

Dynamics of the anatomical variability of *Artemisia herba-alba* in Algeria

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Article info

Received 07.07.2018

Received in revised form
19.08.2018

Accepted 23.08.2018

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Maghni, B., Hellal, B., & Maatoug, M. (2018). Dynamics of the anatomical variability of *Artemisia herba-alba* in Algeria. *Biosystems Diversity*, 26(3), 239–244. doi: 10.15421/011836

In this work, the anatomical variability in *Artemisia herba-alba* Asso was studied based on 90 individuals taken from three different bioclimatic zones of Algeria (inferior semi arid, arid and Saharan) as well as the relationships of the dynamics of this variability with the distribution and adaptation of the species. The interpretation of the results of this study through the analysis of variance and the ascending hierarchical classification allowed us to detect a very high intra- and inter-specific anatomical variability, as well as the presence of a parenchyma of water reserves observed in individuals of each studied region. The existence of relationships between environmental conditions and the dynamics of anatomical variability have been established. The broad morphological structural variability thus determined and the genetic variability observed concur well with the results of our two other previous studies. This anatomical polymorphism could help in the selection of the most effective ecotypes to restore this species in degraded steppe ecosystems of Algeria and North Africa. Thus, this choice will allow us to keep their seeds in a bank of grains in order to preserve the species for possible future use.

Keywords: anatomical polymorphism; bioclimatic stages; ecotypes; adaptation; distribution; ecotypes

Introduction

Artemisia herba-alba is one of the species of major interest in the settlement of ecologically fragile areas. This characteristic is justified by its capacity to adapt in these environments governed by different types of stress, especially abiotic. This species is a natural means of fighting against erosion and desertification (Benjilali & Richard, 1980). Its morphological and physiological characteristics make it a species well adapted to arid climates. The seasonal dimorphism of its foliage allows it to reduce the transpiring surface and thus prevent water loss (Ourcival, 1992). This Asteraceae species grows in desert wadi beds in North Africa and South-West Europe (Pouget, 1980). As with other species of *Artemisia*, wormwood is an important source of biological compounds with biocidal and allelopathic activities (Chauhan et al., 2010). This aromatic species is widely used in North Africa's traditional medicine as an expectorant, analgesic, antispasmodic, stomachic, vermifuge, diarrhetic and sedative (Benmansour et al., 2015).

Various specialists including (Le Houérou, 1969; Djebaili, 1978) agree in saying that pastoral surfaces and especially their ecological potential, including production, have declined dramatically in recent decades. Forage production loss is estimated at 75% (Djebaili, 1978), which raises the serious problem of meeting the requirements of livestock in the context of the massive reduction in natural vegetation cover. Several authors have concurred that this species has a very high morphological and genetic variability, exemplified by the existence of ecotypes in this taxon, such as sagebrush in the Middle East (Zohary, 1973). Similarly, two varieties of this taxon have been identified, based on chromosome counts (Ferchichi, 1997). The results obtained from the morphological study (foliar and floral morphology) of 120 individuals of *A. herba-alba* taken from from three stations in western Algeria indicate a very high intra- and inter-population polymorphism (Maghni et al., 2017). In addition to its very high mineral richness, *A. herba-alba* has a highly sought after index for pharmaceutical properties. Some other studies pinpointed a high chemical polymorphism of essential oils from *A. herba-alba* defining different chemotypes for this plant (Salido et al., 2004; Lamiri et al., 1997).

Its essential oils possess powers, antiseptic, antibacterial and antifungal, which have led to its application in many fields, therapeutic,

cosmetic and the agro-food industry. Its chemical composition, of great economic interest, has been the subject of several phytochemical studies. Despite the great economic value of this plant, a diachronic study of land use trends in the steppes of southwestern Algeria between 1978 and 2004 showed a regression of white wormwood steppes from 130,000 ha in 1978 to 13,000 ha in 2004. Thus, white wormwood steppes are replaced by indicator species of vegetation degradation (Tarik & Arslan, 2005).

It is essential to implement a strategy of protection and restoration of these steppe habitats. The realization of all programmes of action must be based on a sound knowledge of the environment and the study of its floristic component. Any operation included in this framework requires better information on the variability of the different regions of *A. herba-alba* and the dynamics of this variability according to the dominant environmental conditions. Thus, the work presented deals with this problem. It consists of the anatomical study of variability in *A. herba-alba* in different bioclimatic stages and its relation to the distribution and adaptation of the species.

Material and Methods

Location of study areas. Our choice of study sites was based on annual rainfall, in a north-south progression, where we identified three regions, each of which represents a different bioclimatic stage. The choice of different populations was based on the density of the species and the variability of the ecological conditions. The first study site is located in the region of El-Faidja located in the southeast of the province of Tiaret, which is located in the west of the country more than 300 km southwest of the capital Algiers (Fig. 1, Table 1). The second site is situated 193 km from the first study site in the south of the area of Oued Morra in the north of the province of Laghouat (Fig. 1, Table 1). The third site is situated in the area of Hassi Er' Mel, 550 km of south of the capital Algiers. The distance between the second and the third sites is 205 km (Fig. 1, Table 1). The geographic, altitudinal and edaphic data of the three populations at each study site are presented in Table 1.

