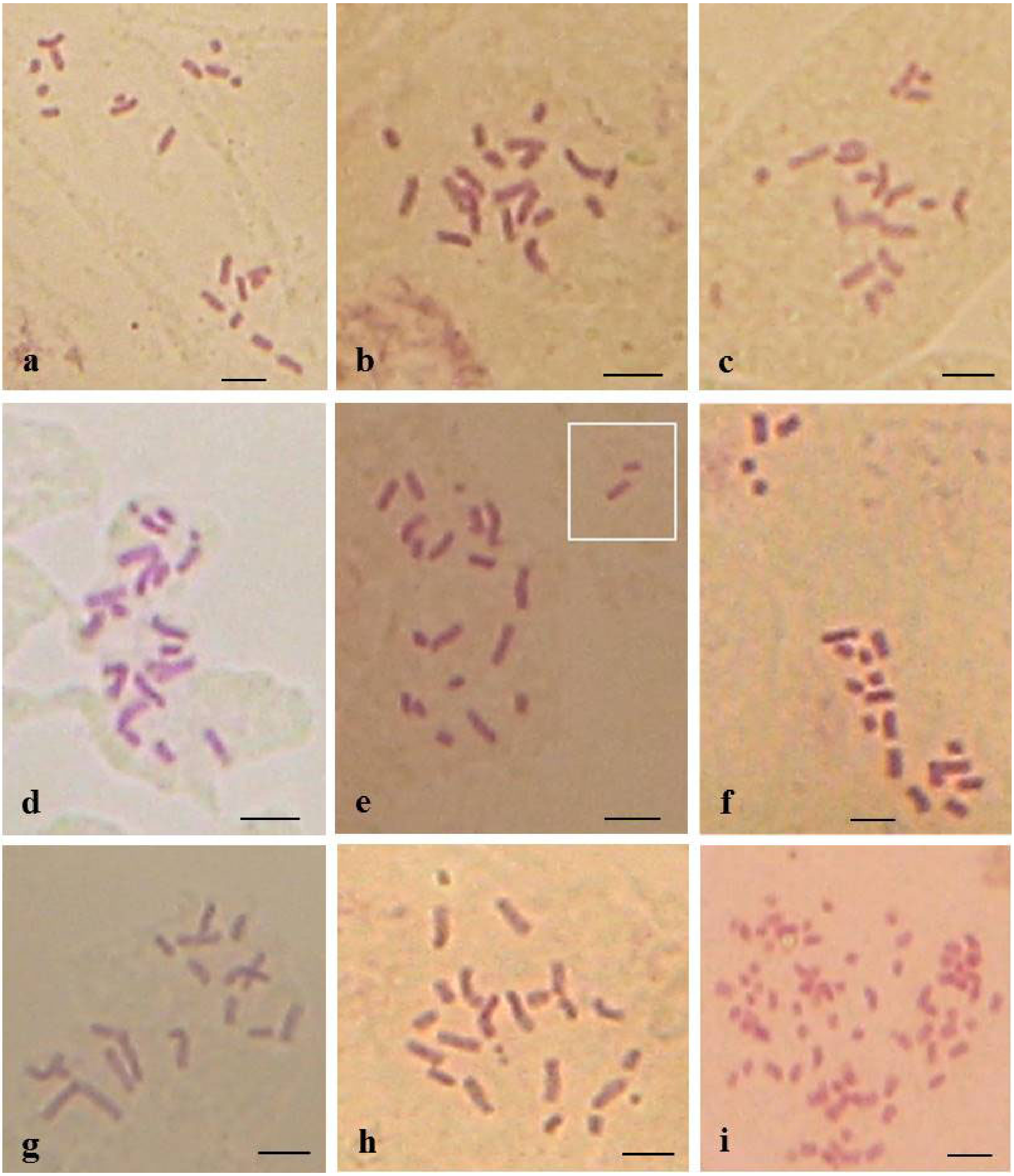

Figure 2. Mitotic metaphase plates: *A. acutifolius* 2n = 20 (a, b), *A. albus* 2n = 20 (c, d), *A. horridus* 2n = 20 (e, f), *A. officinalis* 2n = 20 (g) and (h) 2n = 20 + 1, *A. altissimus* 2n = 60 (i). Scale bars = 10 µm.

The plant is dioecious, woody with a short rhizome and edible turions, slightly bitter. The stem is flexuous 0.25 to 1 m long, green, smooth, and branched. The leaves are reduced to small scales, triangular brownish. The cladodes are subcylindrical, 19.9–86.2 mm long, thick, rigid, persistent and strongly spiny, cladodes in fascicles

Table 3. Chromosome numbers and karyotype parameters of the studied *Asparagus* species.

