

EVALUATING MORPHOLOGICAL VARIABILITY OF *ARTEMISIA HERBA-ALBA* ASSO FROM WESTERN ALGERIA

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ABSTRACT

This work was interested on the study of the morphological variability of 120 individuals of *Artemisia herba-alba* Asso coming from three stations in western Algeria, which was assessed through 15 quantitative and qualitative characters. Analysis of variance (ANOVA) and hierarchical cluster analysis (HCA) results showed significant morphological variability inter and intra-population. This phenotypic variability is explained by a high genetic polymorphism determined at another genetic study that we conducted in parallel on individuals of the same site using molecular markers types ISSR. This study also revealed a large variability within *A. herba alba* which could help investigation on a large collection of individuals and therefore select the most efficient ecotypes for re-introduction this species in steppes and highlands of Algeria.

Key words: *Artemisia herba-alba* Asso; Morphological variability; steppe; Algeria.

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1. INTRODUCTION

In recent decades, steppe ecosystems are highly unbalanced due to an alarming degradation that characterizes these environments. That is related to the variability intra and inter annual climatic elements and factors anthropozoïques by changing the middle of the operating



systems' overgrazing, cereal, overexploitation. The various specialists including [1,2,3] agree to saying that pastoral surfaces and especially their ecological potential including production declined dramatically in recent decades. Forage production loss is estimated at 75% by [2] which raises the serious problem of coverage of livestock needs with a massive reduction in vegetation cover naturel.

Artemisia herba Alba, known as “wormwood” is a perennial small shrub with pubescent leaves. This Asteraceae species grows in desert wadi beds in North Africa and South-West of Europe [5]. As other species of *Artemisia*, wormwood is an important source of biological compounds with biocidal and allelopathic activities [6]. This aromatic species is widely used in North African's traditional medicine as expectorant, analgesic, antispasmodic, stomachic, vermifuge, diarrheic and sedative [7]. This species most advocated to the reconstitution of pastoral ecosystems degraded Mediterranean bioclimate. Its morphological and physiological characteristics make it a species well adapted to arid climate. The seasonal dimorphism of its foliage allows it to reduce the transpiring surface and thus prevent water loss [8,9]. Several authors have concurred that this species has a very high morphological and genetic variability. Thus, [10] mentioned the existence of ecotypes in this taxon, studying groups in sagebrush in the Middle East. Similarly, [11] revealed the existence of two varieties based on chromosome counts.

In Algeria, *A. herba-alba* covers three million hectares of the steppes and its potential as a forage resource is important. *Artemisia herba-alba* is well adapted to arid climatic conditions and grows in clay and compacted soil with low permeability [12,13]. This species is a natural way to fight against erosion and desertification [14]. However, [15], showed that the sagebrush steppes are replaced by indicator species of vegetation degradation or give way to bare soil that has resulted in scarcity or the disappearance of the species. Therefore, it appears necessary that to take actions aiming repopulation and protection of this spontaneous species. The realization of all protection and restocking programs, sustainable steppe course of wormwood necessarily based on a better understanding of morphological variability offered by this species its vis-à-vis adaptation of physical and biological conditions characterizing ecological areas its distribution. The distinction of parameters within and between populations employed in the present study, morphological order includes those that relate to a caulinaire characterization performed during the growing season on the one hand and the other a study of differences characters of the reproductive tract during the reproductive phase.

2. MATERIAL AND METHODS

2.1. Presentation of the study area

Our study sites known as El-Manseb (Altitude 1326m) are located in the highlands of the South East of the province of Tiaret (001° 48' 40.8'' E, 35° 01' 19.2'' N) in the North West of Algeria. The soil in this area is shallow and rocky or stony part, with generally calcareous-lime nature. Annual rainfall reaches 330mm; the range of temperatures varies between 2.5°C (cold season) and 35°C (warm season). The dry season took places during 6 months from the May to October.

2.2. Sampling of the materiel vegetable.

This study was conducted at the laboratory of plant biotechnology from the Faculty of Science of nature and life, in IbnKhaldun University. We referred to the type of subjective sampling, who seemed the most reliable for the selection of individuals sampled, the individuals are chosen because they appear typical and representative to the observer in his experience or flair [16].

After prospecting the study site we have chosen three populations all taking into account the remoteness of these populations and the existence of ecological barriers and the deference of soil and topographic characteristics (slope, aspect) and according to the criteria that seem representative the morphological variability of 30 individuals taken from each population. Samples collected from the *A. herba alba*.for studying morphological parameters of the sheet were made during the most developed vegetative stage, which corresponds to September. To see the characteristics of the reproductive organs, échantillons were collected during the flowering which spreads out end of October until late December 2012.

Table 1.Geographical and topographic data of the studied population

population	Latitude N	Longitude E	Altitude (m)	Topography	Type of soil
1	35° 01 ' 29.1'' N	001°47 ' 59.4'' E	1015M	Depression	Silty, deep
2	35° 01' 17.9''N	001° 48' 40.8''E	1251M	Plain	Silty,shallw
3	35° 01' 27.6''N	001° 49 ' 03.3''E	1359M	Slope	sandy clay, superficial

2.3. Observation, measurements and analysis of characters

The studied qualitative characteristics relate to the color of the leaves (CF) ; color of the petals (CP) color carpel (CCR); color of stamens (EC); the mode of arrangement of leaves on the stem (DF.R); the mod of disposal of the leaflets on the sheet (CFLF) the distribution of leaflets on the sheet (RLF.F); the presence of hair (PPL); leaf color after removal of the bristles (CFPL); the provision of corolla on the ovary, (COD); the shape of the ovary (CRF); the level of insertion of the stamens (NVIE). The shape of the level between the ovary and the corolla (NVOC).

The quantitative characters are represented by the number of leaflets per leaf (NBFL.F) and the number of small leaflets per leaflets (NBFLL.FL).

