Bulgarian Journal of Agricultural Science, 24 (No 4) 2018, 631–637

Leaf morphological and anatomical traits variation of *Artemisia herba-alba* in a steppe zone of Algeria

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Abstract

Abderabbi, Kh., Adda, A., Benhassaini, H. & Merah, O. (2018). Leaf morphological and anatomical traits variation of *Artemisia herba-alba* in a steppe zone of Algeria. *Bulgarian Journal of Agricultural Science*, *24*(4), 631–637

The present study was interested on the leaf morphological and anatomical parameters characterization and variation of *Artemisia herba-alba* Asso population in a steppe zone of western Algeria. The results showed significant micro- and macro-morphological variability between individuals in the study population. The leaf anatomy brings the presence of a water reserve parenchyma in this species. This parenchyma would be a water accumulation site during dry periods and it is therefore an effective mechanism for drought adaptation. The anatomical parameters studied also show significant variations. The other anatomical parameters of the leaf also have a very pronounced variability. It is shown through this study that morpho-anatomical models are established. Thus, the increase of the water reserve parenchyma thickness is accompanied by an increase of the chlorophyll parenchyma and the stomatal density, and the opposite situation was observed.

Keyswords: Artemisia; adaptation; leaf; morphology; anatomy; water reserve parenchyma

Introduction

Artemesia herba-alba Asso is a perennial small shrub. This species is mainly found in northern Africa and southwest of Europe. In Algeria, it presents a wide geographical distribution covering about 4 million hectares, located mainly in the steppe zone. The plasticity of this species is the reason it occurs in deserts (Pouget, 1980). It therefore constitutes a candidate plant for preserving biodiversity in these regions.

Like all other species of *Artemisia*, great importance is given to wormwood. It is an important source of biological compounds with biocidal and allelopathic activities (Maghni et al., 2017).

In addition to its very high mineral richness, this species has a highly-required index for these pharmaceutical properties (Hellal et al., 2007). This aromatic species is widely used in North African's traditional medicine as expectorant, analgesic, antispasmodic, stomachic, vermifuge, diarrheic and sedative (Benmansour et al., 2016). It is also a way of natural struggle against erosion and desertification. It thus contributes to the soils fixation, vegetable richness and consequently the preservation of steppe ecosystems. In this extensive sheep farming region and with its high fodder value, it constitutes the main source of feed (Aidoud, 1983; Bourbouze and Donadieu, 1987).

However, in recent years the steppe ecosystems are an alarming imbalance due to a significant degradation of vegetation. That is related to the rainfall rarity resulting from climate change and anthropogenic factors (Maghni et al., 2017).

This evolution is inevitably accompanied by genetic erosion affecting different species of these fragile areas. *Artemisia herba-alba* covers four million hectares of the steppes. Therefore, it is the most representative and vulnerable among plant species. The few researchs devoted to the study of its adaptation in arid zones concern two main mechanisms. The seasonal dimorphism of its foliage allows it to reduce the transpiring surface and thus prevent water loss (Openheimer, 1961; Ourcival, 1992) and adaptation of its ability to develop a fairly deep and ramified root system allows efficient water absorption (Ferchichi et al., 2004). However, numerous studies have focused on the valorization of the products of this species in the different use fields.

The realization of protection and restocking programs of wormwood steppe course, require better understanding of variability offered by this species and its drought adaptation. Several authors have concurred that this species has a very high morphological and genetic variability (Maghni et al., 2008; Maghni et al., 2017). In this context, it is essential to define and study the mechanisms involved in adapting this species to dry climates.

Although leaf morphology has been sufficiently established and has served as the key to identification and inventory in botanical studies of the species, little information is available on the implication of these studies as well as those related to its structure, in the mechanisms of its adaptation to dry conditions. The persistence and longevity of the leaves in this species suggest that there are effective mechanisms involved in the regulation of water exchange with the atmosphere and the capacity of its retention.