Climatic characteristics. According to the Ombrothermal Diagram, the dry season in the first region (site 1) is five months. It is seven

months for the second region (site 2) and spreads over the whole of the year in the third region (site 3). The Emberger rainfall quotient of site 1 is 33.38, which allows us to classify this region in the lower semi-arid bioclimatic stage in fresh winter with an annual rainfall of 330 mm. The Oued Morra region (site 2) was classified in the arid bioclimatic

stage in cold winter ($-2.17^{\circ} < m < 2.83^{\circ}$) with a rainfall quotient of 11.36 and an annual rainfall of 117.6 mm. On the other hand, the rainfall quotient of the Hassi Er'Mel region is equal to 5.15, which makes it possible to classify this area in the Saharan bioclimatic stage in fresh winter ($3.32^{\circ} < m < 2.25^{\circ}$) with an annual rainfall of 56.37 mm.

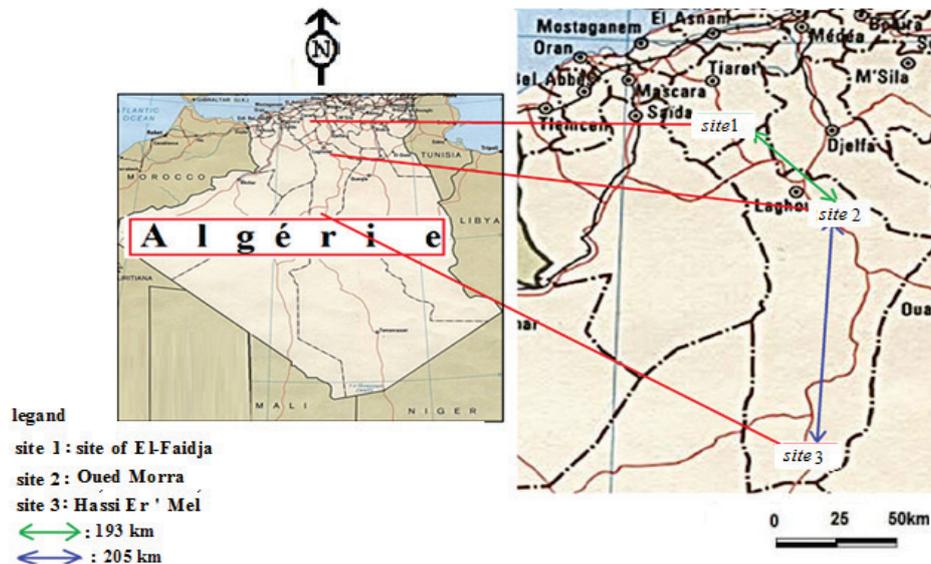


Fig. 1. Location of study sites: site 1 – El-Faidja located 300 km from the capital of Algeria; site 2 – Oued Morra located 193 km from site 1; site 3 – Hassi Er' Mel located 205 km from site 2

Table 1
Geographic and altitudinal data of the three populations at each site

Site 1	Population 1	Population 2	Population 3
Latitude	35° 01' 25.2'' N	35° 01' 19.2'' N	35° 01' 26.7'' N
Longitude	01° 48' 55.5'' E	01° 48' 40.8'' E	01° 49' 02.2'' E
Altitude	1312 m	1326 m	1359 m
Topography	Depression	Gentle slope	Mountain adret
Soil type	Deep, Limestone	Superficial, Limestone	Sandy clay, superficial
Site 2	Population 1	Population 2	Population 3
Latitude	35° 22' 02.5'' N	34° 07' 30.1'' N	35° 05' 87.2'' N
Longitude	01° 20' 20.7'' E	02° 21' 56.9'' E	02° 20' 125'' E
Altitude	1147 m	1170 m	1307 m
Topography	Moderate slope	Depression, wadis	Peak of mountain
Soil type	Rocky-stony	Clay-Deep limestone	Rocky and stony
Site 3	Population 1	Population 2	Population 3
Latitude	33° 07' 75.5'' N	33° 07' 44.3'' N	33° 07' 50.9'' N
Longitude	03° 37' 10.4'' E	03° 37' 06.6'' E	03° 37' 08.6'' E
Altitude	739 m	720 m	725 m
Topography	Plain	Depression	Moderate slope
Soil type	Sandy	Sandy	Sandy and stony

Notes: N – north, E – east, m – meters; site 1 – El-Faidja site in Tiarret province; site 2 – Oued Morra site in the north of Laghouat province; site 3 – Hassi Er' Mel site in the extreme south of Laghouat province.