Species	n	x	A1	A2	Karyotype formula	TCL (µm)	L ± SE (µm)	b	B	S/L	R
A. officinalis	10	2	0.93	0.21	14m + 6sm	51.18	5.11 ± 1.26	3.15	6.52	0.48	2.06
A. albus	10	2	0.91	0.25	18m + 2sm	44.47	4.44 ± 1.12	3.19	6.25	0.51	1.95
A. acutifolius	10	2	0.92	0.20	16m + 4sm	52.07	5.20 ± 1.07	4.21	6.61	0.63	1.57
A. horridus	10	2	0.92	0.11	18m + 2sm	36.04	3.60 ± 0.42	2.91	4.46	0.65	1.53
A. altissimus	30	6	-	-	-	-	-	-	-	-	-

Intrachromosomal (A1), interchromosomal (A2) asymmetry index of Romero Zarco (1986).
Total chromosome length (TCL), mean chromosome length (L) and standard error (SE), minimum and maximum chromosome length (b, B), ratio of shortest/longest pair (S/L), largest/smallest chromosome ratio (R).

1–2. Flowers are yellow purple, grouped 2–6, and clustered at the base of cladodes. The pedicels are shorter, 2.1–3 mm. The perianth is more or less stellate, 2.5–3.5 mm in length. In male flowers, stamens are subequal and shorter than tepals with yellow purplish anthers. The ovary is rudimentary and very developing in female flowers; 3-locular with 2 ovules per locule. The stigmas are trifid. The berries are subglobose, 5–7 mm in diameter, black and then purple with 1 or 2 seeds (Table 2). Flowering from July to August and fruiting early December.

3.3.1. Karyology and meiotic behavior

Examined samples of *A. horridus* reveal 2n = 2x = 20 (Figures 2e and 2f). The karyotype shows structural similarity with that of *A. albus*. All chromosomes are metacentric, only one pair is submetacentric: 18 m + 2 sm. The length of chromosomes varies from 2.91 to 4.46 µm with intrachromosomal and interchromosomal asymmetry indexes A1 = 0.92 and A2 = 0.11, respectively (Table 3). This species is distinguished by irregular meiotic behavior. Various homologous chromosomal associations are observed at metaphase I; most of them are bivalents, others are univalent or multivalents: 0.4 I + 6.1 IIR + 2.4 IIRd + 0.6 III + 0.2 IV. In some anaphase I and telophase I cells, several irregularities in chromosome migration such as laggards, bridges, and micronuclei were observed. The mean number of chiasmata per cell is 16.6.

3.3.2. Habitat and distribution

This taxon is widely distributed in arid and semiarid bioclimates, mainly in NW Algeria (Ksar Chellala, Hassi Fedoul, Emir Khaled, El Ançor). It grows on stony soils in dry places, making a spiny bush with *Calycotome spinosa* and *Ziziphus lotus*. In steppic highlands, it constitutes, beside *Stipa tenacissima*, a striking high bush (Boughezoul and Boussaâda).

3.4. *Asparagus officinalis* L., Sp. Pl. 1: 313 (1753)

Syn.: *A. officinalis* var. *campestris* G.G. in Maire, Fl. Afr. Nord, 5: 217 (1958).

The plant is dioecious, herbaceous, green with a short rhizome. Turions are edible, large and white with a mild taste. The stem is suberect and grows 0.5–2 m long, striate, papillate and extremely branched. The leaves are reduced to small scales, triangular, brownish or whitish. The cladodes are cylindrical, 12.9–26.7 mm long, vigorous, smooth, and grouped 4–6 in fascicles. Flowers are yellow, grouped 1–2 rarely with the cladodes. Pedicel varies from 3.91 to 5.6 mm in length. The perianth is tubular, 6.1–6.5 mm in length. In male flowers, the stamens are subequal with yellow anthers and a rudimentary ovary. In the female flowers, ovary is subglobose, 3-locular with 2 ovules per locule. The stigmas are trifid. The berries are subglobose, 4.5–5.2 mm in diameter and reddish with 1–3 seeds (Table 2). Flowering in April and fruiting in September.

3.4.1. Karyology and meiotic behavior

All individuals of *A. officinalis* show 2n = 20 chromosomes (Figure 2g), some cells with supernumerary chromosomes 2n = 20 + 1 (Figure 2h). The karyotype consists of seven metacentric and three submetacentric pairs: 14 m + 6 sm. The length of chromosomes is 3.15 to 6.52 µm with intrachromosomal and interchromosomal asymmetry indexes A1 = 0.93 and A2 = 0.21 (Table 3). Meiotic behavior is fairly regular; the most common homologous association is bivalents with the following formula: 2 I + 7.5 IIR + 1.5 IIRd. Laggards and bridges of chromatin were observed in some anaphase and telophase I cells. The mean number of chiasmata per cell is 16.5.