The work done is based on these assumptions. It tries to elucidate the anatomical particularities of the leaf and their relationship with its morphological and micro-morphological characteristics. The work concerned sixty individuals from a population located in the El Menseb area, South of Tiaret (Algeria).

Materials and methods

Plant material sampling

The samples of *Artemisia herba-alba* were taken from El-Mansab (Altitude 1326 m) located in the highlands of the South East of the province of Tiaret (001° 48′ 40.8″E, 35° 01′ 9.2″N) in the North West of Algeria. They were collected during the most developed vegetative stage, which corresponds to September month. The study was conducted at the plant biotechnology laboratory of Ibn Khaldoun University of Tiaret (Algeria). All measurements were made on completely differentiated leaves located at the middle part of the stem.

Macro- and micro-morphological parameters studies

The quantitative parameters relate to the number of leaflets per leaf, the number of small leaflets per leaflets and the distance between the leaflets in millimetre (mm). The qualitative parameters concern the leaves colour and the arrangement of leaves on the leaf axis. For the colour, three level scales was used, dark green (1), intermediate green (2) and pale green (3). Two modes of disposition are retained, opposed and alternate.

The micro-morphological parameters measured on the impression of the blade epidermis concerned the density of the stomata and the length of the ostiole. Leaves fixed with FAA (ethanol, formaldehyde, acetic acid) for 12 hours are thoroughly washed with distilled water. After drying, the blade surface is cleaned by applying an adhesive tape. The impression of the epidermis is noted on a collodion film applied on the surface of the leaf blade, removed after drying and glued on slides and observed by an Optica microscope equipped with a micrometer.

Anatomical parameters studies

The anatomical parameters were studied on histological sections of the leaf lamina. A leaflet of the median part of the leaf axis is taken and immediately fixed in FAA (formalin, acetic-acid, alcohol) during 12 h. The samples are then washed and gradually dehydrated by ethanol (50°, 70° and 100°). The samples are then impregnated and included in paraffin blocks and cut with a microtome (LEICA RN 2145) at a thickness of 7 μm. After dewaxing sections were then stained with methyl green and carmine N40. The measures concerned the thickness of the chlorophyll parenchyma, the water reserve parenchyma thickness, the number of cell lines of water reserve parenchyma and their diameter and the thickness of the outer lining of the epidermal cells.

Results

Leaf quantitative-morphological parameters

All selected parameters are variable across the studied individuals (Table 1). Nevertheless, the polymorphism expressed levels diverge between the selected parameters. The results indicate a significant change rate of the leaves number per foliar branch. This number ranges from a minimum of one leaf per branch to a maximum of 7 leaves per foliar branch. The sample of the 60 individuals studied indicates that most of them have a three leaf number, presenting a ratio of 32%.

The leaflets number per leaf fluctuates between extreme values of 2 and 8. However, the numbers of 3 and 4 are greatly repeated in this individual’s sample, where a rate of 35% of individuals expressed 4 leaflets per leaf. Finally, the

Table 1
Effects of individual's nature on the expression of the leaf morphological characters and their variation degree

Settings	MS (DF = 59)	F-Test	Coefficient of variation (%)
Number of leaves	2.105	1.973**	32
Number of follicles per leaflets	5.877	6.411**	35
Distance between leaflets	3.941	4.416***	31