Sampling of plant material. After surveying our three study sites, we selected three populations inside each site, taking into account the space between these populations and ecological barriers, as well as the differences of soil and topographic characteristics (slope, exposure). Thirty individuals were taken from each site. These samples of *A. herba-alba* were taken during the most developed vegetative stage.

Anatomical sections. After fixing the samples in a solution composed of ethanol and acetic acid, they were washed for a duration equal to that of the fixation (24 h). Then, to dehydrate the samples, they were placed into ethanol baths (70°, 90° and 100°). Afterwards, they were impregnated in a bath of toluene plus paraffin, followed by a first paraffin bath for 12 hours at 60 °C and a second bath at 60 °C for only 5 minutes. Finally, the samples were formed into blocks of rectangular shape and left to solidify. The sections were made at the extremities of the small leaflets with a microtome (Leica RM 2145) with a thickness of 7 µm. After removal of paraffin and double staining, these sections were examined through a photo optical microscope (Optika Gr 40).

Characters selected. The anatomical parameters chosen for this study were those which would be least affected by variation in environmental conditions. The anatomical characters thus chosen were the diameter of the reservoir cells, number of lines of reservoir cells, number of conductive bundles, diameter of conductive vessels, external cell-wall thickness of epidermal cells, epidermal cell dimensions (length and width) and cell dimensions of the palisade parenchyma (length and width).

The existence of the anatomical variability observed through the observations of the anatomical sections and the differences in the measurements of the characters noted in each site and also between the different sites has been confirmed through three statistical tests, the first is Multivariate Tests of Significance, the second is a Multivariate Analysis of Variance (MANOVA), ANOVA and the Ascending Hierarchical Classification (CHA).

Results

From the different values of the obtained anatomical measurements we performed significant variance analysis for each trait in all individuals at the three study sites (Table 2).

Table 2
Analysis of variance of all anatomical features

	SC	dl	MC	SC	dl	MC	F	p
External cell wall thickness of epidermal cells	27.7	2	13.8	204.6	87	2.35	5.88	0.0040
Length of epidermal cell	50.6	2	25.3	266.5	87	3.06	8.26	0.0005
Width of epidermal cell	82.1	2	41.1	230.1	87	2.64	15.52	<0.0001
Length of the chlorophyll cells	229.1	2	114.6	6766.1	87	77.77	1.47	0.2349
Width of the chlorophyll cells	119.7	2	59.8	437.8	87	5.03	11.89	<0.0001
Number of lines of reservoir cells	28.1	2	14.0	190.3	87	2.18	6.41	0.0025
Diameter of conductive vessels	520.1	2	260.1	623.9	87	7.17	36.26	<0.0001
Number of conductive bundle	2.5	2	1.2	33.1	87	0.38	3.27	0.0426

The test showed that there is a very high variability of anatomical traits studied at the scale of each site and between the three sites studied. Indeed, the number of variants for each character varies from one site to

another and the values of the measurements performed are also very different among the three bioclimatic stages studied.

Multivariate Tests of Significance for all the individuals studied (90) from the three sites, thus present a highly significant effect which reflects the high degree of anatomical polymorphism found through the comparison of mean differences of values for the characters studied.

The Manova test (Fig. 2) confirms the difference in appearance of traits studied in individuals at the scale of each population and each site. We find that the carcasses are distributed in a very different way at each site, which presents a region different to the others by their characteristics, climatic, edaphic and topographical.

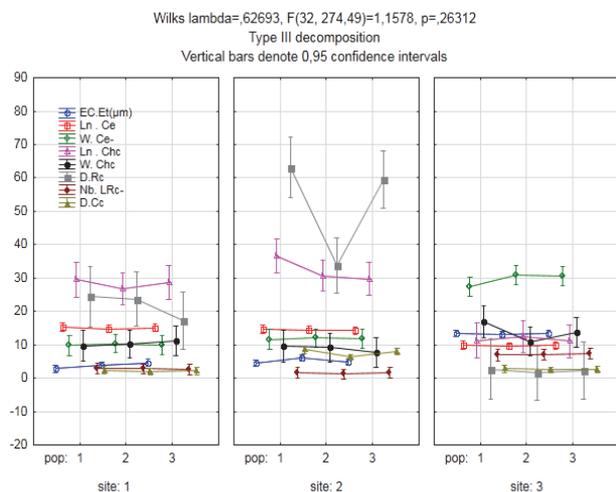


Fig. 2. Manova test of the three study sites

Diameter of conductive vessels. The inter-population polymorphism is very marked at the level of two populations (1 and 3) of the second site, where we found that individuals with a single conducting bundle show the largest diameter of vessels with values fluctuating between 12.0 and 14.5 μm (population 1) and between 12.0 and 13.7 μm (population 3). However, individuals with three conducting bundles expressed small diameter vessels of 5 and 8 μm . In contrast to the two preceding populations, the second population is the least polymorphic for this character and has only small diameters (5 and 8 μm) in all of its individuals, all possessing three conduction bundles.