All of its characteristics are observed under a binocular microscope and photographers. The analyzes of the expression of the characteristics frequencies in individuals from each population, comparison of means by analysis of variance (ANOVA) and the hierarchical ascending classification allowed us to evaluate the variability within and between populations.

Table2. Qualitative characters selected in the vegetative stage

characteristics	associated variables and codes
Color of leaves	dark green (1) intermediate green (2) clear green
Presence of trichoma	Strong presence(1) average p(2) Weakp(3)
Leaf color	leaf after trichoma removal green bright color(1) dull-green (2)
Arrangement of leaves on the stick	Alternate (1) opposite (2)
Arrangement of the leaflets on the leaves	Alternate (1) opposite (2)
distribution of leaflets on the leaf along the leaf	(1) at the end (2)
Number of leaflets per leaf	03fl (1) 05fl(2) 06fl(3) 07fl(4)
Number of small leaflets per leaflets	02fll(1) 03fll(2)

Table3. Qualitative characters selected in the flowering stage

characteristics	associated variables and codes
Color of petals	red(1) orange(2) yellow(3)
Colors of carpel	Yellow(1) brown(2)

Color of stamens White(1) Yellow(2)

Insertion mode of corolla on the ovary Vertical(1) Oblique(2)

Form of Corolla Domed(1) Shortly domed(2)

Levels insertion of stamina compared to the carpel Low level (1) Highlevel(2)

Level form between the corolla and ovary Plane(1) Incline(2) Convex(3) Concave(4)

3. RÉSULTATS ET DISCUSSION

The evaluation of rates of change in leaf and floral morphological traits in individuals of three populations showed a discrepancy in their occurrence frequency of occurrence (Tab. 4).

Table 4. Frequencies of appearance of the color of the sheet, in the three populations.

Caractère	Variante	Fréquence		
		d'apparition du caractère chez les individus (%)		
		Pop 1	Pop 2	Pop 3
CF	Dark green	28	50	13
	Intermediate green	34	17.5	67
	Clear green	38	32.5	20
PPL	Strong presence	37	37.5	30
	Average presence	36	15	43
	Weakp	27	47.5	17
CFPL	Green bright color	94	90	83
	Dull-green	06	10	17
DF.R	Alternate	50	77	37
	opposite	50	23	63
DFLF	Alternate	72	31	20
	Opposite	28	69	80
RLF.F	Along the leaf	24	60	20
	At the end	76	40	80
NBFL.F	03leaflets	18	08	08
	05leaflets	64	60	60

	06 leaflets	08	07	17
	07 leaflets	10	25	15
NBFL.LFL	02 small leaflets	30	31	29
	03 small leaflets	70	69	71
CP	Red	60	32	00
	Orange	28	68	47
	Yellow	12	00	53
DCO	Vertical	28	37	27
	Oblique	72	63	73
FRC	Domed	74	75	80
	Shortly domed	26	25	20
NVOC	Plane	08	10	33
	Incline	62	78	57
	Convex	18	10	07
	Concave	12	2	03
CE	White	88	67	73
	Yellow	12	33	27
NVIE	Low level	60	65	13
	Highlevel	40	35	87
CCR	Yellow	00	00	60
	Brown	100	100	40

The analysis of the results shows that the color of the sheets (CF) is distinguished into three groups among individuals in each of the three studied populations (Fig.1). The three levels distinguishing between the different groups are represented by the bright green, middle green and green tent. The frequency of appearance of the three colors is homogeneity in the population 1 (Tab.4). Nevertheless, the population level 2, there is a dominance of dark green (50%) while 67% of individuals in the population 3 carry the green intermediate color. This characteristic is a criterion for distinguishing intra- and inter-population revealing a rather accentuated polymorphism in this species. According to the work of [17], the morphological

characteristics of the sheet are highly polymorphic in this case and therefore constitute a highly sought criterion in estimating the genetic variability of different populations.

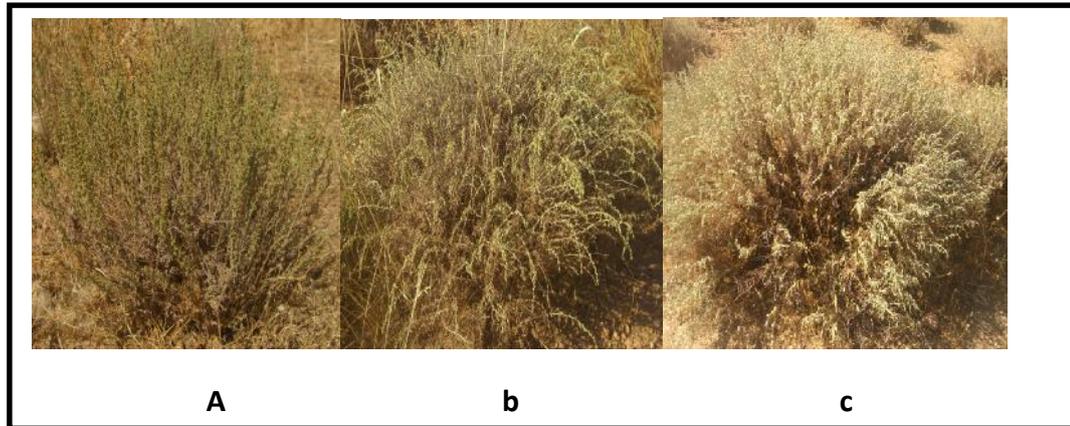


Fig. 1.Color polymorphism leaves; a- dark green; b- intermediate green; d- clear green

The color on the surface of the limb after removal of trichoma (CFPL) (Fig.2) also presents a test for distinguishing between individuals from the same population and between the three studied populations (Tab.4). The distribution of individuals within the same population, based on this criterion is demonstrated divergent account of the populations concerned. After removal of trichoma, the leaves had expressed mainly a green bright color with a level of 94% of the studied effective, against only 6% of people who are characterized by a dull green color.

