

# Morpho-anatomical diversity of five species of the genus *Asparagus* (Asparagaceae) from Algeria

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**Abstract** – Five species of the genus *Asparagus* are recognized in the flora of Algeria: *A. acutifolius* L., *A. albus* L., *A. horridus* L., *A. officinalis* L., and the endemic *A. altissimus* Munby. The chorology of each of these species is fairly well known. In this study, morphological variation and the anatomical features of the cladodes have been evaluated in respect to each taxonomic unit and ecogeographical distribution and they suggest distinct adaptive strategies. Analyses have been performed on twenty-nine natural populations sampled along the east-west bioclimatic gradient of Northern Algeria. Multivariate analysis based on the main diagnostic descriptors underlines the interspecific differentiation particularly with respect to the stigma type, bifid *versus* trifid, shape of flowers, color of berry, and the number of cladodes in a fascicle. For each species, the anatomy of the cladodes is unique, unlike that of stems and roots. Interspecific differentiation was observed in the form of cross-sections of the cladode, thickness of the cuticle, shape of epidermal cells, number of vascular bundles and presence of raphides. Morphological and anatomical traits of the cladode constitute important interspecific criteria within the genus *Asparagus*.

**Keywords:** adaptation, Algeria, anatomy, *Asparagus*, cladodes, morphology, taxonomy

## Introduction

The genus *Asparagus* (Asparagaceae) includes more than 200 species distributed in the arid and subarid regions of Africa, Europe, Asia and Australia (Chen and Tamanian 2000). Most species of *Asparagus* have great economic value, particularly *A. officinalis* L., cultivated as a vegetable crop all over the world. Some wild Mediterranean species such as *A. acutifolius* L., *A. horridus* L., *A. aphyllus* L. and *A. albus* L., are traditionally collected and sold in local markets (Pieroni 2005, Boubetra et al. 2017a, Mantovani et al. 2019).

The genus is noteworthy for its diversity of herbaceous, shrubby and climbing forms. Plants are provided with underground rhizomes from which the aerial shoots arise. All species are characterized by cladodes that correspond to green photosynthetic stems; the true leaves are reduced to small scales. In Mediterranean forests, the lianascent species grow preferentially in shady and wet ecological niches (Schnitzler and Arnold 2010). In contrast, shrubby species are encountered in open habitats especially in steppe areas, exhibiting tolerance to high temperatures and aridity.

Among the Asparagaceae (*sensu* Angiosperm Phylogeny group III 2009), species may display different phyllocladodes from flattened or cylindrical forms (Kubitzki and Rudall 1998, Fukuda et al. 2005). For instance, species of the genus *Ruscus*, develop a leaf-like organs, termed precisely phylloclades. They differ from cladodes in consisting of a stem with multiple internodes (Bell 2008). It is through the variation of these photosynthetic organs (cladodes leaves, stems, phyllocladodes) that species express adaptation and physiological responses to environmental changes. Molecular phylogenetic studies on *Asparagus*, indicated that cladodes evolve from a leaf-like (flattened) to a rod-like (cylindrical) form, suggesting a rapid radiation particularly in arid regions (Fukuda et al. 2005, Kubota et al. 2012). The genes involved in the evolutionary pathway of the transformation of the leaf-like form to axillary shoots were identified by Nakayama et al. (2012). Their cooption into a gene network is well documented in Nakayama et al. (2013).

Although numerous studies have been devoted to the biochemical characteristics of some *Asparagus* species, the morpho-anatomical variations in response to changes in

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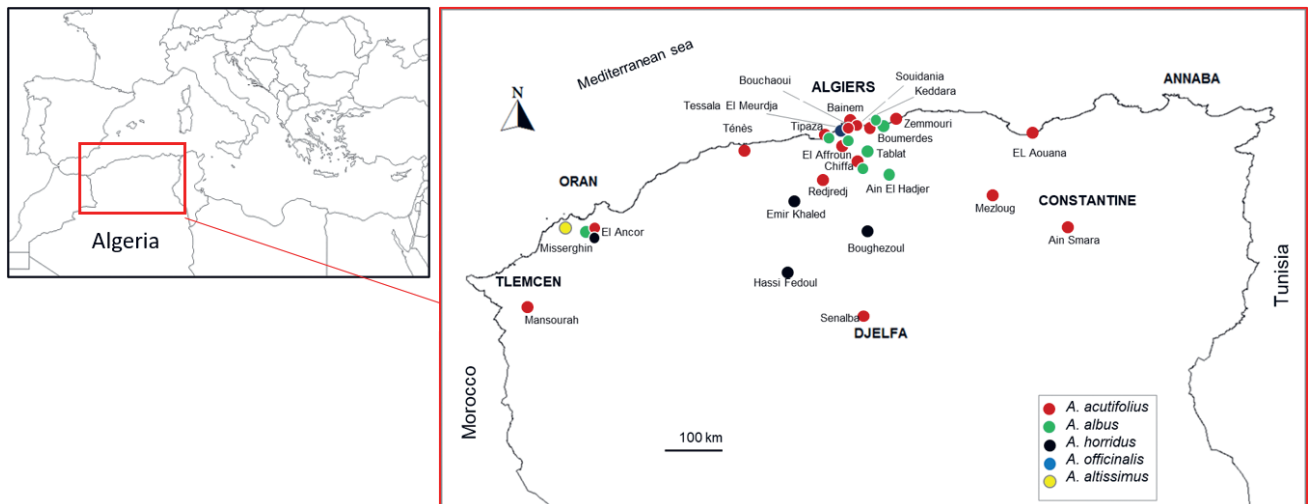


Fig. 1. Locations of the sampled populations of the five species of the genus *Asparagus* in northern Algeria.

environmental conditions have been poorly documented. In addition, current researches are more focused on roots and rhizomes for the detection of bioactive molecules.

In the flora of Algeria, five species have been recorded: *A. acutifolius* L., *A. albus* L., *A. horridus* L., (= *A. stipularis* Forsk.), *A. officinalis* L., and the endemic *A. altissimus* Munby. All these species are diploids with  $2n = 2x = 20$ , and grow in contrasting ecological conditions in northern parts of Algeria, except the endemic *A. altissimus* which is hexaploid with  $2n = 6x = 60$  (Boubetra et al. 2017b). The most widespread species, *A. acutifolius*, is encountered from humid to arid bioclimates, while *A. horridus* and *A. albus*, are less common and have a predilection for open habitats, dry, sandy and stony soils. *A. altissimus*, is a narrow endemic to NW Algeria and Morocco. *Asparagus officinalis* is very rare and seems a remnant of ancient cultures. However, spears of wild asparagus (*A. acutifolius*, *A. horridus*, *A. albus*) are widely harvested for food by local people. Therefore, wild species constitute a very interesting potential as a genetic resource for breeding programs.