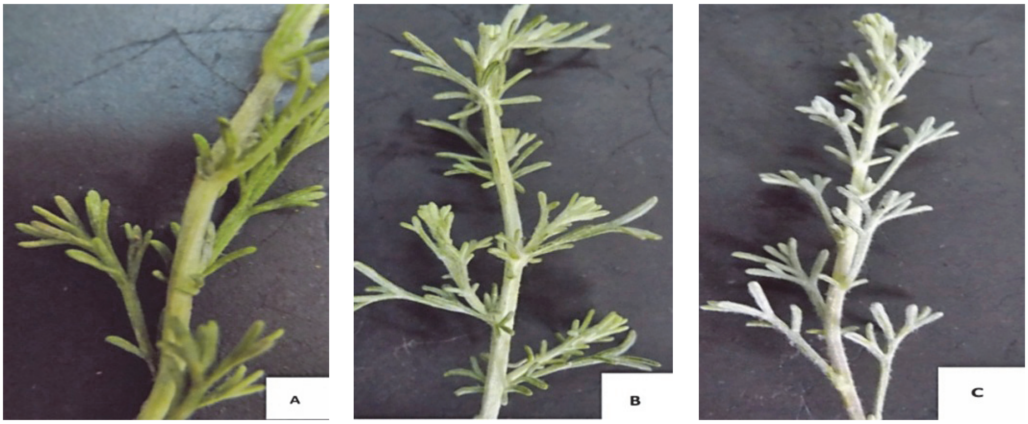

Fig. 1. The vegetation colours encountered in the studied individuals – dark green (A), intermediate green (B) and light green (C)

Table 2
Leaf qualitative parameters distribution among the individuals studied

Settings	Variations	Individual's distribution (%)
leaves color	Dark green	35
	Intermediate Green	25
	Light green	40
Leaflets arrangement mode on leaf axis	Opposite	47
	Alternate	53
Leaves arrangement mode on the foliar branch	Opposite	33
	Alternate	67

distance separating the leaflets on the same sheet, expresses values varying between the extremes of 1 and 8 mm.

Leaf qualitative-morphological parameters

A high polymorphism concerns the color of the vegetation and the mode of insertion of leaflets and leaves, among the individuals studied. Three colour levels were observed at the level of the studied population (Fig. 1). The frequencies of appearance among individuals are of the order of 40% for light green, 35% for intermediate green and 25% of the leaves are of a dark green color.

Two modes of arrangement of the leaves are met in this population, alternate and opposite. The results indicate that the alternate mode is more represented with a rate of 67% of individuals concerned. The opposite disposition mode characterizes the rest of the individuals (33%).

For the arrangement of the leaflets on the leaf axis, the alternate and opposite modes respectively present rations of 53 and 47% among the individuals studied (Table 2).

Micro-morphological parameters

The characteristics retained in this parameters category relate to the stomata density and the ostiole length (Fig. 2). The stomata density show great variation across the studied individuals (Table 3). So, the average values vary between 55 to 152 stomata/mm². The ostiole length values are also variable (Table 3), where they range from 24.5 to 35.5 µm.

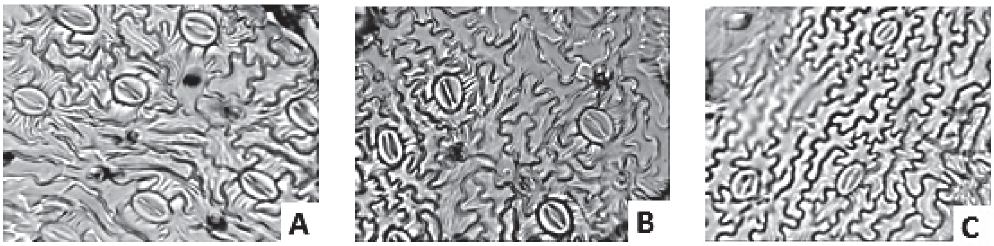

Fig. 2. Photos showing the imprints of the epidermis with the presence of the stomata – high presence (A), mean presence (B), low presence (C)

Table 3
The mean square, the F-test and the degree of influence of individuals studied on the variations of the anatomical and micro-morphological parameters

Parameters	MS (DF = 59)	F-Test	Coefficient of variation (%)
WRPT	2926	4.142***	48
NWRPC	2.910	7.077***	32
WRPCD	58.10	1.508*	30
CPT	219.1	6.747**	26
RP	2.376	3.329***	50
SD	1914	1330**	24
OL	19.58	14.96***	9