It is noted that the frequencies of the foliage color and abundance of trichoma at the surface of the leaf, are very close. Then it would be obvious to establish a strong bond between these two parameters. Changes in the color of the sheets are strongly conditioned by the importance of the presence of the bristles to the surface of this body. The same findings were observed with two other populations.

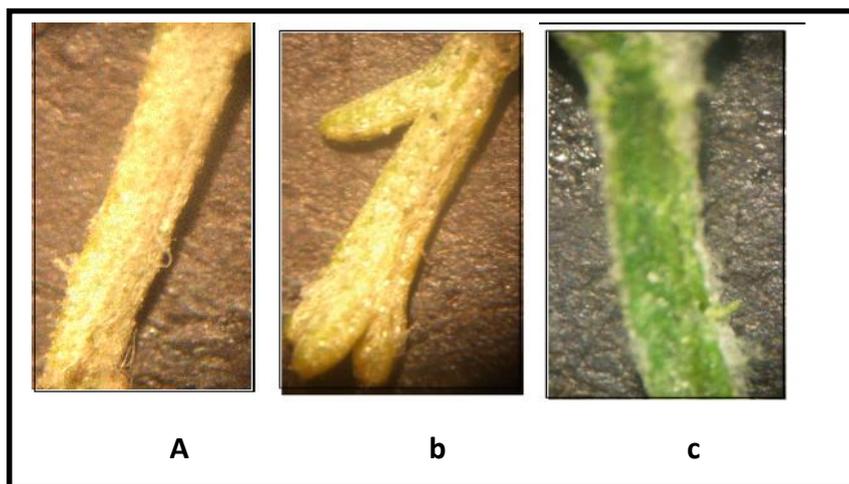


Fig.2. Presence of trichoma on the leaves: a- Strong presence; b- average presence; c- live green leaf after trichoma removal

The dispositions modes of leaves on the stem (DF.R) encountered in the three populations are two types, alternate and opposite (Tab.4) (Fig.3). We consider that this parameter would be highly heritable and therefore slightly influenced by changes in environmental conditions. The results show that the three studied populations materialize both modes of insertion of the leaves. Nevertheless, the frequency representation of the two characters by individuals from each population proves to be different. Population 2 has a majority expression of mode switches (77%), population 3 is defined by a dominant expression of the opposite mode (63%). While in the population 1 representation of the two modes appears equitable.

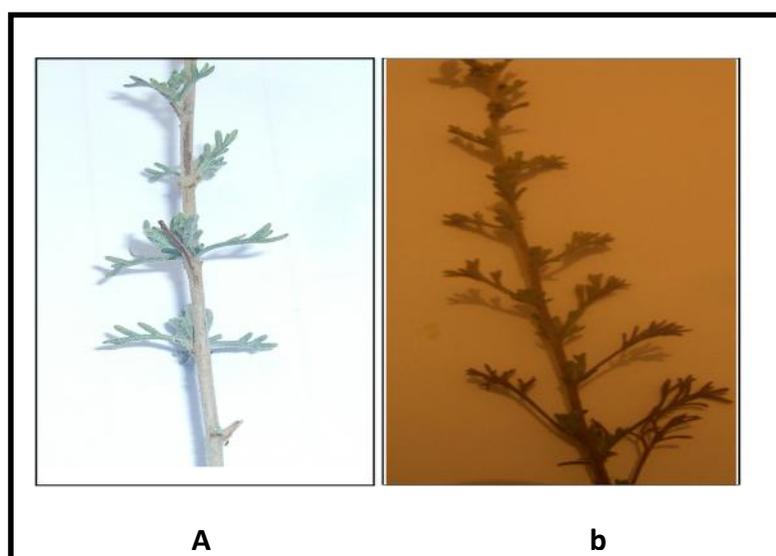


Fig.3. Mode of arrangement of leaves on the stick: a- Oppose ; b- Alternate
The expression of the arrangement of the leaflets on the leaves (DFLF)((Fig.4a,4b).
between individuals of the three populations is very distinct because population 1 is for

controlling the alternate guide (72%), As opposed to other populations with much the opposite mode .

The inter- and intra-population polymorphism based on the expression of the distribution of leaflets on the leaf (RLF.F) (Fig.4c,4d). shows differences between individuals of the three populations. Indeed the distribution of leaflets mode at the end of the sheet is more presented at both populations 1 and 3 with 76% and 80% of individuals carrying this character however, the second population is characterized much more by the mode of distribution of the leaflets on along the sheet (60%).

The analysis of variance (ANOVA) with the result of the estimation of the number of leaflets per leaf shows very important variations of expressions among the three populations. Certainly, the variations induced by the genetic variability are significant at $p < 0, 05$ The number of leaflets per leaf is 3, 5.6 and 7 leaflets per leaf but at very different percentages, the number of leaflet 05 is more dominating in the three populations. However, that polymorphism in the number of leaflets per inter-leaflet population is low ($p > 0.05$). The number of leaflets per leaflets met in the three populations is varied between 2 and 3 foliolules (Tab.4). The frequency of appearance of these two numbers of leaflets in the three populations, indicates that the character three leaflets per leaflet is represented by 70% of individuals (population 1), 69% (2 people), 71% (population 3) . Nevertheless, the character leaves 2 per leaflet is expressed in 30% of individuals (population 1), 31% (population 1), 29% (population 3). This indicates heterogeneity of distribution of a number of foliolules per leaflet to the level of each population