The aims of this study were to evaluate morphological, floral and anatomical variations among the natural populations of the five *Asparagus* species occurring in Algeria. Anatomical examinations have been performed on cross sections of the cladodes in order to understand the ecological affinities and adaptive strategies of these species.

## Material and methods

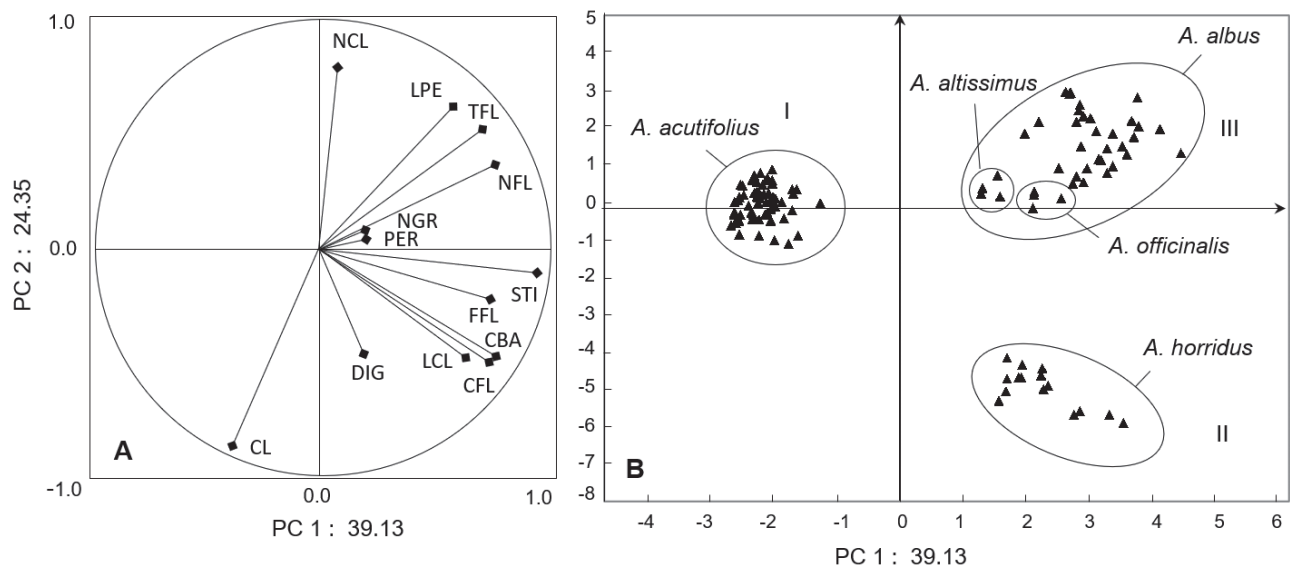
### Examined specimens

Twenty-four locations were selected in northern Algeria in humid, subhumid, semiarid and arid zones corresponding to the main vegetation types of forest, shrub formation, open habitat and steppic highlands. The taxonomic determination of the specimens was made according to the Flora of North Africa (Maire 1958) and Algeria (Quézel and Santa 1962). Overall, twenty-nine populations representative of the five studied species, recognized in the flora of Algeria, were sampled, some of them occurring in sympatry (Fig. 1, On-line Suppl. Tab. 1).

Specimens for morphological and anatomical analysis were sampled in reproductive stage with their whole vegetative structures. Seeds collected in the field were sown in the experimental station of the National Institute of Forest Research (Baraki, Algiers) in order to start a living collection of the studied species. Vouchers were deposited in the Official Herbarium of ENSA (Algiers, Algeria).

Tab. 1. Morphological and floral characters used in the multivariate analyses of five species of the genus *Asparagus* from Algeria.

Abbreviations	Characters	Qualitative values and measurement units	
1	CL	Shape of cladodes	Smooth 0
			Spiny 1
			Strongly spiny 2
2	LCL	Length of cladodes	mm
3	NCL	Number of cladodes in a fascicle	
4	CFL	Color of flower	Yellow 0
			White 1
			Purplish 2
5	TFL	Type of flower	Dioecious 0
			Hermaphrodite 1
6	FFL	Shape of flower	Campanulate 0
			Stelate 1
			Tubular 2
7	NFL	Number of flowers	
8	STI	Type of stigma	Bifid 0
			Trifid 1
9	LPE	Pedicel length	mm
10	PER	Perianth length	mm
11	CBA	Color of berry	Red 0
			Black 1
			Purple 2
12	NGR	Number of seed per berry	
13	DIG	Diameter of seed	mm



**Fig. 2.** Principal components analysis of 29 natural populations of five *Asparagus* species from Algeria based on 13 morphological and floral traits. A – loading of the 13 variables on the principal components (see Table 1 for abbreviations), B – overall scatter plot of 120 individuals representative of all species.

### Analysis of the variation of the vegetative and floral characters

The morphological evaluation was carried out on three or four individuals per population directly taken in their natural environment. A total of five quantitative and eight qualitative morphological and floral characters were selected following the diagnostic criteria for species delimitation, and self-observations (Tab. 1). The global raw data matrix consisting of 120 individuals belonging to the five studied species, and 13 variables, was first subjected to principal component analysis. This PCA was performed on the basis of the correlation matrix of the variables, generated after standardization of the raw matrix data. This analysis was used to estimate the relative contribution of each variable to the main principal components. To detect the phenetic grouping and relationships among the species studied, a UPGMA dendrogram was constructed using the Euclidean distances of the mean values per population.

In order to assess the impact of the bioclimatic conditions on the morphological variability, another PCA was focused on the populations of *A. acutifolius* which have the largest biogeographical distribution. This analysis was based on the average values of each variable for each population and included the main parameters of the Mediterranean bioclimate and the altitude (On-line Suppl. Tab. 1). For all the analyses we used Statistica software (ver. 12.0).

### Anatomical analyses

Anatomical examinations were performed on cross sections of cladodes freshly collected and conserved in ethanol 70°. The cross sections were made using a microtome. The technique consists of making thin paraffin sections (about 10 µm). Several successive steps are required: fixation, inclusion in paraffin, microtome sections, staining, assembly and observation. The technique of double staining with suc-

cessively methyl green (7') and Congo red (10') was used. The methyl green stains the lignified walls and the Congo red stains the cellulosic walls. Cross sections were photographed with a Zeiss Axiostar-Plus microscope equipped with a Canon digital camera.