3.4.2. Habitat and distribution

This taxon is rare in Algeria and would be a remnant of ancient cultures since the colonial period. It is scattered in very small and isolated populations along the edge of fields and roadsides, particularly in the plain of Mitidja near Algiers (Tessala El Merdja) in association with bulbous and annual herbs such as *Allium ampeloprasum*, *Foeniculum vulgare*, *Smilax aspera*, *Tamus communis*, and *Clematis flammula.*

3.5. *Asparagus altissimus* Munby, B Soc Bot Fr 2: 287 (1855)

Syn.: *A. altissimus* var. *typicus* Maire; *A. altissimus* var. *foeniculaceus* (Lowe) Maire; *A. altissimus* var. *asperulus* Maire. in Maire, Fl. Afr. Nord, 5: 223 (1958).

The plant is a hermaphroditic green vine with short rhizome and bitter turions. The stem is very climbing, flexuous, grows up to 1–5 m and extremely brunched. The leaves are reduced to small scales, membranous, triangular and brownish. The cladodes are subcylindrical, 14.89–19.7 mm long, linear, grouped in fascicles of 7–10. Flowers are white, fragrant and solitary with a long pedicel, 3.29–3.91 mm. The perianth is campanulate, varying from 3.87 to 4.1 mm in length. The stamens are shorter with yellow anthers. The ovary is subglobose, 3-locular with 2 ovules per locule. The stigmas are trifid. The berries are globose, 5–6 mm in diameter, reddish with 1 or 2 seeds (Table 2). Flowering from August to October and fruiting in December.

3.5.1. Karyology

Mitosis of somatic cells shows a high number of chromosomes representing a hexaploid level of 2n = 6x = 60 (Figure 2i). On the whole, the chromosomes are very small compared to the previous four species. Meiotic configurations were difficult to interpret.

3.5.2. Habitat and distribution

This taxon was firstly described as endemic to the northwest of Algeria at Misserghin near Oran, which represents the locus classicus. It grows in disturbed habitats bordering fields and roadsides under the cover of *Eucalyptus* and *Casuarina* hedges.

4. Discussion

Twelve *Asparagus* taxa have been described for the North African flora (Maire, 1958). The five species recognized for Algeria (Quézel and Santa, 1962) have been subjected in this study to morphological and cytogenetic analysis: *A. acutifolius*, *A. albu*s, *A. horridus*, *A. altissimus*, and *A. officinalis*.

4.1. Taxonomic and biogeographical remarks

A. acutifolius, a typical Mediterranean species, is undoubtedly the most frequent in Algeria. It grows in the undergrowth of forests in various biogeographic sectors from humid to arid areas. Populations of *A. acutifolius* show high polymorphism for the length and shape of cladodes in correlation with drought and temperature, the two main ecological factors. Morphological variability was also described for Sardinian populations by Urbani et al. (2007). The differentiation among populations led to the description of infraspecific taxa such as var. *gracilis* Baker and var. *achallii* Valdés. However, these varieties are rejected by the current nomenclatural status, like the African Plant database (accessed November, 2016) and the *World Checklist of Selected Plant Families* (Govaerts, 2016).

A. albus is much less common in Algeria than *A. acutifolius*. Although this species constitutes small populations scattered in coastal formations, it is widespread from the Tellian Atlas to the steppic high plains, mainly on dry and stony soils.

Compared to the two former species, *A. horridus* (syn. *A. stipularis*) is expanded throughout the southern side of the Mediterranean basin (Urbani et al., 2007) with a predilection for sandy soils of stabilized dunes and sometimes for rocky places (Heneidy and Bidak, 2004). In Algeria, *A. horridus* is more frequent in the NW than in the NE biogeographic sectors, growing on dry and rocky soils predominantly of arid and semiarid bioclimates.

Since its discovery by Munby (1855), *A. altissimus* has been quoted by Maire (1958) and Quézel and Santa (1962). This endemic species seems to be a polymorph in reference to the length of cladodes, which led Maire (1958) to describe three varieties. The var. *typicus* is narrowly located in the locus classicus, precisely at Misserghin near Oran in NW Algeria. The two others presumed varieties, var. *foeniculaceus* and var. *asperulus*, would be widespread from S and SW Morocco to the Western Sahara (Ozenda, 2004). In fact, the taxonomic status of these latter two varieties has been rejected by the African Plant database and by the *World Checklist of Selected Plant Families* (Govaerts, 2016). In Morocco, *A. altissimus* is considered as an important source in the traditional pharmacopoeia (Bellakhdar, 1998).