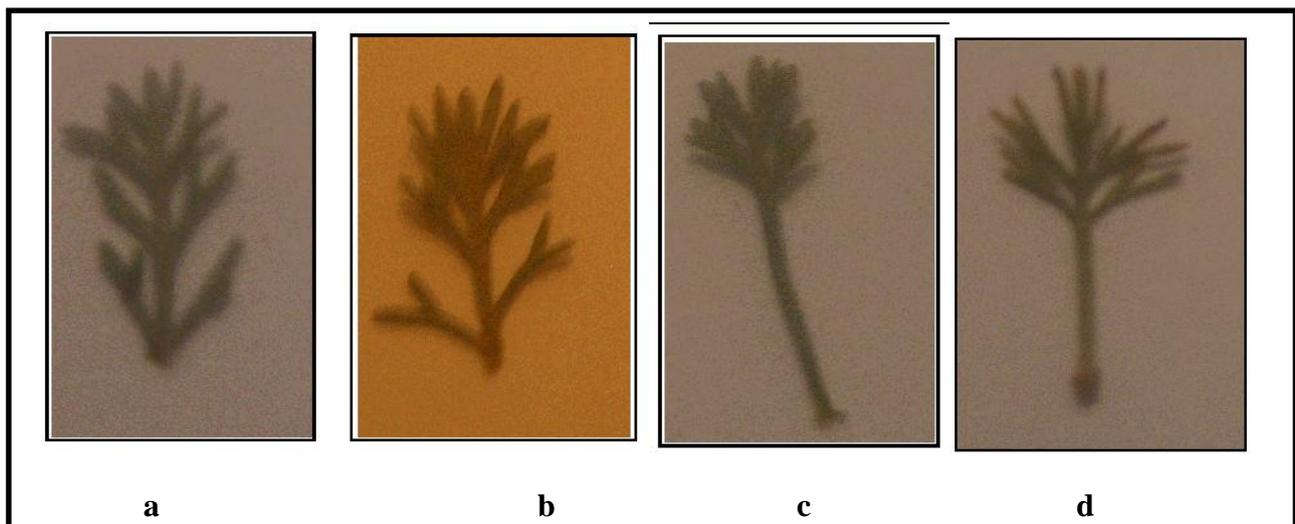


Fig.4. Distribution of leaflets on along the leaf and their disposal: a- Oppose; b- Alternate ;c and d: division of leaflets at the end of the leaves.

The expression of the color of the petals (CP)(Fig.5).Show very divergent behavior between individuals of the three populations. Indeed Population 1 is the most polymorphic for this character. It is recognized by the distribution of three different colors; red, orange and yellow. This hang populations 2 and 3 show only two colors which the general color between the two is orange, but the population 2 manifested the red color that population3 lack it and is distinguished by the presence of yellow color. Thus the color red is specific to the two populations 1 and 2 while the yellow color is unique to the two populations 1 and 3.

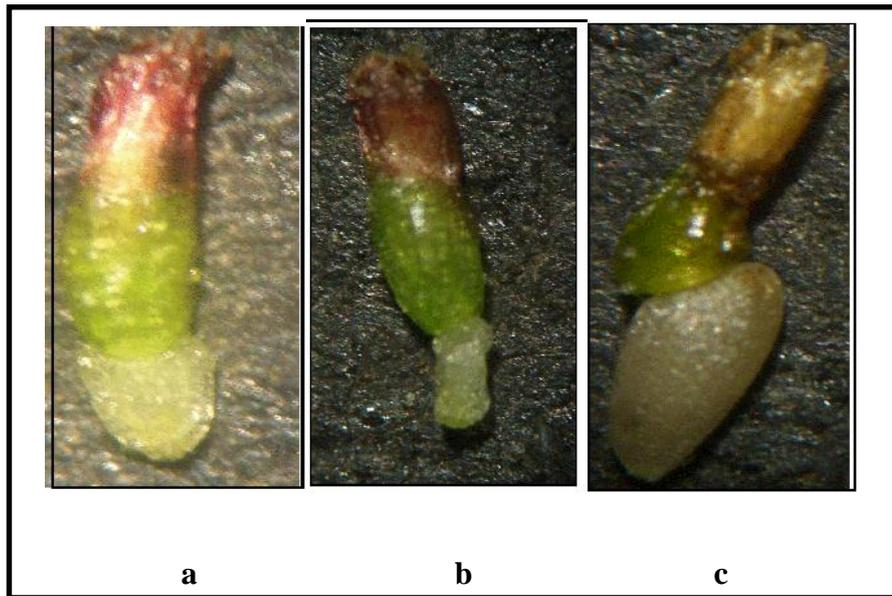


Fig.5. Colored petals Polymorphism; a- red ; b- orange; c- yellow

Two modes of insertion of the corolla on the ovary (COD) met in the three populations, either oblique or vertical (Fig.6a,6b) .The oblique mode is mainly manifested in the three populations but with different proportions. Indeed the frequency of occurrence of this character in the three study populations was 72%, 63% et73% .However[18] reported that the Corolla is inserted very obliquely on the ovary.

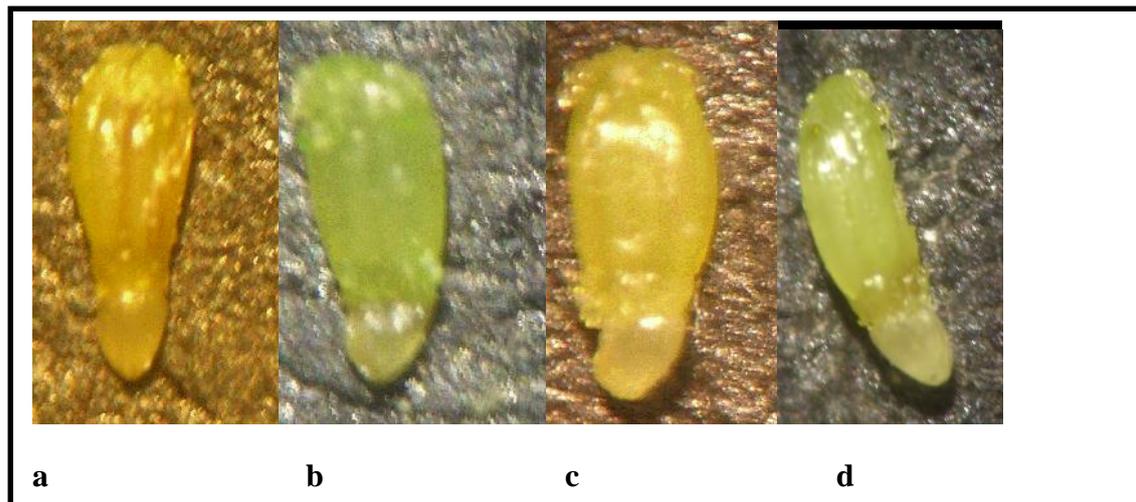


Fig.6. Insertion mode of corolla on the ovary: a- Vertical; b- Oblique; Form of Corolla: c- Domed; d- Shortly domed