The third study site (Hassi E'rmel) revealed a high level of intra-population variability (Table 2). Indeed, within each population there are very significant variations in the diameter of the vessels (Fig. 3a). Individuals in population 1 showed small values of diameters of 5 and 9 μm and large values up to 14.2 μm . Nevertheless, very low values were recorded for population 2. These are the vessel diameters of individuals with three bundles forming a triangle (Fig. 6c). The same observation was made for population 3, which presents the most polymorphic with three intervals of diameter of the vessels. The first contains very small values of vessel diameters (2 and 3 μm), the second has small values of 5 and 7 μm , and finally the third range contains the most extreme values between 11.5 and 14.6 μm .

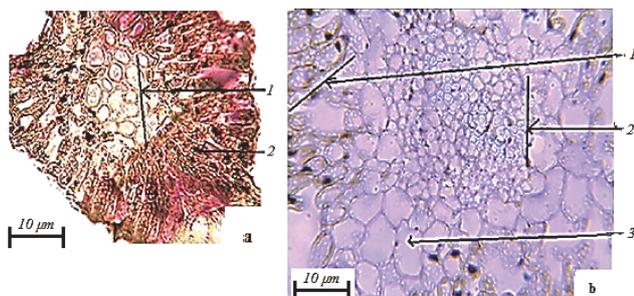


Fig. 3. a: (Hassi Er'mel site); 1 – conductive bundle, 2 – palisade parenchyma cell; b: transversal section at the leaflet of *A. herba-alba* (El Faidja-Tiaret site): 1 – palisade parenchyma, 2 – conductive bundle, 3 – reserve cells

Analysis of variance for all individuals from three study sites indicated a highly significant effect ($P < 0.001$). The results of the expression of this characteristic at site 1 (Fig. 2, 3b) show that individuals have smaller diameters. The values of diameters measured are 1 and 3 μm . This characteristic is very important because it allows very good circulation of the sap.

External cell wall thickness of epidermal cells. Analysis of variance of external cell wall thickness of epidermal cells of all the individuals in the three sites studied showed a significant effect ($P < 0.05$) (Table 2). At site 1, the thicknesses had values between 1.5 and 3.3 μm (population 1). With population 2, the walls had values of 2 and 3 μm . Population 3 is the most polymorphic of the populations. Therefore, the thicknesses expressed by the individuals of this population reached values of 4.5 μm .

It was at the second site (Oued Morra) that we noted extreme values of external cell wall thickness of epidermal cells. (Fig. 2). These values are found only in individuals which carry a single line of reservoir cells and a single conducting bundle with large diameter vessels (20% of individuals in each of two populations 1 and 3).

At the Hassi E'rmel site, the external walls of the epidermal cells are less thick compared to the other sites (Fig. 2). The values noted vacillated between 1.5 and 3.8 μm within the three populations.

Epidermal cell dimensions. The measurements concerned the length and width of the coating cells and the determination of their forms. These parameters also show expression variations between the three sites studied, within each population (Table 2), and not at the inter-population inside the same area. Variations are greater in length than the width of the epidermal cells within each population. Indeed, the individuals of each population of the first site showed epidermal cells with more extreme length varying between 15 and 34 μm . In the second position, site 2 with 11 to 18 μm of length and finally, the third site which had individuals with short epidermal cells (11 to 15 μm). Variance analysis in the length of the epidermal cells at the three studied sites showed a very highly significant effect ($P < 0.001$) so revealing a very important inter-site variability in the epidermal cells.

As to the form of the epidermal cells, we found two different forms; bomb cells of two internal and external ribs at sites 1 and 3 (Fig. 5a) and convex cells on the external and concave side of the internal side in individuals from site 2 (Fig. 4a).

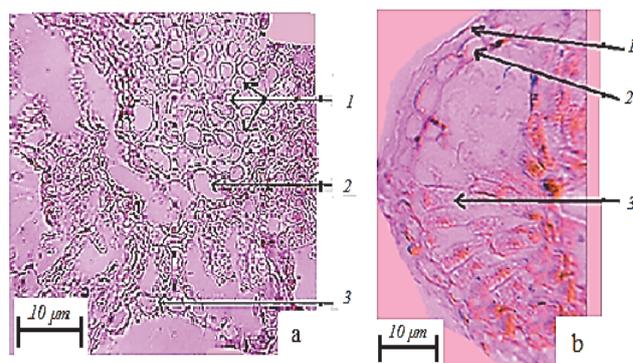


Fig. 4. a: transversal section at the leaflet of *A. herba-alba* (Oued Morra-Aflou site): 1 – external cell wall of epidermal cells, 2 – epidermal cell, 3 – palisade parenchyma cells; b (Oued Morra-Aflou site): 1 – conductive vessels, 2 – reserve cell, 3 – epidermal cell

Cell dimensions of the palisade parenchyma. Palisade parenchyma consists of two lines of elongated cells perpendicular to the epidermis at the three studied sites. These cells manifest very different dimensions of length ($P < 0.001$) but not significant effect for the width. Indeed, at site 1 (Fig. 5b), the length of the chlorophyll cells in the individuals of population 1 was from 13 to 34 μm and their width was from 8 to 13 μm . For populations 2 and 3, the studied cells had identical dimensions which fluctuated from 14 to 41 μm (for the length) and between 6 to 14 μm (width). The palisade cells of site 2 were very elongated with lengths superior to 48 μm observed in the three populations (Fig. 2, 5c). At the Hassi Er'mel site (Fig. 5a), the intra-population polymorphism of this characteristic is very important, with cell lengths varying between 18 and 45 μm inside each population.