A. officinalis is very rare in Algeria. This taxon is represented by isolated populations remaining from cultivation since the colonial period. It has only been cited in some floristic lists from NW Algeria.

The taxonomic status of *Asparagus* taxa is sometimes confusing. In the Algerian floras (Maire, 1958), *A. acutifolius*, *A. horridus*, and *A. officinalis* were included within sect. *Euasparagus* Baker, while the two others, *A. albus* and *A. altissimus*, were placed within sect. *Asparagopsis* Kunth. This subdivision was based on morphological and flower sexuality criteria, separating the dioecious taxa from hermaphroditic.

In a molecular phylogenetic study, Norup et al. (2015) identified several clades with different bootstrap values. One of those, named the Canary Island Clade, brings together sequences belonging to putative gynodioecious species, *A. acutifolius* and *A. altissimus*, in particular. However, the herbarium specimens used by these authors for representing *A. acutifolius* and *A. altissimus* could be confused with *A. nesiotes* and *A. scoparius*, respectively, as already noted by Valdés (1979) in a revision of the genus *Asparagus* in Macaronesia. Indeed, *A. acutifolius* is deemed only dioecious and would be lacking in the Macaronesian Islands (Valdés, 1979, 1980), whereas *A. altissimus*, endemic to NW Africa, is known to be hermaphroditic (Maire, 1958; Quézel and Santa, 1962; Valdés, 1979).

Our findings indicate that dioecy and hermaphroditism characterize the Algerian populations of these two taxa.

4.2. Karyotype, meiotic behavior, and polyploidy

Karyological analysis revealed that four out of the five Algerian taxa are diploids (2n = 2x = 20). Only one species, the endemic *A. altissimus*, is hexaploid with 2n = 6x = 60. The diploid level seems to be the most common within the genus *Asparagus* with a well-conserved base number of x = 10 (Moreno et al., 2008; Harkess et al., 2016).

The chromosome number 2n = 20 found for *A. acutifolius*, *A. albus*, *A. horridus*, and *A. officinalis* is in accordance with previous numerations for many other taxa such as *A. retrofractus* L., *A. scoparius* Lowe, *A. verticillatus* L., *A. plumosus* Baker, *A. racemosus* Willd., *A.* cf. *pyramidalis*, *A. arborescens* Willd., *A. umbellatus* Link., *A. fallax* Svent., and *A. asparagoides* L. (Bozzini, 1959; Borgen, 1969; Bramwell et al., 1976; Kar and Sen, 1985; Urbani et al., 2007; Mukhopadhyay and Ray, 2013).

To our knowledge, the karyotype parameters of *A. acutifolius* and *A. albus* are presented here for the first time. The karyotypes of these two species are similar and consist mainly of metacentric chromosomes from 3.19 µm to 6.61 µm in length (Figures 3a and 3b). Compared to those of the diploids *A. plumosus*, *A. racemosus*, and *A. pyramidalis* (Kar and Sen, 1985; Jena and Das, 2003; Mukhopadhyay and Ray, 2013), our results show much higher chromosomes and a low interchromosomal asymmetry index (A2). The karyotype of other diploids such as *A. arborescens*, *A. umbellatus*, *A. fallax*, *A. scoparius*, and *A. asparagoides* (Ramos-Martinez, 1989) consist also of metacentric but relatively small chromosomes.

Concerning *A. horridus*, the chromosome number (2n = 20) has been quoted in many Mediterranean areas, sometimes with differentiation in the size and morphology of chromosomes (Figure 3c). For example, specimens of *A. stipularis* (=*A. horridus*) from the Canary Islands (Ramos-Martinez, 1989) show a smaller chromosome with total length of 26.72 µm versus 36.04 µm. Moreover, the karyotype of the Canarian samples consists of five metacentric and five submetacentric pairs of chromosomes against nine and one respectively in our own material.

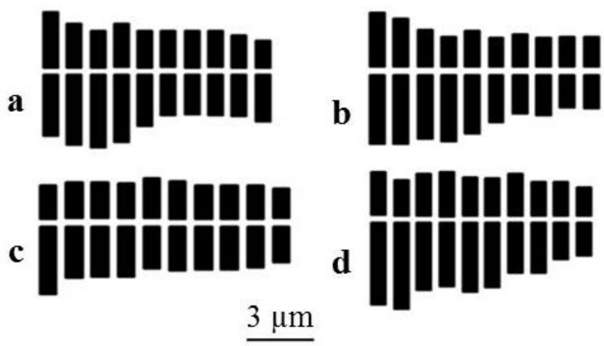

Figure 3. Idiograms of *A. acutifolius* (a), *A. albus* (b), *A. horridus* (c), and *A. officinalis* (d).