WRPT: water reserve parenchyma thickness, NWRPC: number of water reserve parenchyma cells strata, WRPCD: water reserve parenchyma cells diameter, CPT: chlorophyllian parenchyma thickness, RP: water reserve parenchyma/chlorophyll parenchyma ration, SD: stomata density, OL: ostiole length
***significant at 0.1% level, **significant at 1% level, *significant at 5% level

Anatomical parameters

The leaf structure presents the organization of a chlorophyllian parenchyma and an water reserve parenchyma (Fig. 3). The chlorophyllian parenchyma is located on the periphery of leaf structure and the water reserve parenchyma is located deeper nearby the vascular bundles.

The average results show that the thickness of the water reserve parenchyma varies considerably through the studied individuals. Indeed, obtained values fluctuate between 28 and 169 μm. This thickness is conditioned by the number of cell layers and their diameters. The cells layers number is delimited by the extreme values of 2 and 6 and their diameters vary between 15 and 33 μm. The importance of the chlorophyllian parenchyma, evaluated by its thickness in the leaf structure, also presents a high variability. The thickness values of this parenchyma vary considerably which extend in an interval delimited by extreme values of 19 and 55 μm. Another structure parameter relates to the relationship between the two parenchymas, water reserve and chlorophyllian. It would inform about the relationship between photosynthetic activity and the water-carrying capacity at the leaf level. The values in this report vary between 1 and 5.

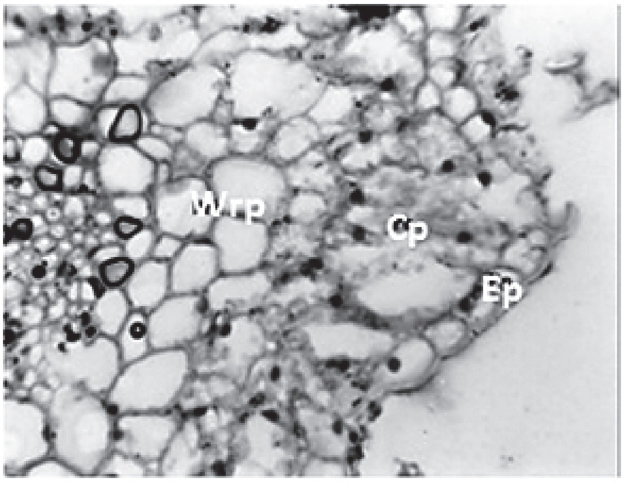

Fig. 3. Cross-section at leaflet level showing the localization of the water reserve parenchyma and chlorophyllian parenchyma (Ep: epidermis; Cp: chlorophyllian parenchyma, Wrp: water reserve parenchyma)

Discussions

Wormwood develops different strategies of drought adaptation. The main mechanisms concern the development of the root system (Floret and Pontannier, 1982; Le Floch, 1989), the reduction of the aerial vegetative mass (Aidoud, 1988; Maghni et al., 2017), the regulation of water loss by deposit trichomes (Agrawal, 2004; Dalin et al., 2008) and reduction of stomatal density (Schoch, 1972; Ouyahya, 1996). The work presented relates to another adaptation strategy to dry conditions, it is about the existence of a tissue organization used for the accumulation of water and assimilated to a water reserve parenchyma. The anatomical study carried out reveals the structural disposition of a cells group deprived of chloroplasts and accumulates water.

The anatomical organization of the species leaf imposes specific morphological and micro-morphological characteristics. These particularities develop different strategies of environment adaptation among the population individuals studied.

A rather large polymorphism concerning the morphological and micro-morphological criteria has been observed in the sample of the studied variability. Among the variability which characterizes the traits studied is that which concerns the mode of arrangement of the leaves on the foliar axes, where two dispositions are encountered opposite and alternate. This characteristic is of strong heritability whose development is weakly influenced by the environment and that any variation in its expression is explained by a genetic variability characterizing this species. The vegetation colour also shows a pronounced variation.