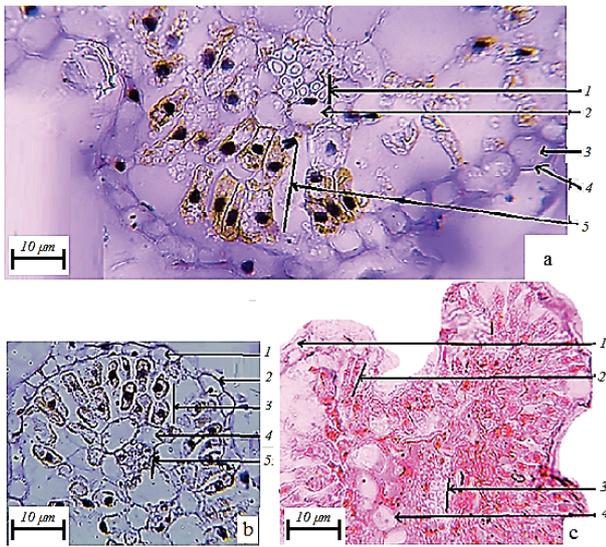


Fig. 5. *a* – part of a transversal section of the leaflet of *A. herba-alba* Asso (Hassi Er'mel site): 1 – conductive bundle, 2 – reserve cell, 3 – epidermal cell, 4 – external wall of the epidermic cell, 5 – palisade parenchyma; *b* – El Faidja – Tiaret site: 1 – epidermal cell, 2 – external wall of the epidermic cells, 3 – palisade parenchyma, 4 – reserve cell, 5 – conductive bundle; *c* – Oued Morra – Aflou site: 1 – external wall of epidermis cells, 2 – palisade parenchyma, 3 – conductive bundle, 4 – reserve cell

Number of conductive bundles. Three variants of the number of conducting bundles are also found among the individuals of the three studied sites, a single bundle (Fig. 6a), three bundles in the same straight line (Fig. 6b) and three bundles forming a triangle (Fig. 6c). All individuals at site 1 showed three bundles arranged in the same line. Similarly, the second site showed a dominance of this character at the level of its three populations with respectively 80%, 100% and 80% of the individuals carrying this character.

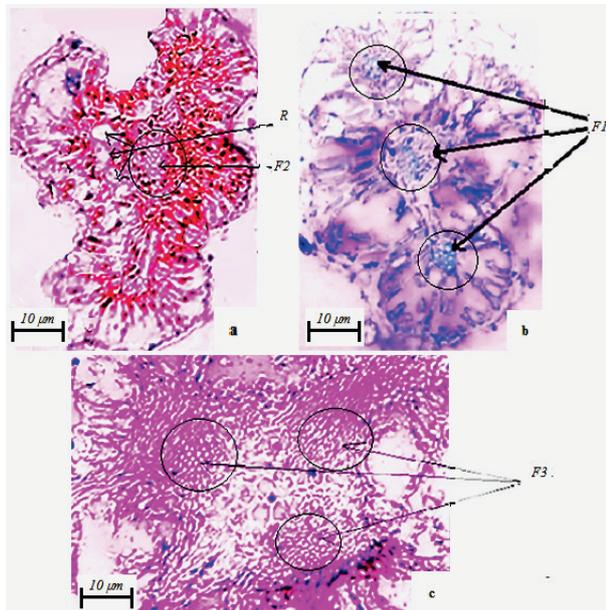


Fig. 6. Central part of a transversal section at the level of the foliole of *A. herba-alba*: *a*. F2 – single conducting bundle, R – reserve parenchyma cells (Oued Morra – Aflou site); *b*. F1 – three conductive bundles in the same straight line (El Faidja – Tiaret site); *c*. F3 – three conductive bundles forming a triangle (Hassi Er'Mel site)

Site 3 is the most polymorphic for the character studied by presenting the three variants observed with different proportions of expression at intra and inter-population level. Indeed, the characteristic three bundles in the same straight line is most manifested in the three popula-

tions with respectively 80%, 60% and 70% against the character of three bundles forming a triangle that is represented by 30% of the individuals of population 2 and by 20% of individuals in population 3. Finally, only 10% of individuals in each population presented a single conducting bundle).