A. officinalis is undoubtedly the most studied species for economic reasons. Loptien (1979), Sheidai and Inamdar (1992), and Mukhopadhyay and Ray (2013) found a karyotype consisting of chromosomes mostly submetacentric. In our specimens, the karyotype appears to be more symmetric (Figure 3d), with slightly smaller chromosomes in total length (51.18 µm versus 61.76 µm). In addition, we have observed an occasional supernumerary chromosome (2n = 20 + 1). Aneuploidy seems to be very rare within the genus *Asparagus*; this sporadic event was already reported in diploid *A. officinalis* from India, with individuals having 1 to 6 additional chromosomes (Sheidai and Inamdar, 1992). In the tetraploid context, this species exhibits occasional aneuploidy with 2n = 44 (Kondo et al., 2014).

Polyploidy, the major mechanism for adaptation and speciation, occurs in *Asparagus* probably in association with sexual dimorphism (Kar and Sen, 1985; Sheidai and Inamdar, 1997; Castro et al., 2013). Most ploidy levels encountered in natural populations were 4x and 6x, some species having both 2x and 4x, others with 4x and 6x cytotypes. However in some cultivars of *A. officinalis* a polyploid series from 3x to 10x has been observed (Moreno et al., 2008).

The tetraploid level (4x) is found in *A. aphyllus* L., *A. cooperi* Baker, and *A. pastorianus* Webb & Berth., with a wide range of geographical repartition from West Europe to India (Bozzini, 1959; Borgen, 1969; Kar and Sen, 1985). Within the *A. acutifolius* polyploid complex, 4x populations are not frequent and are primarily encountered in southern parts of Europe, eastwards to SE Bulgaria (Bozzini, 1959; Valdés, 1980). In contrast, the 2x populations are widespread in Eurasia and mentioned only in the south of France (Vernet, 1971) and in Sardinia (Urbani et al., 2007). A similar situation arises in the geographical distribution of 2x and 4x populations of *A. officinalis* (Bozzini, 1959; Valdés, 1980).

In Algeria, for both *A. acutifolius* and *A. officinalis*, we found only 2x cytotypes. The predominance of diploid compared to tetraploid cytotypes have been previously established for some Algerian species belonging to the families Alliaceae and Hyacinthaceae (Hamouche et al., 2010; Azizi et al., 2016; Khedim et al., 2016).

However, the hexaploid level (2n = 6x = 60) found for the endemic *A. altissimus* is rare. It has been quoted mainly in the Eurasian region for some species such as *A. densiflorus* Kunth, *A. sprengeri* Regel, and *A. robustus* Hort. from India (Kar and Sen, 1985; Mukhopadhyay and Ray, 2013); *A. persicus* Baker from Russia (Tamanyan and Pogosyan, 1979); *A. gonocladus* Baker from Sri Lanka