We met two forms of corolla (FRC) (Fig.6c, 6d) .Either bomb, or little bomb. The three populations have nearly the same frequencies of the two forms of the corolla (Tab.4). Indeed populations; 1, 2, 3, have respectively, 74%, 75% and 80% of the bomb as against 26%, 25% and 20% of form No bomb.

Observations made about the character indicating the form of level between the corolla and ovary (NVOC) showed the existence of four forms characterizing the level that lies between the corolla and ovary (Fig.7). These forms are: Plane, Incline, Concave and Convex. At the level of the three populations is the inclined level, which is the most represented with respectively 80%, 78%, 57% of individuals followed by other forms of level plane, concave and convex.

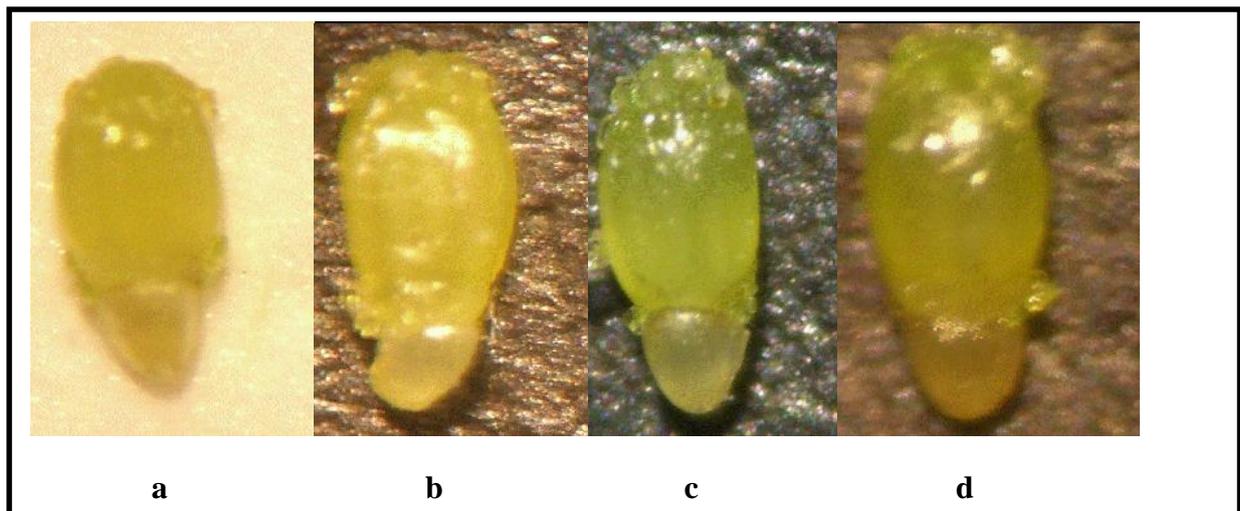


Fig.7. Level form between the corolla and ovary: a-Plane; b-Incline; c- Convex ;d Concave

Two colors stamens (EC)(Fig.8a,8b), differ through the individuals of three populations (Fig.28), mainly white and yellow in the second degree. In each population, the majority of individuals are carriers of white stamens Flower with respectively 88%, 67% and 73%. Against 12%, 33% and only 27% of individuals who are characterized by the yellow color.

Two levels of insertion of the stamens compared to carpel (NVIE) met in all individuals of the three study populations, high and low level (Fig.8c,8d). The distribution of individuals according to this characteristic (Tab.25), at populations, 1 and 2 is characterized by the dominance of the high level with 60% and 65%. However, inside the population 3, it is the low level is in a majority with a rate of 87% against only 13% with a High Level.

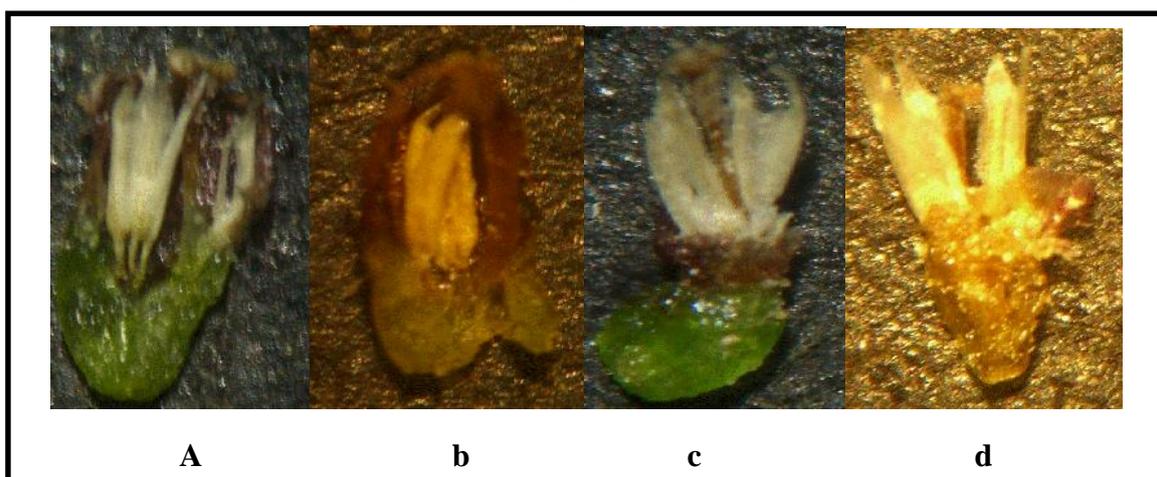


Fig.8. Color of stamens : a - White ; b-Yellow ; Levels insertion of stamina compared to the carpel: c – Low level ; d- level High.