## Results

### Interspecific variation for morphological and floral traits

In aim to assess the morphological characters involved in the variability and the interspecific relationships, a PCA

**Tab. 2.** Loading of the morphological and floral characters on the first three PCA axes of five species of genus *Asparagus* from Algeria (see Table 1 for abbreviations). PCA loadings > 0.60 are indicated in bold.

Characters	PC1	PC2	PC3
LCL	<b>0.65</b>	-0.47	0.05
NCL	0.02	<b>0.78</b>	0.36
CL	-0.40	<b>-0.85</b>	0.14
TFL	<b>0.74</b>	0.54	0.26
CFL	<b>0.79</b>	-0.49	0.28
FFL	<b>0.78</b>	-0.20	-0.44
NFL	<b>0.79</b>	0.37	0.32
STI	<b>0.97</b>	0.08	-0.03
LPE	0.56	<b>0.62</b>	-0.16
PER	0.22	0.05	0.84
DIG	0.22	-0.45	0.29
CBA	<b>0.81</b>	-0.49	0.04
NGR	0.18	0.08	-0.54
Eigenvalue	5.08	3.16	1.72
Total variance (%)	39.13	24.35	13.29
Cumulative variance (%)	39.13	63.49	76.79

followed by UPGMA analysis, were applied to all populations

In the overall PCA, we first examined the relative contribution of each variable on the first two principal components, then the distribution of individuals (Fig. 2). The loading of the variables, the eigenvalues and the cumulative variance to the principal components are given in Tab. 2. Together, the two first components describe 63.58% of the overall variances with 39.13% and 25.35% for PC1 and PC2 respectively.

As shown in Fig. 2A, eight variables displayed a high absolute contribution to PC1 in respect to their correlation: pedicel length (LPE), type of flower (TFL), number of flowers (NFL), type of stigma (STI), shape of flower (FFL), color of berries (CBA), color of flowers (CFL) and length of cladode (LCL). The highest contribution being that of the type of stigma bifid *versus* trifid. Compared to the second axis, only the number of cladodes (NCL) and the seed diameter (DIG) are discriminative. The length of the perianth (PER) and the number of seeds per berry (NGR) show no significance in either PC1 or PC2.

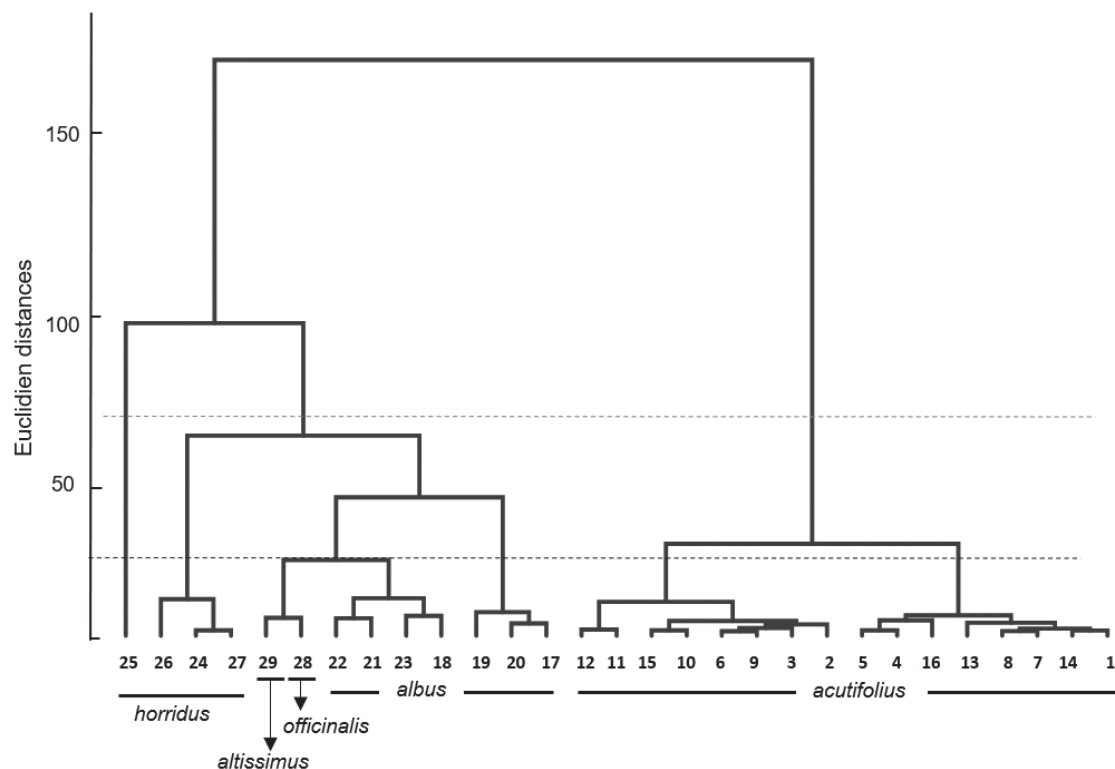
The scatterplot of individuals on the two first PCs display three main well-separated groups (Fig. 2B). Compared to PC1, a first group (I) is isolated in the negative part and corresponds to *A. acutifolius*. By contrast, towards the positive values of axis 1, the next two groups (II, III) concern individuals of other species. These last two groups are distinguished from each other in respect to PC2. One corresponds to the individuals of *A. horridus*, the other to those

of *A. albus*. The individuals belonging to *A. altissimus* and *A. officinalis* occupy a neighboring position to *A. albus*.