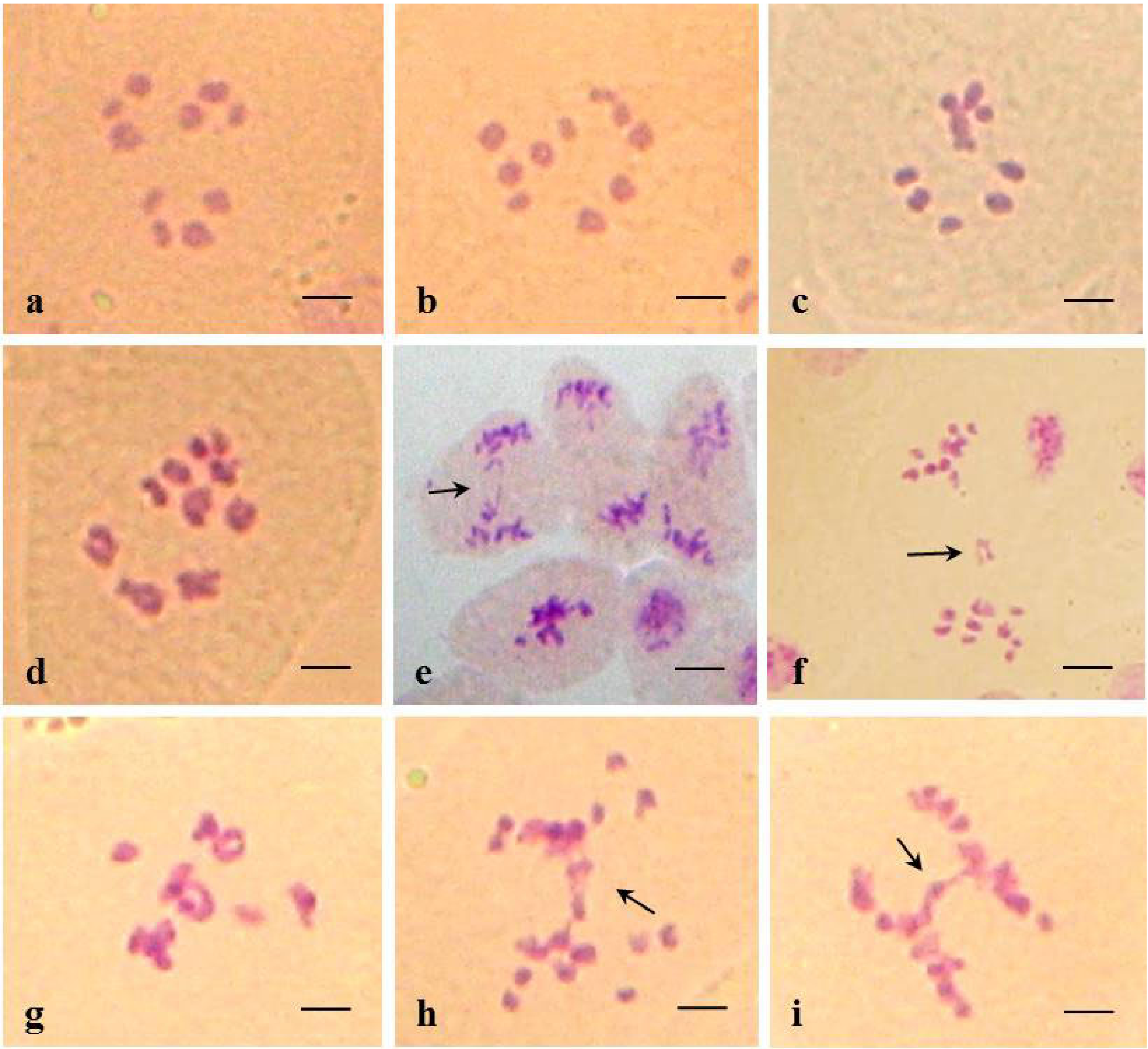

Figure 4. Meiotic chromosome associations at diakinesis, metaphase I, and anaphase I in four *Asparagus* species: *A. acutifolius* (a, b) and *A. albus* (c) with 10 IIR; *A. horridus* with 10 IIR (d), laggards and chromatin bridges (e, f); *A. officinalis* with 10 II ring and rod bivalents (g) and bridges (h, i). Arrows indicate laggards and bridges. Scale bars = 10 µm.

(Sheidai and Inamdar, 1992); and *A. maritimus* Mill. from Slovenia (Stajner et al., 2002). In the West Mediterranean region, the 6x level has been reported for *A. nesiote* subsp. *purpurienses* A.Marrero & A.Ramos, an endemic to Gran Piton Island of Macaronesia (Bramwell et al., 1976). In addition, a hexaploid population of *A. pastorianus* was discovered by Ramos-Martinez (1989) in a restricted area of Fuerteventura Island of the Canarian Archipelago. Furthermore, an exceptional high ploidy level (12x) was recently quoted for the newly discovered *A. macrorrhizus* endemic to Spain (Regalado et al., 2016).

Finally, the scattered endemic polyploids species in the NW Mediterranean region seem to be linked to past polyploidization events occurring in this area.

On the other hand, abnormalities involving univalents, bridges, and laggards (Figures 4a–4i) were sometimes observed in *A. officinalis* and *A. horridus*. In previous meiotic analysis of *A. officinalis*, Sheidai and Inamdar (1992) and Sheidai (2001) suggested that these abnormalities at anaphases I and II may be due to a genomic heterogeneity resulting from being a long way off from cultivation. Within the tetraploid *A. racemosus*, the occurrence of abnormalities would be linked to autopolyploidy (Nathar et al., 2013). Indeed, the unreduced gametes observed in some *Asparagus* species (Camadro, 1994), deriving probably from nondisjunction, would be at the origin of autopolyploidy.

In conclusion, the new chromosome number reports for the genus *Asparagus* in Algeria indicate a likely predominant diploid species with results showing a differentiation in genome size and, on the other hand, a regular meiotic behavior. These data indicate that diploids from North Africa may be the putative ancestral genome of *Asparagus* species, particularly that of *A. acutifolius*. The newly discovered hexaploid chromosome number of the endemic *A. altissimus* highlights the occurrence of polyploidy in NW Africa. This hexaploid, well adapted to semiarid bioclimates, may also be useful for genetic improvement of *Asparagus*. Further studies should be carried out on the different cytotypes for understanding the phylogenetic and evolutionary relationships of the genus *Asparagus* in North Africa.