The expression of the character color of the carpel (JRC)(Fig.9), shows a wide discrepancy between the population3 on the one hand and the two other populations on the other hand (Tab.4). Certainly, at both populations1 and 2, all individuals carried flowers to carpel yellow (100%). However, only 40 % of the individuals of the population3 expressed the carpels yellow. While the most of the individuals in this population showed a brown color of carpels(60%) which is specific for him.

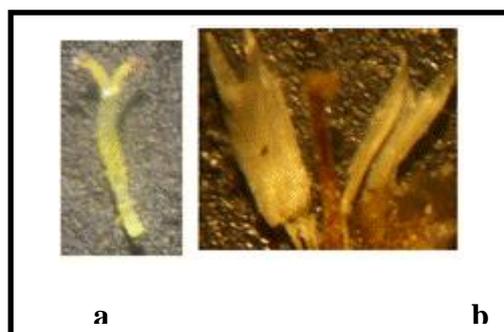
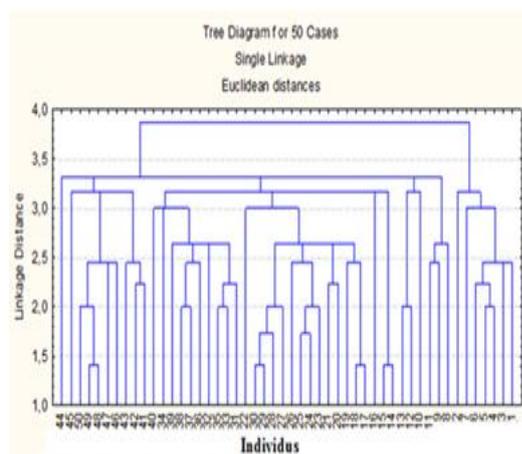
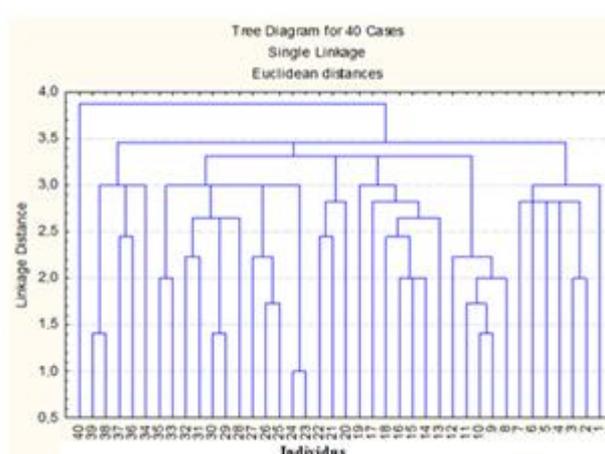


Fig.9. Colors of carpel: a- Yellow; b-brown

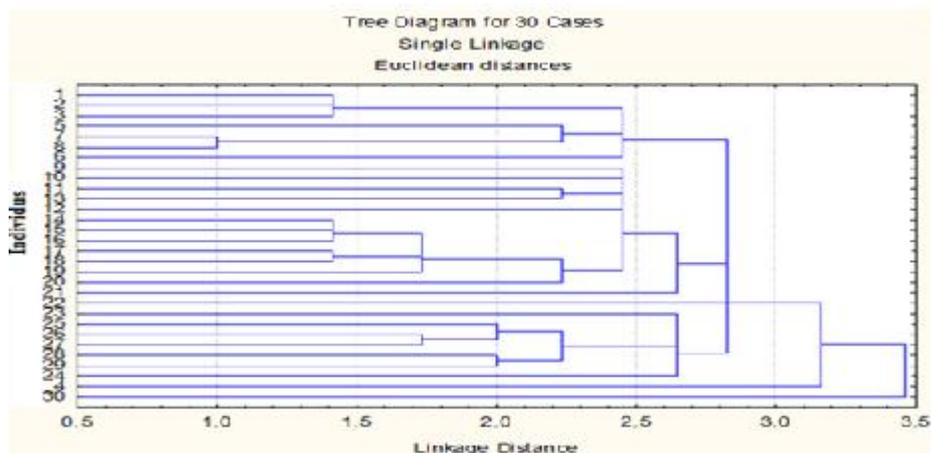
The study of the dendrogram obtained by the establishment of the hierarchical clustering (ACH) Confirms variability within and between -population, recognized in previous analysis in this work. In fact, individuals of the populations 1, 2, 3 (50,40 and 30 individuals) are distributed according leaf morphological parameters respectively in 37,29 and 19 separate groups(Fig.10a,10b,10c), and are divided according to the floral morphological parameters respectively in 38, 29 and 21 different groups(Fig.12a,12b,12c). The division of individuals from each population into different groups, indicating that the site studied is characterized by a high variety based on morphological polymorphism of the air vegetative part.



a



b



c

Fig.10. Hierarchical classification of individuals in the population1 according to morphological parameters of the leaves. a: in the population 1; b : in the population 2; in the population 3