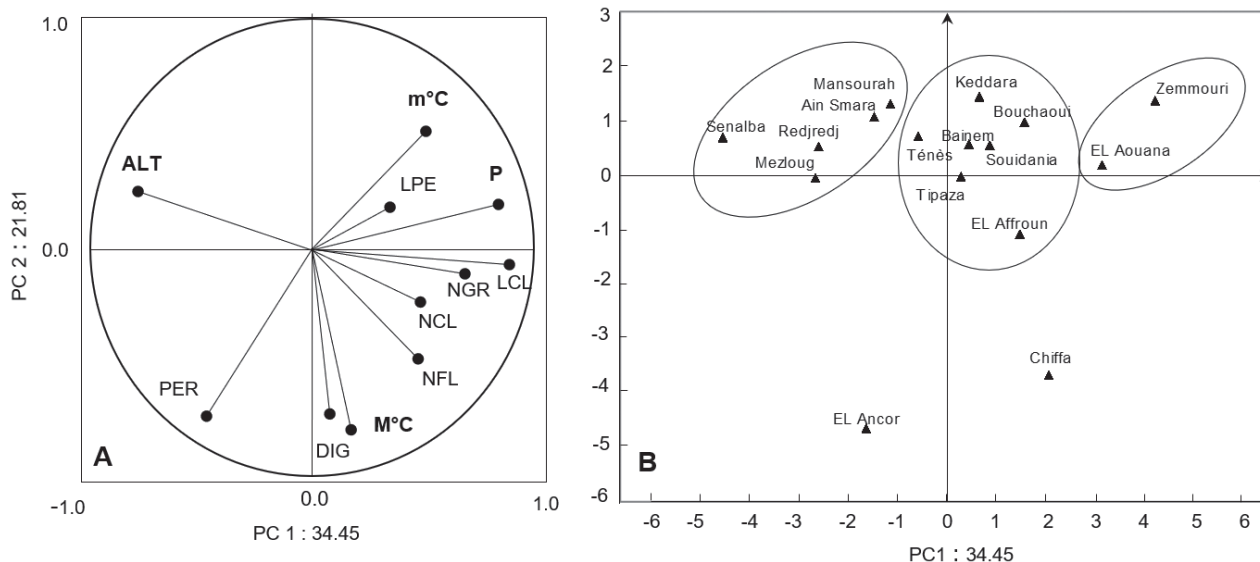
This principal component analysis makes it possible to discriminate the relevant diagnostic criteria of the five studied species. For instance, the color of berries and type of flowers discriminate all the species. The berries are red in *A. albus*, *A. officinalis* and *A. altissimus*, black in *A. acutifolius* and purple in *A. horridus*. The flowers are campanulate in *A. acutifolius*, stellate in *A. albus*, *A. horridus* and *A. altissimus*, and tubular in *A. officinalis*. The last two species, *A. altissimus* and *A. officinalis*, show affinities in relation to their smooth cladodes. On the other hand, *A. horridus* and *A. acutifolius* are outstanding, by one strongly spiny cladode and by bifid stigmas, respectively.

A hierarchical analysis using the matrix of the means values by population of the 13 morphological and floral traits, brings out the same grouping of populations as in the previous PCA (Fig. 3). At the distance  $d < 100$ , two main clades are clearly distinct from each other. The first clade brings together the populations belonging to *A. albus* and *A. horridus* which constitute small distinctive groups. Although developing in open and semi-arid habitats, the populations of these two species show unexpected inter-population variability. The other two species *A. officinalis* and *A. altissimus*, show close relationships with *A. albus* due to the smooth cladodes.

Within *A. acutifolius*, two clusters are quite well separated (at the distance  $d < 55$ ). The first one is mainly composed of coastal populations from Tipaza, El Aouana and



**Fig. 3.** Dendrogram analysis showing relationships among 29 Algerian populations of the genus *Asparagus*. Analysis was performed on the matrix of mean values of 13 morphological and floral traits. The dotted lines correspond to the distances allowing the main clusters to be distinguished.



**Fig. 4.** Principal components analysis focused on 16 populations of *A. acutifolius* showing the relationships between the morphological and floral traits and the main bioclimatic parameters. A – loading of the variables on the circle of correlations, B – scatter of the mean values of each population.

Zemmouri. The second cluster concerns populations from the inland collecting sites at higher altitudes such as Senalba (southern slope of the Saharan Atlas) Mezloug, Ain Smara (east of the Tellian Atlas), Redjredj and Keddara (central part of the Tellian Atlas).

In order to estimate the impact of climatic factors on morphological variability, we performed another PCA focused on the 16 populations of *A. acutifolius*. Indeed, unlike *A. albus* and *A. horridus*, populations of *A. acutifolius* are encountered in very diverse habitats from wetlands to semi-arid and arid areas. This PCA takes into account the five main parameters of the Mediterranean bioclimate (Fig. 4).

In contrast to the two species *A. albus* and *A. horridus*, populations of *A. acutifolius* are encountered in very diverse habitats of undergrowth and open areas from wetlands to semi-arid and arid zones. In this aim, a second PCA was focused on the 16 populations of *A. acutifolius* taking into account the five main parameters of the Mediterranean bioclimate (Fig. 4). The PCA has been carried out on the average of the values of each variable for each population as well as the annual precipitation (P), the altitude (Alt.), the average of the maximum in summer ( $M^{\circ}C$ ) and the minimal temperatures in winter ( $m^{\circ}C$ ) (On-line Suppl. Tab. 1). The first two axes PC1 and PC2 describe 55.26% of the total information with 34.45% and 21.81%, respectively (Fig. 4A). The length of the cladodes, LCL, and, to a lesser extent NGR, NCL and NFL, are located on the positive part of PC1 and highly correlated with the precipitation P. In contrast, the altitude (Alt) is located on the negative part of this axis. PC2 shows, on one hand, a positive correlation between the diameter of the seeds (DIG) and the maximum temperature in summer  $M^{\circ}C$  and, on the other hand, a negative correlation with the minimum temperature in winter ( $m^{\circ}C$ ).

Two groups of populations are opposed with respect to PC1 (Fig. 4B). The populations from continental, semi-arid

to arid bioclimates and higher altitudes (Senalba, Redjredj, Ain Smara and from Mezloug in the East to Mansourah in the West area) constitute a first group. The second one concerns populations from northeast coastal stations as Zemmouri and El Aouana located in subhumid and humid bioclimate. All other populations are from subhumid bioclimate (Keddara, Bainem, Bouchaoui, Tipaza, Souidania) and are distributed in an intermediate situation between the two previous groups within the biogeographical sector of Algiers.

#### Anatomical analysis of the cladodes

The cross sections of the cladodes of the five studied species show a different structure. Interspecific variations have been observed relatively to the shape of section, the thickness of the cuticle, the shape of epidermal cells, the number of layers of palisade cells, the number of vascular bundles and the presence of raphides (Fig. 5, On-line Suppl. Tab. 2).

Circular cross sections are found in three species, *A. acutifolius*, *A. officinalis* and *A. altissimus* (Fig. 5A, I, K). The first one is characterized by uniseriate epidermis with isodiametric cells strongly cutinized (Fig. 5B) with the presence of stomata along this epidermis (Fig. 5C). Just below the epidermis, the palisade parenchyma consists of two layers of elongated cells rich in chloroplasts. Spongy parenchyma varies highly in shape, and the cells may be isodiametric or elongated. At this level, some cells, called idioblasts, contain raphides (calcium oxalate crystal) in the form of rods (Fig. 5D). In the pith, the cells are strongly sclerified, and only two vascular bundles have been observed.