Acknowledgments

This work was financially supported by the University of Sciences and Technology Houari Boumediene (USTHB, Algiers, Algeria). It was conducted in the framework of the program "Asparagales" of the Team Biosystematics, Genetics, and Evolution (Project: Cnepru n° F00220100043).

References

African Plant Database (2016). Conservatoire et Jardin botaniques de la Ville de Genève and South African National Biodiversity Institute, Pretoria (Version 3.4.0). Available online at http://www.ville-ge.ch/musinfo/bd/cjb/africa/ (accessed November 2016).

Amirouche R, Misset MT (2009). Flore spontanée d'Algérie: différenciation écogéographique des espèces et polyploïdie. Cah Agric 18: 474-480 (in French).

APG IV (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot J Linn Soc 181: 1-20.

Azizi N, Amirouche R, Amirouche N (2016). Karyological investigations and new chromosome number reports in *Bellevalia* Lapeyrouse, 1808 and *Muscari* Miller, 1758 (Asparagaceae) from Algeria. Comp Cytogen 10: 171-187.

Bellakhdar J (1998). La pharmacopée marocaine traditionnelle: Médecine arabe ancienne et savoirs populaires. Saint-Etienne, France: Ibis (in French).

Borgen L (1969). Chromosome numbers of vascular plants from the Canary Islands with special reference to the occurrence of polyploidy. Nytt Mag Bot 16: 81-121.

Bozzini A (1959). Revisione cito-sistematica del genere *Asparagus* L. Caryologia 12: 199-264 (in Spanish).

Bramwell D, Pérez de Paz J, Ortega J (1976). Studies in the flora of Macaronesia. Some chromosome numbers of flowering plants. Bot Macar 1: 9-16.

Camadro EL (1994). Second meiotic division restitution (SDR) 2n pollen formation in diploid and hexaploid species of *Asparagus*. Genet Resour Crop Ev 41: 1-7.

Castro P, Gil J, Cabrera A, Moreno R (2013). Assessment of genetic diversity and phylogenetic relationships in *Asparagus* species related to *Asparagus officinalis*. Genet Resour Crop Ev 60: 1275-1288.

Clifford HT, Conran JG (1987). Asparagaceae. In: George AS, editor. Flora of Australia. Canberra, Australia: Australian Government Publishing Service, pp. 140-142.

Fellingham AC, Meyer NL (1995). New combinations and a complete list of *Asparagus* species in southern Africa (Asparagaceae). Bothalia 25: 205-209.

Govaerts R (2016). World Checklist of Selected Plant Families Asparagaceae. Kew, UK: Royal Botanic Gardens. Available online at http://apps.kew.org/wcsp/ (accessed November 2016).

Hamouche Y, Amirouche N, Misset MT, Amirouche R (2010). Cytotaxonomy of autumnal flowering species of Hyacinthaceae from Algeria. Plant Syst Evol 285: 177-187.

Harkess A, Mercati F, Abbate L, McKain M, Pires JC, Sala T, Sunseri F, Falavigna A, Leebens-Mack J (2016). Retrotransposon proliferation coincident with the evolution of dioecy in *Asparagus*. G3-Genes Genom Genet 6: 2679-2685.

Heneidy SZ, Bidak LM (2004). Potential uses of plant species of the coastal Mediterranean region, Egypt. Pak J Biol Sci 7: 1010-1023.

Jena S, Das AB (2003). Karyotype variation and genomic characterization in five monocotyledonous mangrove associate from Orissa coast. Iran J Bot 10: 7-13.

Kanno A, Yokoyama J (2011). *Asparagus*. In: Kole C, editor. Wild Crop Relatives: Genomic and Breeding Resources: Vegetables. Berlin, Germany: Springer, pp. 23-42.

Kar DK, Sen S (1985). Chromosome characteristics of *Asparagus*-sapogenin yielding plant. Cytologia 50: 147-155.

Khedim T, Amirouche N, Amirouche R (2016). Morphological and cytotaxonomic data of *Allium trichocnemis* and *A. seirotrichum* (Amaryllidaceae) endemic to Northern Algeria, compared with *A. cupanii* group. Phytotaxa 243: 247-259.

Kondo K, Smirnov SV, Kucev M, Shimakov AI (2014). A chromosome study in *A. officinalis* L. in Mt. Altai. Chromosome Botany 9: 123-124.

Kubota S, Konno I, Kanno A (2012). Molecular phylogeny of the genus *Asparagus* (Asparagaceae) explains interspecific crossability between the garden *Asparagus* (*A. officinalis*) and other *Asparagus* species. Theor Appl Genet 124: 345-354.

Kumar M, Kumar Naik PS, Chhocar V (2015). Genetic variations in *Asparagus racemosus*, an endangered medicinal herb endemic to India using RADP markers. Br Biotechnol J 10: 1-11.