The colour variations encountered are explained by the trichrome density, particularly waxes, on the leaves surface and young stems. The light colour with a whitish appearance indicates an abundance of epicuticular waxes. The high waxes density is used as a mechanism for regulating the water loss through the different types of transpiration, sought in climates characterized by drought and high temperatures (Kappen et al., 1972;

Table 4
Relationships between leaf morphological and anatomical parameters

	NWRPC	WRPCD	WRPT	CPT	RP	SD	OL	LN	NF
WRPCD	0,201**								
WRPT	0,763***	0,701***							
CPT	0,155**	0,085	0,192**						
RP	0,666***	0,582***	0,840***	-0,312**					
SD	0,235**	0,110	0,192**	0,710***	-0,186**				
OL	-0,064	-0,061	-0,045	-0,023	-0,041	0,019			
LN	0,006	0,034	0,072	-0,008	0,047	0,006	0,081		
NF	0,080	-0,044	0,080	-0,036	0,102	-0,075	0,283**	0,161**	
DF	-0,243**	-0,114	-0,221**	-0,187**	-0,106	-0,156**	-0,055	0,139	-0,029

WRPT: water reserve parenchyma thickness, NWRPC: number of water reserve parenchyma cells layers, WRPCD: water reserve parenchyma cells diameter, CPT: chlorophyllian parenchyma thickness, RP: water reserve parenchyma/chlorophyll parenchyma ration, SD: stomata density, OL: ostiole length, LN: leaves number, NF: follicles per leaflets Number, DF: distance between leaflets
***significant at 0.1% level, **significant at 1% level

Camefort, 1996). Among the micro-morphological parameters studied concerns the stomata density on the leaf surface which presents a very important variability. Some studies (Schoch, 1972; Camefort, 1996; Raven et al., 2007) demonstrate that the reduction of the density is an effective criterion for dry environments adaptation of different species ecotypes.

The anatomical characterization of the ecotypes concerned by this study shows the existence of a fairly important variability in the expression of the various parameters. A very important observation emerges from this study which concerns the organization of a water reserve parenchyma within the leaf structure. The existence of such a tissue or-