In *A. officinalis*, the cuticle is thin and the epidermal cells are rounded and not of the same size with some larger cells (Fig. 5J). Stomata were observed all around the cross section. The palisade parenchyma is composed of two lay-

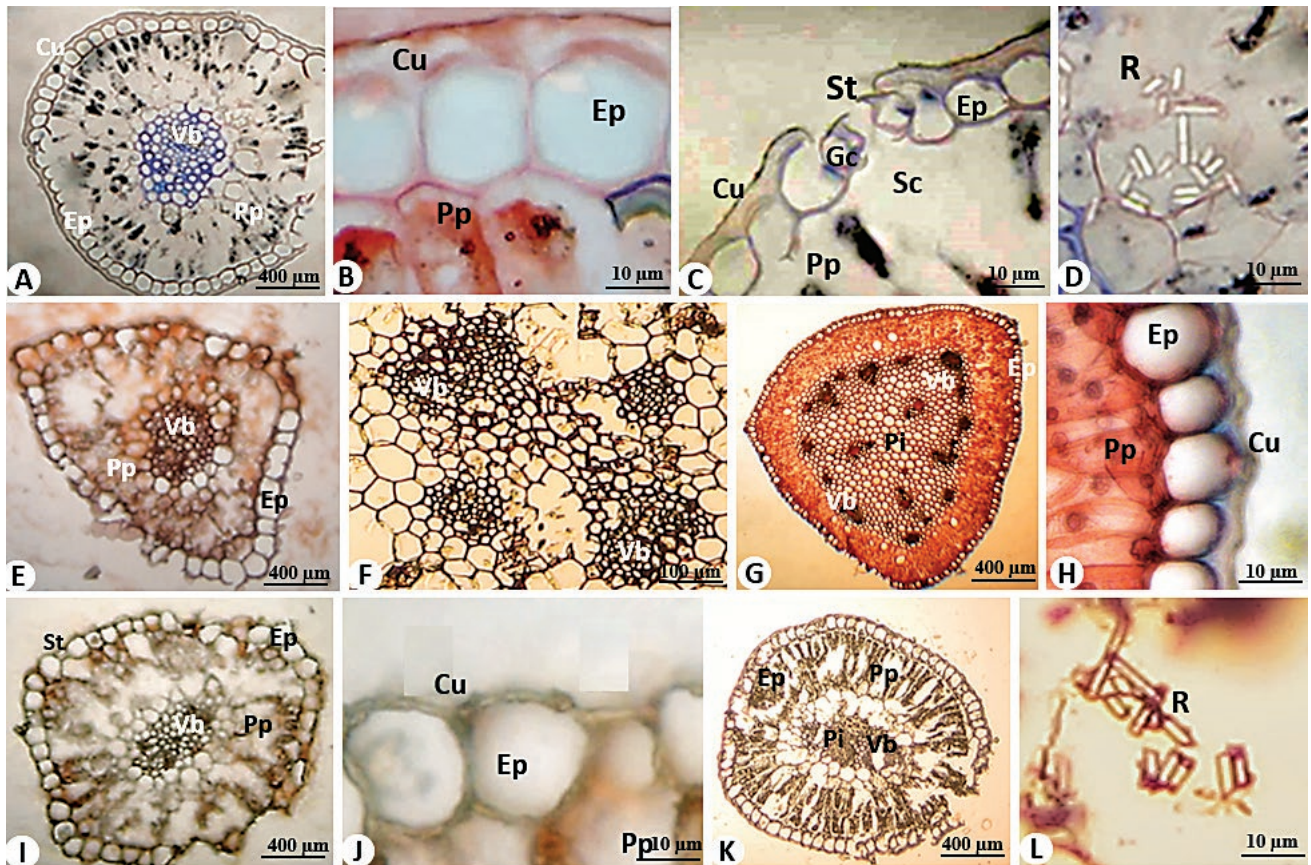


Fig. 5. Cross sections of cladodes of the five Algerian *Asparagus* species. A. *acutifolius*: A – circular section, B – epidermis and cuticle, C – stomata, D – raphides. A. *albus*: E – triangular section, F – vascular bundles. A. *horridus*: G – triangular section, H – epidermis and cuticle. A. *officinalis*: I – circular section, J – epidermis. A. *altissimus*: K – circular section, L – raphides. Cu: cuticle, Ep: epidermis, Pp: palisade parenchyma, St: stomata, Gc: guard cell, Sc: substomatal chamber, R: raphides, Pi: pith, Vb: vascular bundles, Ph: phloem.

ers of elongated cells. In the pith, two vascular bundles are observed and surrounded by a single layer of spongy parenchyma.

In the endemic *A. altissimus*, the epidermis is made up of a single layer of square cells also covered by thick cuticle with numerous stomata. The palisade parenchyma is composed of two layers of elongated cells. The cells of spongy parenchyma are irregular and some of them contain raphides (Fig. 5L). Four vascular bundles are observed.