Levan A, Freda K, Sandberg AA (1964). Nomenclature for centromeric position on chromosomes. Hereditas 52: 201- 220.

Loptien H (1979). Identification of the sex chromosome pair in *Asparagus* (*Asparagus officinalis* L.). Z Pflanzenzücht 82: 162-173.

Maire R (1958). Flore de l'Afrique du nord. Vol. 5. Paris, France: P. Lechevalier (in French).

Moreno R, Espejo JA, Cabrera A, Gil J (2008). Origin of tetraploid cultivated *Asparagus* landraces inferred from nuclear ribosomal DNA internal transcribed spacers' polymorphisms. Ann Appl Biol 153: 233-241.

Mukhopadhyay S, Ray S (2013). Chromosome and marker-based genome analysis of different species of *Asparagus*. Cytologia 78: 425-437.

Munby G (1855). Notice sur quelques plantes d'Algérie. B Soc Bot Fr 2: 282-289 (in French).

Naidu MT, Kumar OA, Venkaiah M (2014). Taxonomic diversity of lianas in tropical forests of Northern Eastern Ghats of Andhra Pradesh, India. Not Sci Biol 6: 59-65.

Nathar VN, Dhoran VS, Gudadhe SP (2013). Meiotic analysis and pollen viability in *Asparagus racemosus* var. *javanica* (Kunth) Baker. Ann Pl Sci 2: 108-113.

Norup MF, Petersen G, Burrows S, Bouchenak-Khelladi Y, Leebens-Mack J, Pires JC, Peter Linder H, Seberg O (2015). Evolution of *Asparagus* L. (Asparagaceae): Out-of-South-Africa and multiple origins of sexual dimorphism. Mol Phylogenet Evol 92: 25-44.

Ozenda P (2004). Flore et végétation du Sahara. Paris, France: Centre National de la Recherche Scientifique (in French).

Quézel P, Santa S (1962). Nouvelle Flore de l'Algérie et des Régions Désertiques Méridionales. Paris, France: Centre National de la Recherche Scientifique (in French).

Ramos-Martinez A (1989). Aportaciones al conocimieto cariologico del género *Asparagus* L. (Liliaceae) en las Islas Canarias. Bot Macar 18: 3-14 (in Spanish).

Regalado JJ, Moreno R, Castro P, Carmona Martin E, Rodriguez R, Pedrol J, Larrañaga N, Guillén R, Gil G, Encina L (2016). *Asparagus macrorrhizus* Pedrol, Regalado et López-Encina, an endemic species from Spain in extreme extinction risk, is a valuable genetic resource for asparagus breeding. Genet Resour Crop Ev (in press).

Romero Zarco C (1986). A new method for estimating karyotype asymmetry. Taxon 35: 526-530.

Rudall PJ, Engleman EM, Hanson L, Chase MW (1998). Embryology, cytology and systematics of *Hemiphylacus*, *Asparagus* and *Anemarrhena* (Asparagales). Plant Syst Evol 211: 181-199.

Schnitzler A, Arnold C (2010). Contribution of vines to forest biodiversity in the Mediterranean basin. Ecologia Mediterranea 36: 7-23.

Sheidai M (2001). Notes on the occurrence of unreduced meiocytes in some species of *Asparagus* L. Nucleus 44: 36-41.

Sheidai M, Inamdar AC (1992). Polyploidy in the genus *Asparagus* L. Nucleus 35: 93-97.

Sheidai M, Inamdar AC (1997). Cytomorphology of *Asparagus* taxa using multivariate statistical analysis. Nucleus 40: 7-12.

Stajner N, Bohanec B, Javornik B (2002). Genetic variability of economically important *Asparagus* species as revealed by genome size analysis and rDNA ITS polymorphisms. Plant Sci 162: 931-937.

Tamanyan KG, Pogosyan AI (1979). Cytotaxonomic study of Caucasian species of *Asparagus* L. (Liliaceae). Bot Zhurn 64: 398-403.

Urbani M, Becca G, Ledda MG (2007). Notes on systematics and chorology of *Asparagus* L. (Asparagaceae) in Sardinia (Italy). Bocconea 21: 267-271.

Valdés B (1979). Revision del genero *Asparagus* (Liliaceae) en Macaronesia. Lagascalia 9: 65-107 (in Spanish).

Valdés B (1980). *Asparagus* L. In: Tutin TG, Heywood VH, Burges NA, Valentine D, editors. Flora Europaea, Vol. 5. Cambridge, UK: Cambridge University Press, pp. 71-73.

Vernet P (1971). La proportion des sexes chez *Asparagus acutifolius* L. B Soc Bot Fr 118: 345-358 (in French).