Diameter of reserve cells. The characterization of the water reserve parenchyma has focused on the number of cell lines and the size of their volumes, estimated through the diameter of the cells. At site 1, the cell diameter varied between 15 and 30 µm (population 1), between 24 and 34 µm (population 2) and between 9 and 30 µm (population 3). Within the second site, the diameters were delimited by extreme values of 90 and 101 µm in the individuals of the two populations 1 and 3 (Fig. 2) and by average values of 30 and 55 µm (Fig. 5c). Finally, at the third site, the diameters of these cells differed only substantially from one population to another and had values between 18 and 26 µm.

Number of lines of reservoir cells. The overall results show that the values of the number of lines of cells fluctuated between 1 and 5 at site 1 but we also found that 20% of population 3 did not have a reserve parenchyma. Analysis of variance showed highly significant effect ($P < 0.001$). At the third site, the number of lines of cells varied between 2 and 4. Nevertheless, the majority of individuals at the second site had only one line of reserve cells. Indeed, 100% of the population 2, 75% of the population 1 and 70% of the population 3 had the previous characteristic and the rest of the individuals presented three lines of reserve cells.

Hierarchical classification of the individuals of the three sites according to the anatomical parameters of the leaf. All individuals (90) are distributed through 25 distinct groups (Fig. 7).

Discussion

Diameter of conductive vessels. We found that the character of three conducting bundles forming a triangle is strongly related to the presence of very small diameter vessels. This characteristic is peculiar to the individuals of population 3 of site 3, because this latter population is the only one which includes the individuals with three bundles forming a triangle. Conversely, the largest diameters are always present in individuals with a single conducting bundle (site 2 and 3).

External cellwall thickness of epidermal cells. The expression of external cellwall thickness of epidermal cells is highly variable among the individuals of each studied population within each site, indicating a very high intra-population polymorphism. This characteristic plays an essential role in limiting water losses through transpiration. Because site 3 is characterized by a dry climate, this characteristic of the walls being less thick presents an anomaly allowing more water loss by transpiration. This can be only explained by reasons closely linked to water supply, because in the arid region morning mists and dew provide the water to the plants, which have to catch it by exposing more stomata to the external environment.

Diameter and number of lines of reservoir cells. The spatial importance of this parenchyma characteristic would condition the plant's ability to preserve its hydration. The study of the results obtained shows the existence of a large variation of expression of this characteristic in the individuals of three studied areas. The comparison of the degrees of variation of expression of this characteristic shows that among the three sites studied, site 1 is the most polymorphic site. The number of cellular files of this parenchyma is paramount in the adaptation process of the plant to drought conditions. It makes it possible the plant's ability to preserve its hydration.

Hierarchical classification of the individuals according to the anatomical parameters of the leaf. This distinction in different groups for each population of each site indicates very pronounced intra-population variability. Distribution study of the individuals of the three sites based on these characteristics makes it possible to distinguish a rather marked polymorphism. It is observed that the groups of individuals from the three sites are characterized by a significant heterogeneity, which minimizes the impact of the ecological factors on the expression of the common morphological characteristics chosen in this study when it is possible to pronounce a high genetic heritability and that these anatomical characteristics will be at the origin of a structural polymorphism and are certainly involved in the adaptation function of individuals.

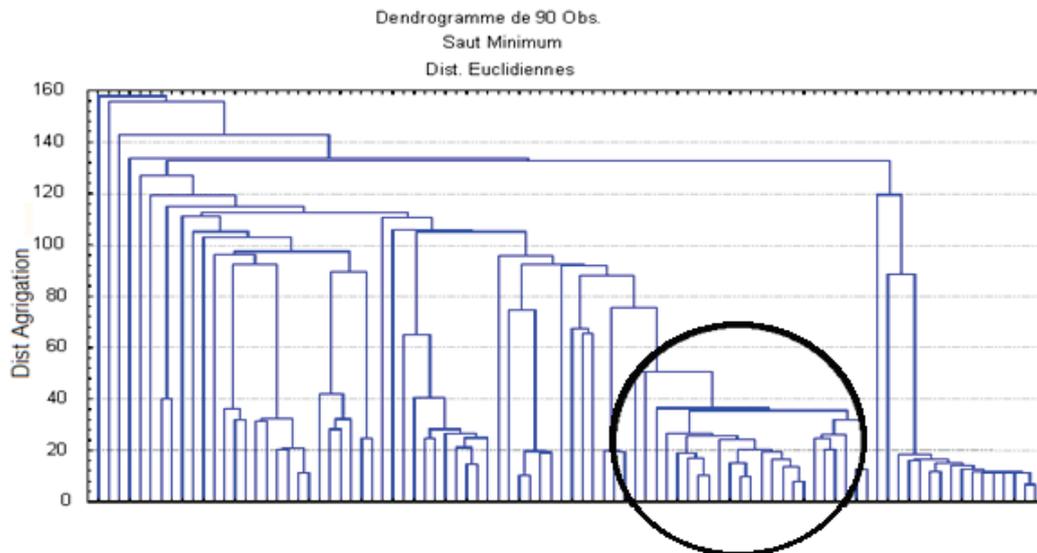


Fig. 7. Hierarchical classification of the individuals of the three sites (S1 + S2 + S3) according to anatomical parameters