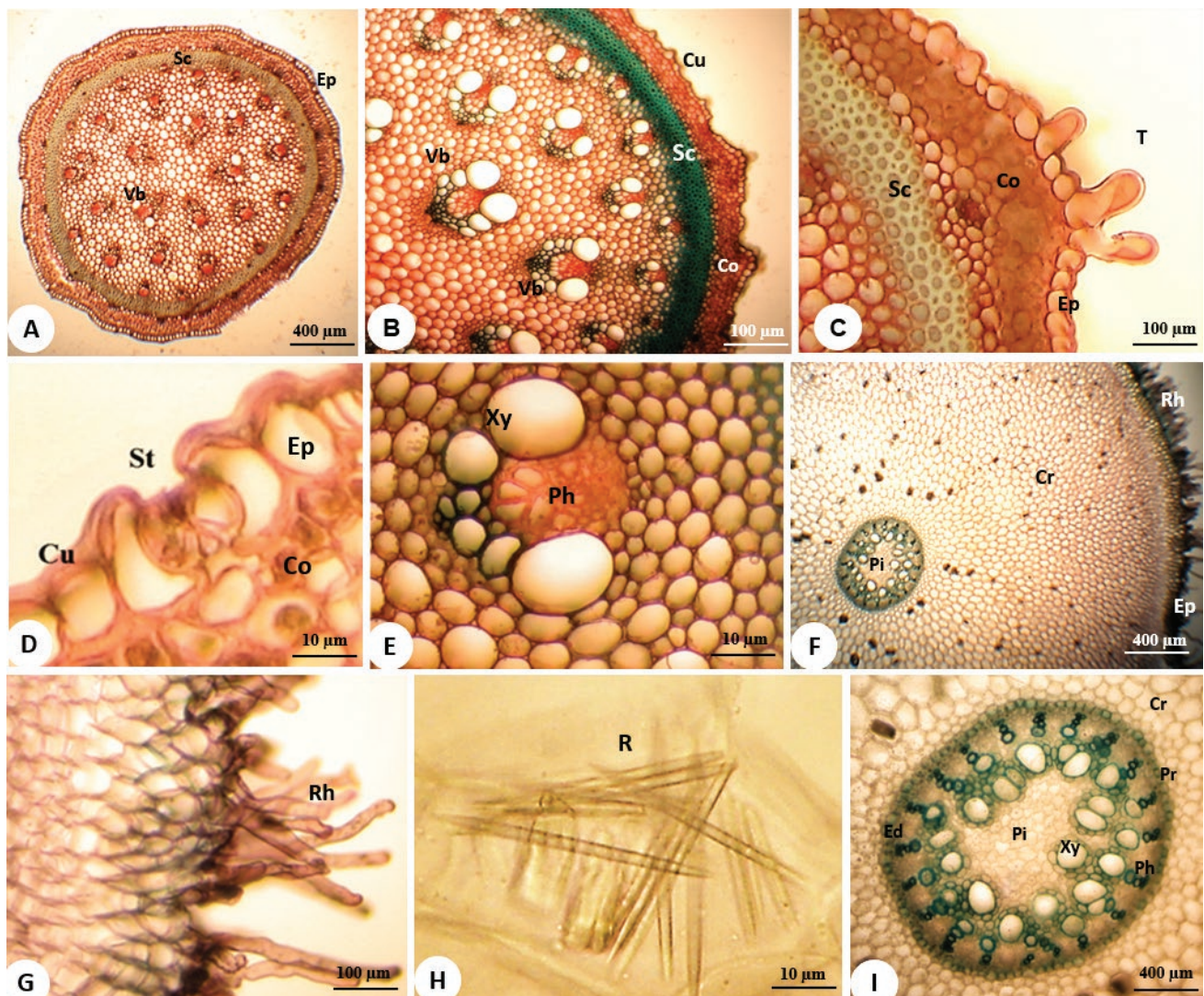
In the two other species, i.e. *A. albus* and *A. horridus*, the cross section is triangular (Fig. 5E, G). The epidermal cells in *A. albus*, are rounded to irregular in shape with variable size. They are covered by a thin cuticle (Fig. 5E). Stomata were also observed. The palisade parenchyma contains elongated cells arranged in three layers whereas the spongy parenchyma consists of relatively large, thin-walled cells. In the pith, the cells are sclerified with four vascular bundles (Fig. 5F). In *A. horridus*, the epidermal cells are rounded with a thick cuticle (Fig. 5H). In the palisade parenchyma, the cells were rearranged in several layers with small intercellular spaces. The pith occupies the greatest part and contains numerous vascular bundles arranged in a ring. Here, in each bundle, the xylem is directed toward the center of the cladode.

## Discussion

### Taxonomic relationships

Algeria occupies a central biogeographic position in North Africa, characterized by an impressive east-west bioclimatic gradient from humid to arid lands. In this Mediterranean region, strong topographic and soil heterogeneity explains the highly contrasting habitats and the floristic richness. In this ecogeographical context, wild species of the genus *Asparagus* occur in various ecological conditions, under forest cover as well as in open and dry habitats of the steppe vegetation of the highlands, showing high tolerance to drought and high temperatures (Boubetra et al. 2017a, b).

*Asparagus acutifolius* is widespread and quite common in moist and shady biotopes of woodlands and shrublands of humid and semiarid bioclimates. *Asparagus albus* and *A. horridus* were less common in this research; both are linked to dry and stony soils mostly in arid and semiarid habitats of NW Algeria. Sometimes, they occur in sympatry in steppe highlands making high xerophytic shrubs. *Asparagus altissimus* is endemic to northwestern Algeria consisting of small populations of scattered individuals along hedges on saline and dry rather sandy soils. *Asparagus*



**Fig. 6.** Illustrations of some cross sections of stems and roots from Algerian specimens of *Asparagus*. Stem of *A. horridus*: A – global view of the section, B – arrangement of cribro-vascular bundles. C – trichomes in the stem of *A. acutifolius*. D – stomata in the stem of *A. altissimus*. E – vascular bundle in stem of *A. horridus*. Root of *A. acutifolius*: F – general view of the section, G – root hairs, H – raphides within cells of the cortex, I – general view of vascular cylinder. Cu: cuticle, Ep: epidermis, St: stomata, Co: collenchyma, Pi: pith, Vb: vascular bundles, Xy: xylem, Ph: phloem, Cr: cortex (parenchyma cells), Rh: root hairs, R: raphides, Pi: pith, Ed: endodermis, Pr: pericycle.

*officinalis* is very rare and appears to have naturalized as isolated individuals on the edges of cultivated fields.

Despite the wide distribution of the species of the genus *Asparagus* in the Mediterranean region and their ecological and economic interest, few studies have been performed to elucidate the morphological variability, particularly for wild *A. acutifolius*, *A. albus* and *A. horridus*. Current researches are focused mainly on the cultivated species *A. officinalis* for its economic importance. Studies based on molecular markers, aim to assess genetic diversity among germplasm including the related wild species from Europe and Asia (Mousavizadeh et al. 2021). Other studies combine both morphological and molecular data (Irshad et al. 2019, Chen et al. 2020). Morphological variability, growth and production of spears may be correlated to environmental factors (Altunel 2021).

Concerning wild species, a multivariate analysis performed on Indian populations of *A. racemosus* Willd.

(Chithra and Siril 2017), showed that significant characters were relative to plant height, the diameter and color of stem, length and number of cladodes in fascicle. In the Iranian species, *A. officinalis*, *A. persicus* Baker, *A. verticillatus* L., and *A. breslerianus* Schult. & Schult., the length of cladodes and seed number per fruit are the most discriminating characters (Mousavizadeh et al. 2015). In these species, the number of seeds per berry varies from 1 to 6, unlike the Algerian species where the number of seeds is 1-2, exceptionally 3 in an individual of *A. officinalis*.

Despite the morphological similarities among some species of the genus *Asparagus*, Chen et al. (2020) have shown that individual variations could be linked to environmental factors. The analysis of the genetic variation of Italian populations of *A. acutifolius*, evaluated by the ISSR markers (Sica et al. 2005), indicated an inter-population diversity according to their geographical origin. A study on the systematics and the chorology of *A. acutifolius*, *A. albus* and *A.*

*horridus* in Sardinia, allowed Urbani et al. (2007) to confirm the presence of these species and to exclude *A. aphyllus* L., from the flora of this island on the basis of anatomical criteria of cladodes. In Iran, the wild asparagus polyploids (8x, 10x) are adapted to saline and dry lands (Mousavizadeh et al. 2022). These results agree with the repartition of the endemic hexaploid (6x) *A. altissimus* which occurs preferentially on saline soils.