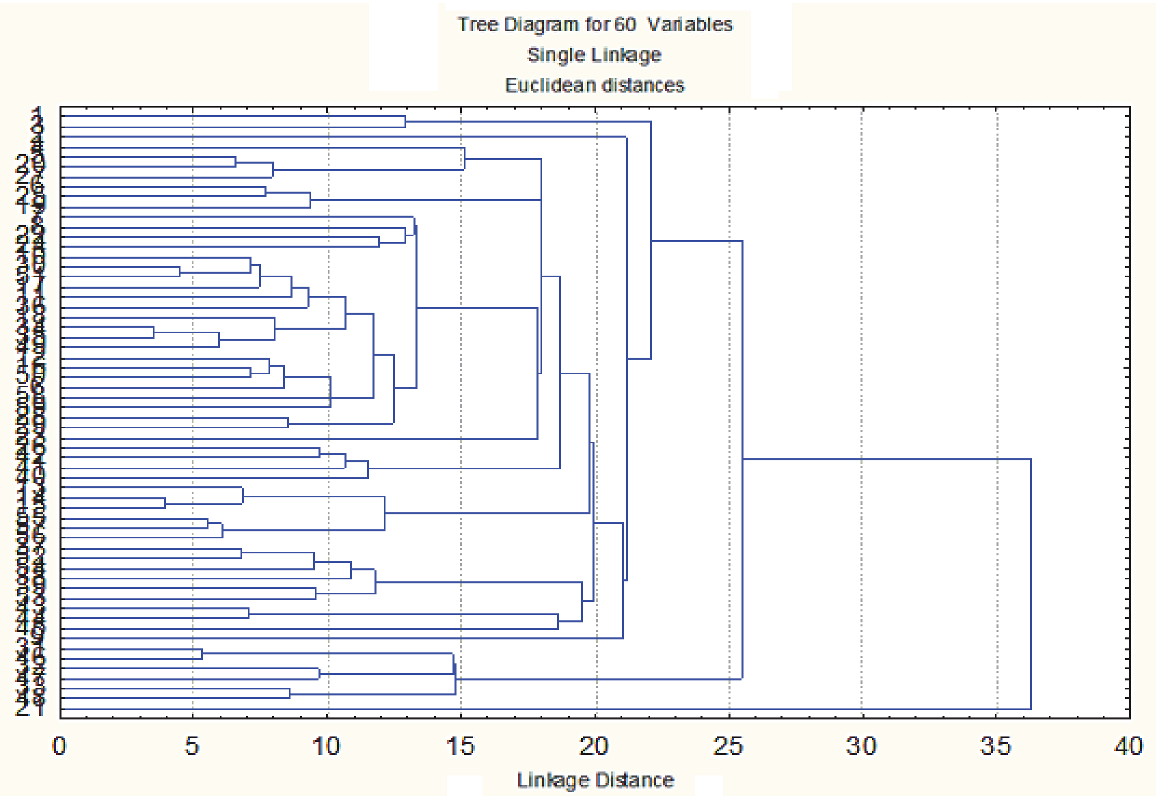

Fig. 4. Hierarchical classification of individuals according to the macro-, micro-morphological and anatomical parameters of the leaf

ganization at the level of wormwood reduced size leaves would be a very important contribution in the diversification of drought tolerance mechanisms. Various works (Ferchichi, 1997) have shown that the existence of water reserve parenchyma effectively contributes to the plants drought adaptation (Ferchichi et al., 2004; Openheimer, 1961). This tissue organization presents variations across the leaves of the studied ecotypes. Thus, the variations concerning it and which are evaluated by its thickness, are conditioned by the number of cellular layers (0.770***) and their diameters (0.772***). The results obtained also indicate that the importance of the water reserve parenchyma depend on the importance of the chlorophyllian parenchyma where the thicknesses of the two parenchyma are positively correlated ($r = 0.191^{**}$). According to the works of Fahn and Brido (1963), Smail-Saadoun (2005) and Muhaidat et al. (2007), the photosynthetic activity is correlated positively with the importance of the chlorophyllian parenchyma. This indicates that in this situation the maintenance of a high photosynthetic activity by an abundance of chlorophyllian parenchyma in drought conditions would be supported by an optimum water state fed by an abundant presence of water reserve parenchyma.

The results obtained show the existence of a positive and significant correlation between the importance of the water reserve parenchyma and the stomata density ($r = 0.2^{**}$) (Table 4). This relationship demonstrates that if the stomata density increases and which would favour the increase of the loss through stomatal transpiration (Gates, 1995), it would be compensated by an increase in water storage capacity by the importance of the water reserve parenchyma.

Concerning the abundance of the aerial vegetative mass, evaluated by the distance separating two consecutive leaves on the same leaf axis, it is shown to be negatively correlated with a significant level with the thickness of the water reserve parenchyma ($r = -221^{**}$). The increase of aerial vegetative mass is ensured by optimum water nutrition (Schonfeld et al., 1988; Muller and Whitsitt, 1996), which justifies the obtained relationship.

The combination of morphological and anatomical parameters results in distinguishing 5 individuals group in the population (Fig. 4). This distinction would present the different models of plants according to their adaptation strategies to the respective environmental conditions.

Conclusion

From this work, we note that the foliar anatomical parameters in the wormwood are characterized by a very important variability. A feature of primordial importance in the process of adaptation of the species to dry conditions, relates to the existence of a water reserve parenchyma. This parenchyma is deeply in the foliar structure and it seems to condition an ecotype profile adapted to the conditions of scarcity of hydric resources. It is also confirmed that this structural distinction imposes a set of morphological and micro-morphological parameters at the level of the individuals studied and issued from the population of El Menseb.

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