Characteristics at each study areas. At each bioclimatic stage, a different structural form of adaptation appears. Indeed, individuals of *A. herba-alba* in the lower semi-arid (site 1) carry the largest number of files of reservoir cells to store the water that is available in large quantities compared to other sites. Thus, as individuals with a thick epidermal cell wall, their reserve cells are small. On the other hand, individuals with a less thick wall have very large reservoir cells. The characteristic of the arid climate (site 2) is the presence of a single conductive bundle accompanied by one line of reserve cells. We found that individuals with this characteristic external wall of epidermal cells are very thick with extreme values up to 8.3 μm in order to limit water losses by transpiration.

The Hassi Er'mel site is the most polymorphic with respect to the character of the number of conducting bundles by presenting three variants, the peculiarity presented in some individuals of this site is the presence of three conductive bundles forming a triangle and that the values of the vessels diameter are less than two micrometers. This characteristic proves to be very important because it allows increase in the hydraulic resistance of the conducting vessels and consequently a better rise of the sap.

Comparison between the different study areas. In a north-south progression, a very high polymorphism of the anatomical characters of *A. herba-alba* leaf is recorded at the lower semi-arid bioclimatic stage (site 1), followed by a less important variability at site 2 indicating a discontinuity of the variability in the lower arid bioclimatic stage monitored by an evolution of the expression of the characters observed at the site of Hassi Er'mel (site 3).

We have found that the third area with a Saharan climate (Hassi Er'mel, site 3) has more polymorphism of the anatomical characteristics of the leaf, presenting very high expression of variations for five characters and which differs from other sites by the presence of the variant three conductive bundles in the position of triangle that is characteristic of this site. In second position comes site 1 (El Faidja – Tiaret) representing the lower semi-arid bioclimatic stage which has a high anatomical polymorphism very close to that of site 3. Site 2 (Oued Morra – Nord de Laghouat) is the least polymorphous site compared to the others.

It is very interesting to take into account the geographical location of the populations studied to explain the anatomical variability observed previously within each site. Thus, to describe the different models of speciation commonly used in biology, we can take into account either the nature of the mechanisms involved in the formation of the species or the geographical status of the populations that we observe (Losos & Glor, 2003). The case of the situation of population 3 of site 1 and population 3 of site 3 which are isolated from the other populations behind a geographical barrier (mountains) is interesting. It is found that these two populations exhibited a large anatomical variability. Then, following the establishment or the appearance of a geographical barrier

on either side of this barrier, the two separated populations must be of equivalent size in order to have a genetic composition and a number of close individuals. The genetic frequencies of each trait can be maintained between the two populations. The differentiation between these two populations will only be due to ecological or environmental differences, which will impose different forms of selection and different mutations inside the genome of each population. Genetic drift will also intervene in the differentiation of these two newly formed populations.

It also appears that differences in edaphic characteristics have a direct effect on the variations of the anatomical structures of the different individuals of *A. herba-alba* across the three sites studied. Indeed, the soil type at the three sites 1, 2 and 3 respectively varies between loamy-deep and sandy clay, shallow (site 1), rocky-stony and clay-loamy (site 2) and is sandy or sandy-stony in site 3.

The wide geographical distribution suggests that populations of different origins will, in view of the contrasting edaphic-bioclimatic conditions of their environments of origin, have different phylogenetic behaviours (Ferchichi et al., 2004). Indeed, the existence of ecotypes at the level of this taxon has been studied for groupings with *A. herba-alba* in the Middle East (Zohary, 1973). Similarly, the existence of two varieties based on chromosomal counts has been demonstrated. This same author explained that on the morphological level the two forms are very close, so that for a botanist there is only one taxon (Ferchichi, 1997). The differences that distinguish them are not superior to those which exist between populations, even between individuals of the same population. Therefore, the karyological evolution preceded the morphological evolution, but it is likely that during evolution, and over time, these "geographical" chromosomal types did not become taxa with a distinct morphology (Ferchichi, 1997).

Sixty polymorphic loci were scored using four primers revealing a high level of genetic polymorphism among *A. herba-alba* accessions (Haouari & Ferchichi, 2008). Correlation analysis revealed no direct relation between morphological traits, geographic distance and genetic distance. Correlogram analysis showed a patchy distribution of the genetic variability of *A. herba-alba* accessions revealing the contribution of local ecological and geographic conditions on variability.