### Anatomical diversity of the cladodes

Compared to the stems and roots (Fig. 6), the morphology and anatomy of the cladode in the genus *Asparagus*, show a striking diversity and distinctive interspecific features. Undoubtedly the structure of the cladodes has a taxonomic significance. It would also be linked to the ecological conditions with regard to the chorology and geographic distribution of each species (Boubetra et al. 2017a, b).

The cross section is circular in all the species, except *A. albus* and *A. horridus*, which show a triangular shape. Circular sections have also been observed in *Asparagus* species from Bulgaria such as *A. tenuifolius* Lam., and *A. acutifolius* (Raycheva and Stojanov 2013). The triangular shape is rare, having been reported only in *A. adscendens* Roxb., (Kawale et al. 2014). Various other shapes are specific to this genus such as the oval in *A. brachyphyllus* Turcz., and *A. schoberioides* Kunth (Ito et al. 2006), irregular elliptic in *A. lycicus* P.H. Davis and *A. persicus* (Güvenç and Koyuncu 2002). In *A. officinalis*, *A. maritimus* (L.) Mill., and *A. verticillatus*, the shape varies from irregular elliptic to stellar with unclear angles (Raycheva and Stojanov 2013). Samples of *A. officinalis* from Algeria are remarkable for their circular shape.

The interspecific variability of cross-section of the cladodes was also observed within other genera such as *Ruscus* where the shape is rectangular in *R. aculeatus* L., and elliptical in *R. colchicus* Yeo (Güvenç et al. 2011). Furthermore, the epidermal cells also exhibit a variability. For the Algerian specimens of *A. acutifolius* and *A. officinalis*, the cells are respectively isodiametric and rounded, whereas they are rectangular in Bulgarian populations (Raycheva and Stojanov 2013), barrel-shaped in *A. brachyphyllus* (Bercu 2008) and longitudinal in *A. racemosus* (Durai Prabakaran et al. 2015).

In addition, these cells are covered with a cuticle varying in thickness depending on the species. Anatomical studies performed on *A. asparagoides* (L.) Druce show that the cuticle thickness correlates with the shape of the section of the cladode (Coles et al. 2006). According to Tamanian (1982), this variability expresses adaptation to ecological conditions. Under the epidermis of each species two or three layers of palisade cells with different lengths are situated. These results are consistent with those obtained on *A. adscendens* (Kawale et al. 2014) and *A. racemosus* (Durai Prabakaran et al. 2015). The most numerous and the longest palisade cells were observed in *A. verticillatus*, with four rows (Raycheva and Stojanov 2013).

In *A. tenuifolius*, *A. maritimus* and *A. officinalis*, the parenchyma is represented by two rows of slightly prolonged

cells (Raycheva and Stojanov 2013). These results correlate with our studied species except for *A. albus* and *A. horridus* whose show three and several layers respectively. Compared to species of the genus *Ruscus*, the palisade and spongy parenchyma are replaced by layers of cells containing chloroplasts (Güvenç et al. 2011).

The vascular system is delimited by the assimilation parenchyma by one cell row. In our study, raphides are remarkable in this parenchyma, and are present only in cladodes of *A. acutifolius* and *A. altissimus*. Their presence in some taxa may have a taxonomic significance as mentioned also by Prychid and Rudall (1999), also shown in the genus *Ruscus* (Güvenç et al. 2011). The cladodes of the Algerian samples of *A. acutifolius* are distinguished by the constant presence of two vascular bundles while in Turkish and Bulgarian specimens, this number were four and five respectively (Güvenç and Koyuncu 2002). Two vascular bundles have also been reported for *A. brachyphyllus* and *A. officinalis* (Begum et al. 2017). In *A. horridus* from Algeria, the vascular bundles are can number up to 20 and occupy all the pith, as in the case of *A. aphyllus* (Güvenç and Koyuncu 2002). As suggested by Raycheva and Stojanov (2013), the anatomical characters of the cladodes particularly the number of vascular bundles can be correlated with the ecological environments.

### Conclusion

This study constitutes the first report on morpho-anatomy for the genus *Asparagus* in Algeria. The results highlight the taxonomic importance of morphological and floral characters as well as the anatomical features of the cladodes, mainly the shape, the number of vascular bundles and the raphides. They also provide perspective for a better understanding of the diversity of species and populations in relation to environmental conditions, particularly for *A. acutifolius*, which is widespread in highly contrasting bioclimatic conditions.

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### References

- Altunel, T.A., 2021: Morphological and habitat characteristics of *Asparagus* (*Asparagus officinalis* L.) and socio-economic structure of producers. Turkish Journal of Agriculture - Food Science and Technology 9, 1092–1099.
- Angiosperm Phylogeny Group III, 2009: An update of the Angiosperm phylogeny group classification for the orders and