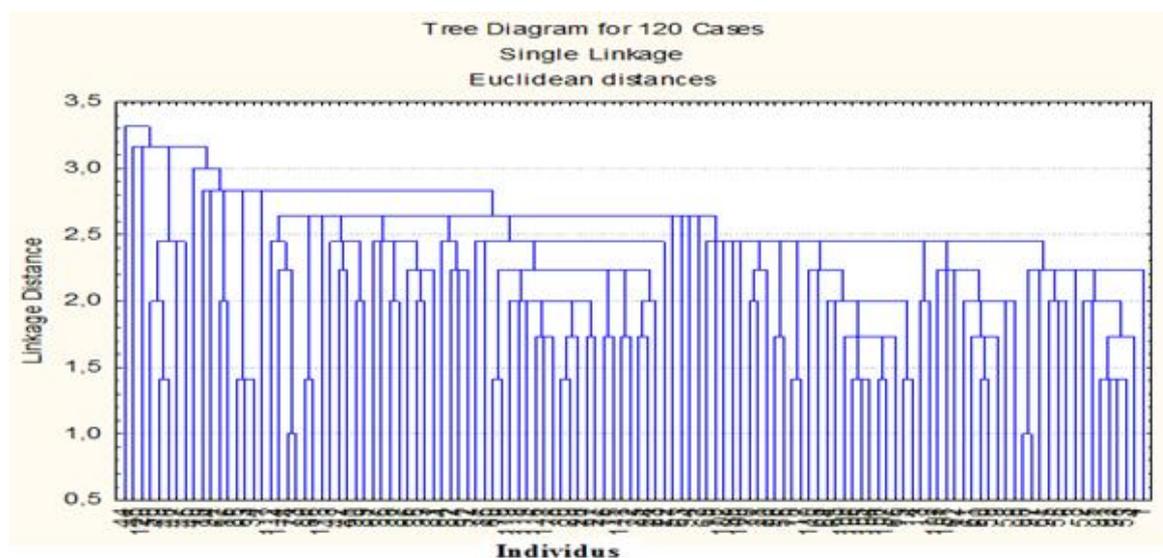


Fig.11. Hierarchical classification of individuals of the three populations according to morphological parameters of the leaves

The classification (ACH), including all individuals (120) from the three populations demonstrates that individuals differ according leaf traits in 68 different groups (Fig.11).. This distinction is made out indifferently origins of individuals (three populations). This indicates that the differences and similarities of expression of the various foliar parameters characterizing the variability of different accessions are only slightly conditioned by the geographical distribution of individuals. The observation of these groups shows the existence of 46 groups with one individual from different populations, two individuals 13 groups, 04

groups (3 people), and 2 groups, each one bringing seven individuals. In the end two groups each are bringing the highest number (11) individuals. In spite of this highest number, these two groups are characterized by a more marked heterogeneity, as they bring together individuals from three populations studied. Indeed the first contains 50% of individuals (population 3), 30% (population 1), 20% (population 2). The second group comprises 55% (3 people), 30% (population 2) and 15% (population 1).

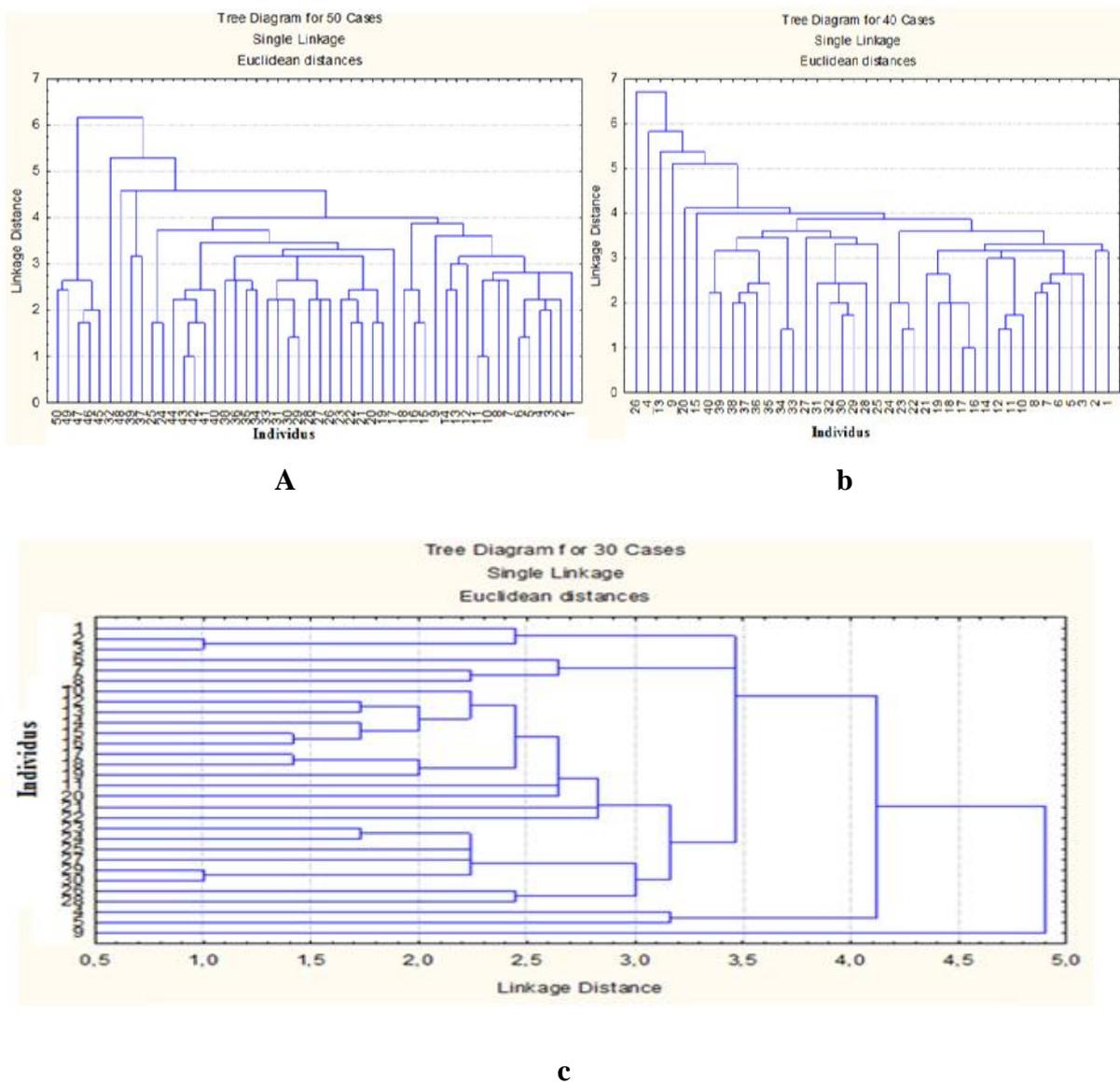


Fig.12. Hierarchical classification of individuals of the population(a) ; population(b) ; population(c) according floral morphological parameters