The expression of morphological characters remains under the simultaneous action of the genotype and the environmental medium and the appearance of phenotypic variability in individuals of the same population (intra-population variability) subjected to the same edaphic conditions is due mainly to genetic diversity (Maghni et al., 2017). Thus, the determined phenotypic polymorphism is explained by a genetic polymorphism demonstrated by the use of ISSR molecular markers for the analysis of genetic material, carried out on a sample of 12 individuals from the same study site. This genetic analysis demonstrated that *A. herba-alba* is characterized by a very marked genetic polymorphism, evidenced by amplifiers of stand 37 bands of different sizes including

78.4% of these bands which were polymorphic and the use of ISSRs to determine polymorphism. Genetics of white wormwood appears to be a very good choice (Maghni et al., 2016). Therefore, ISSR molecular markers based on di-, tetra- or penta-nucleotides repeated at the inter-microsatellite zones constitute one of the most effective markers used to estimate the genetic variability of species (Wang et al., 2009). The genetic variability thus demonstrated is explained mainly by the spontaneous mutations affecting this species. Genetic mixing would thus be limited, except through accidental hybridization, due to the autogamy characterizing this species (Maghni et al., 2016). This variability consolidates the results of the high anatomic polymorphism, which was observed at site 1 and at the scale of the other sites 2 and 3.

Conclusion

The histological sections of the white wormwood leaf show the presence of a central parenchyma at the level of the limb structure, which could be described as a parenchyma of water reserves. In our autogamous *A. herba-alba* species, the inter-population anatomic variability reflects the diversity of environmental conditions, but it is possible that performing mutants are better adapted to the environment and rapidly diffuse through strong autogamy (Lande & Barrowclough, 1987). Thus, the morphological polymorphism demonstrated within our study sites and thus confirmed during our genetic study on the same species of the same site (Maghni et al., 2016) is explained essentially by the spontaneous mutations affecting this species. It is considered that the assessment of the genetic diversity of natural resources is an essential prerequisite for defining strategies for their management or for genetic improvement. This study also indicated a high variability in *A. herba-alba*, which could help the choice of the most effective ecotypes for the reintroduction of this species in the degraded steppe ecosystems of Algeria and thus of North Africa. The choice of populations with high genetic variability will allow us to conserve their seeds in a grain bank in order to preserve our species for possible future use.

Our thanks go to the members of management of forests of Tiaret, Aflou and Laghouat (Algeria) for their help and suggestions when choosing populations at study sites. We thank all engineers of the Biothechnologie Vegetale Laboratory of Ibn Khaldoun University of Algeria.

References

- Agnew, A. D. Q., & Zohary, M. (1974). Geobotanical foundations of the Middle East. *The Journal of Ecology*, 62(1), 349.
- Benmansour, N., Benmansour, A., El Hanbali, F., González-Mas, M. C., Blázquez, M. A., El Hakmaoui, A., & Akssira, M. (2015). Antimicrobial activity of essential oil of *Artemisia judaica* L. from Algeria against multi-drug resistant bacteria from clinical origin. *Flavour and Fragrance Journal*, 31(2), 137–142.
- Djellouli, Y., & Daget, P. (1987). Climat et flore dans les steppes du sud-ouest algérien. *Bulletin de La Société Botanique de France. Lettres Botaniques*, 134(4–5), 375–384.
- Ferchichi, A. (1997). Contribution à l'étude cytotoxonomique et biologique de *Artemisia herba-alba* Asso en Tunisie présaharienne. *Acta Botanica Gallica*, 144(1), 145–154.
- Haouari, M., & Ferchichi, A. (2008). Study of genetic polymorphism of *Artemisia herba-alba* from Tunisia using ISSR markers. *African Journal of Biotechnology*, 7(1), 44–50.
- Lande, R., & Barrowclough, G. F. (1987). Effective population size, genetic variation, and their use in population management. *Viable Populations for Conservation*, 87–124.
- Le Houérou, H. N. (1969). La végétation de la Tunisie steppique (avec référence aux végétations analogues d'Algérie, de Libye et du Maroc). *Annales de l'Institut National de la Recherche Agronomique de Tunisie*, 42(5), 1–622.
- Losos, J. B., & Glor, R. E. (2003). Phylogenetic comparative methods and the geography of speciation. *Trends in Ecology and Evolution*, 18(5), 220–227.
- Maghni, B., Adda, A., & Merah, O. (2017). Evaluating morphological variability of *Artemisia herba-alba* Asso from Western Algeria. *Journal of Fundamental and Applied Sciences*, 9(1), 509.
- Merah, O. (2016). Optimization of DNA Extraction and PCR conditions for genetic diversity study in *Artemisia herba-alba* from Algeria. *Scientific Journal of Genetics and Gene Therapy*, 2(1), 10–12.
- Shang, Z. H., Hou, Y., & Long, R. J. (2012). Chemical composition of essential oil of *Artemisia nanshanica* Krasch. from Tibetan plateau. *Industrial Crops and Products*, 40, 35–38.
- Wang, W.-H., Kaskar, K., Ren, Y., Gill, J., DeSplinter, T., Haddad, G., & Singh, M. (2009). Comparison of development and implantation of human embryos biopsied with two different methods: Aspiration and displacement. *Fertility and Sterility*, 92(2), 536–540.