- families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161, 105–121.
- Begum, A., Sindhu, K., Giri, K., Umera, F., Gauthami, G., Kumar, J.V., Naveen, N., Rao, K.N.V., Ali, S.S., Sri, K., Dutt, R., 2017: Pharmacognostical and Physio-Chemical Evaluation of Indian *Asparagus officinalis* L. *International Journal of Pharmacognosy and Phytochemical Research* 9, 327–336.
- Bell, A.D., 2008: *Plant form: An illustrated guide to flowering plant morphology*. Timber Press, Portland.
- Bercu, R., 2008: Some aspects of *Asparagus brachyphyllus* Tucz. (Asparagaceae) anatomy. *Research Journal of Agricultural Science* 50(3), 7–12.
- Boubetra, K., Amirouche, N., Amirouche, R., 2017a: Le genre *Asparagus* L. en Algérie : Systématique, chorologie et importance en écologie forestière. *Biocénose* 8, 77–81.
- Boubetra, K., Amirouche, N., Amirouche, R., 2017b: Comparative morphological and cytogenetic study of five *Asparagus* (Asparagaceae) species from Algeria including the endemic *A. altissimus* Munby. *Turkish Journal of Botany* 41, 588–599.
- Chen, H., Guo, A., Wang, J., Gao J., Zhang S., Zheng J., Huang, X., Xi, J., Yi, K., 2020: Evaluation of genetic diversity within asparagus germplasm based on morphological traits and ISSR markers. *Physiology and Molecular Biology of Plants* 26, 305–315.
- Chen, X.Q., Tamanian, K.G., 2000: *Asparagus* L. In: Wu, Z.Y., Raven, P.H. (eds.), *Flora of China* Vol. 24. (Flagellariaceae through Marantaceae), 209–216. Science Press, Beijing, Missouri Botanical Garden Press, St. Louis.
- Chithra, M.G., Siril, E.A., 2017: Morphological variability of aerial vegetative characters among 20 Shatavari (*Asparagus racemosus* Willd.) collections from Kerala, India. *Journal of Root Crops* 43(2), 21–32.
- Coles, R.B., Willing, K.L., Conran, J.C., Gannaway, O., 2006: The identification and distribution of western cape form bridal creeper (*A. asparagoides*) in the south east of south Australia and western Victoria. *Plant Protection Quarterly* 21, 104–108.
- Durai Prabakaran, K., Vadivu, R., Jayshree, N., 2015: Pharmacognostical standardization of leaves of *Asparagus racemosus* Willd. *International Journal of Multidisciplinary Research and Development* 2, 332–335.
- Fukuda, K., Ashizawa, H., Suzuki, R., Ochiai, T., Nakamura, T., Kanno, A., Kameya, T., Yokoyama, J., 2005: Molecular phylogeny of the genus *Asparagus* (Asparagaceae) inferred from Plastid *petB* intron and *petD-rpoa* intergenic spacer sequences. *Plant Species Biology* 20, 121–132.
- Güvenç, A., Koyuncu, M., 2002: Studies on the anatomical structure of cladodes of *Asparagus* L. species (Liliaceae) in Turkey. *Israel Journal of Plant Sciences* 50, 51–65.
- Güvenç, A., Coşkun M., Arihan, O., 2011: Anatomical structure of cladodes of *Ruscus* L. taxa (Liliaceae) in Turkey. *FABAD Journal of Pharmaceutical Sciences* 36, 119–128.
- Irshad, M., Idrees, M., Tariq, A., Pathak, M.L., Hanif, M., Naeem, R., 2019: Genetic diversity among *Asparagus* species using morphological characteristics and RAPD markers in Pakistan. *Journal of Biodiversity Conservation and Bioresource Management* 5, 13–24.
- Ito, T., Ochiai, T., Ashizawa, H., Shimodate, T., Sonoda, T., Fukuda, T., Yokoyama, J., Kameya, T., Kanno, A., 2006: Production and analysis of reciprocal hybrids between *Asparagus officinalis* L. and *A. schoberioides* Kunth. *Genetic Resources and Crop Evolution* 54, 1063–1071.
- Kawale, M., Ankoliya, S., Saravanan, R., Dhanani, T., Manivel, P., 2014: Pharmacognostical and physicochemical analysis of *Asparagus adscendens* Buch. Ham. ex Roxb. (Shweta musali). *Journal of Pharmacognosy and Phytochemistry* 3, 131–139.
- Kubitzki, K., Rudall, P.J., 1998: Asparagaceae. In: Kubitzki, K. (ed.), *The families and genera of vascular plants*, 125–129. Springer, Berlin, Heidelberg, New York.
- Kubota, S., Konno, I., Kanno, A., 2012: Molecular phylogeny of the genus *Asparagus* (Asparagaceae) explains interspecific cross ability between the garden *Asparagus* (*A. officinalis*) and other *Asparagus* species. *Theoretical and Applied Genetics* 124, 345–354.
- Maire, R., 1958: *Flore de l'Afrique du nord*. Vol. 5, 215–232. Paul Lechevalier, Paris.
- Mantovani, D., Rosati, A., Perrone, D., 2019: Photosynthetic characterization and response to drought and temperature in wild *Asparagus* (*Asparagus acutifolius* L.). *Horticultural Science* 54, 1039–1043.
- Mousavizadeh, S.J., Hassandokht, M.R., Kashi, A., 2015: Multivariate analysis of edible *Asparagus* species in Iran by morphological characters. *Euphytica* 206, 445–457.
- Mousavizadeh, S.J., Gil, J., Castro, P., Hassandokht, M.R., Moreno, R., 2021: Genetic diversity and phylogenetic analysis in Asian and European *Asparagus* subgenus species. *Genetic Resources and Crop Evolution* 68, 3115–3124.
- Mousavizadeh, S.J., Gil, J., Moreno, R., Mashayekhi, K., 2022: *Asparagus* ploidy distribution related to climates adaptation in Iran. *Environment. Development and Sustainability*, 24, 5582–5593.
- Nakayama, H., Yamaguchi, T., Tsukaya, H., 2012: Cladodes, leaf-like organs in *Asparagus*, show the significance of co-option of pre-existing genetic regulatory circuit for morphological diversity of plants. *Plant Signaling & Behavior* 7, 961–964.
- Nakayama, H., Yamaguchi, T., Tsukaya, H., 2013: Modification and co-option of leaf developmental programs for the acquisition of flat structures in monocots: unifacial leaves in *Juncus* and cladodes in *Asparagus*. *Frontiers in Plant Sciences*, 4, 248.
- Pieroni, A., 2005: Food for two seasons: culinary uses of non-cultivated local vegetables and mushrooms in a south Italian village. *International Journal of Food Sciences and Nutrition* 56, 245–272.
- Prychid, C.J., Rudall, P.J., 1999: Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. *Annals of Botany* 84, 725–739.
- Quézel, P., Santa, S., 1962: *Nouvelle Flore de l'Algérie et des Régions Désertiques Méridionales*. Vol. 1, 208–209. Éditions du Centre National de la Recherche Scientifique, Paris.
- Raycheva, T., Stojanov, K., 2013: Comparative anatomical study of five species of genus *Asparagus* in Bulgaria. *Trakia Journal of Sciences* 2, 104–109.
- Schnitzler, A., Arnold, C., 2010: Contribution of vines to forest biodiversity in the Mediterranean basin. *Ecologia Mediterranea* 36, 5–24.
- Sica, M., Gamba, G., Montieri, S., Gaudio, L., Aceto, S., 2005: ISSR markers show differentiation among Italian populations of *Asparagus acutifolius* L. *BMC Genetics* 6, 17.
- Tamanian, K.G., 1982: Analysis of the taxonomic value of anatomic and morphologic characteristics of the cladodia of Caucasian species of the genus *Asparagus* L. USSR. 1. *Biological Journal of Armenia* 35, 885–892 (in Russian).
- Urbani, M., Becca, G., Ledda, M.G., 2007: Notes on systematics and chorology of *Asparagus* L. (Asparagaceae) in Sardinia (Italy). *Bocconea* 21, 267–271.