The characters in shared between different individuals of the three populations in these two groups are the number of leaflets per leaf, the leaf arrangement on the stem, the arrangement of leaflets on the sheet and the method of distribution. These findings confirm an high inter-population polymorphism of leaf morphological characteristics of white wormwood.

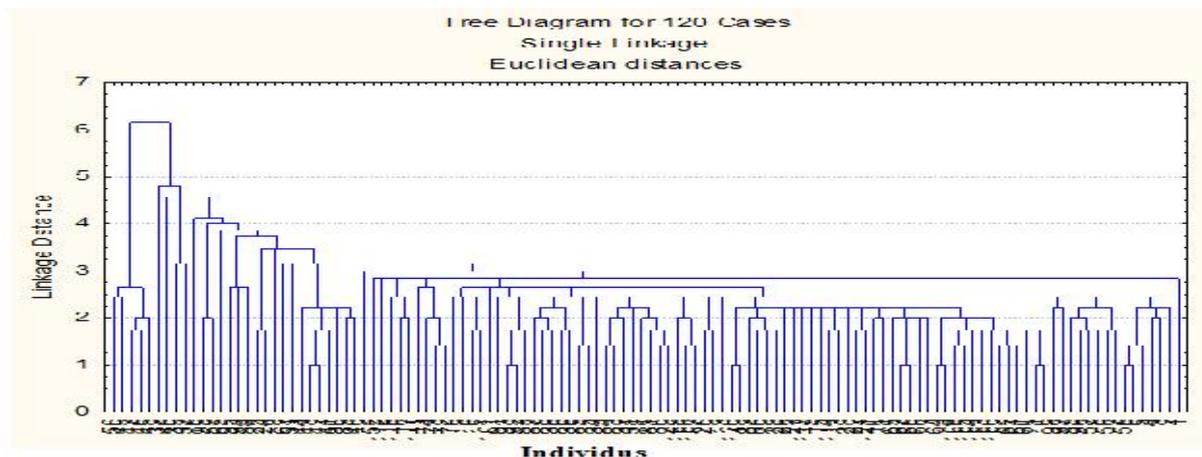


Fig.13. Hierarchical classification of individuals of three populations according to the floral morphological parameters

The dendrogram realized for 120 individuals from three studied populations in terms of flower morphology (Fig. 13) distinguishes, 67 distinct groups, for the aggregation distance in 41 groups, there is only a single individual for everyone. These individuals coming from the various populations. Other individuals divide through the rest of the groups as followings: 14 groups have 2 individuals, 7 groups (3 people), 3 groups (4 people), one group consists of five individuals. In the end he stands out among these groups one group that brings together 13 individuals derived from three populations with six individuals in the population 3, four individuals (population 1) and three individuals (population 2). The similarities between these individuals are; the color of the petals, stamens and carpels and the level of insertion of the stamens. This distinction groups has any realized indifferently the origins of individuals (three populations). This indicates that the differences and similarities of expression of different leaf and floral parameters characterizing the variability of different accessions are only slightly conditioned by the geographical distribution of individuals

According to [19]. The wide geographic distribution us to think that populations have different origins, will have different phytoecological behavior. Certainly, [10] mentioned the existence of ecotypes in this taxon, studying groups in *Artemisia herba-alba* in the Middle East Similarly.

In the same way, [11] revealed the existence of two varieties based on chromosome counts: the variety *communis* with $n = 9$ and variety *Desertii* with $n = 18$. The same author explained as on the morphological level, the both forms are very close, very closely connected, so that, for a field botanist, there is only one taxon. The differences between them are not greater than those which occur between populations or even between individuals of the same population. Thus [11] explained that the evolution caryologique preceded morphological evolution, but it is likely that during evolution, and with time, these chromosomal races "geographical" from becoming taxa with distinct morphology.

The existence of a pronounced inter-population variability of our results indicated that the selected parameters would be of a high heritability, which minimizes the impact of the environment on the expression of these foliar and floral morphological characteristics. Thus, it 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 *Artemisia herba alba* is characterized by a very marked genetic polymorphism, evidenced by a amplifias of stand 37 bands of different sizes including 78.4% of these bands were polymorphic [20].

4. CONCLUSION

The results of the morphological study (leaf and flower morphology) of 120 individuals of *Artemisia herba-alba* Asso from three stations in western Algeria, indicate a very high polymorphism intra and inter-populations. The expression of morphological characters remains under the simultaneous action of genotype and the environmental community. Appearance of variability phenotypical at the individuals of same the population (variability intra-population) subject to the edaphic and climatic is mainly due to genetic diversity. At our species autogams *Artemisia herba-alba* Asso, morphological variability inter population reflects the diversity of environmental conditions but, it is possible that appear powerful mutants, better adapted to the environment and quickly diffuse through strong selfing [21]. Then the morphological polymorphism demonstrated in our study site and thus confirmed in our genetic study on the same species of the same site is mainly explained by spontaneous mutations affecting this species. Genetic exchange would be limited except through accidental hybridizations, because of self-fertilization characterizing this species [20]. This study constitutes a first step to investigate diversity on larger collection of this species.

5. ACKNOWLEDGEMENTS

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