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Full Length Research Paper

Taxonomic study of the Tunisian *Calligonum* genus using the morphological and anatomical traits

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The Tunisian desert harbors an important socioeconomic pastoral species including the *Calligonum*. It has an important ecological and ethological role. An important biodiversity is to be signaled in Tunisia. Variability within the *Calligonum* Tunisian genus has not been a thorough scientific study. Therefore, the morphological and anatomical characteristics may play an important role in the analysis of this variability. The present study is an attempt to gather information and analyze morphological and anatomical natural diversity within the Tunisian *Calligonum* genus to distinguish the three species (*C. azel* Maire *C. arich* Le Houérou and *C. comosum* L'Herit.) cited in the Tunisian flora. The present report deals with three species of *Calligonum* of (Polygonaceae) collected from Tunisian desert using morphological and anatomical traits. 10 plants for each species were used in our study. The degree of similarity among the three species has also been studied through these traits. The gotten dendrogram showed two groups: The first is composed of *C. comosum* and *C. azel* with a similarity of 29% in the morphological an anatomical trait. The second is formed by *C. arich* with a likeness of 15% with the first group.

Key words: *Calligonum*, inter-specific diversity, morphological traits, Tunisian desert.

INTRODUCTION

Desertification or land degradation, presents major at global scales. In Tunisia, which covers 164103 km^2, arid and desert regions form the three quarters of its area (Le Houérou, 1959). The Tunisian desert harbors a great number of very important socioeconomic pastoral species. Most importantly, many autochthonous species deserve to be studied including: those colonizing the Grand Erg Oriental (*Calligonum azel*, *Ephedra alata*, *Calligonum arich*, *Genista saharae*, *Stipagrostis pungens*, *Helianthemum confertum*, *Henophyton desertii*, *Retama raetam* and *Calligonum comosum*é). These species support lower levels of rainfall (<50 mm / year), high temperatures and a high evapotranspiration. In the other hand, these species present an ecological interest as Saharan they the sand, resist wind erosion, enrich the soil with organic matter.

The family of polygonaceae has been always considered as the sister of the family of the Plumbaginaceae, due to their anatomical structure (Carlquist, 2003).

Calligonum genus belongs to the Polygonaceae family, with some 80 species distributed throughout Western Asia, Southern Europe and North Africa (Okasaka and al., 2004). *Calligonum* L. is an ancient genus of the arid desert flora of North Africa. *Calligonum* L. has been playing a key role in the stability of the natural and planting vegetation ecosystems of the desert of the Tunisia. The species belonging to this genus have great potential and importance because of providing different products and services, such as forage, traditional medicines, arresting desert encroachment and stabilizing sand dunes. Therefore, the genus has attracted the

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attention of the scientists.

Only *C. comosum* L'Hérit, *C. azel* Maire and *C. arich* Le Houérou occur in the Tunisian arid zone and the last one is endemic (Le Houérou, 1959).

Those species which deserve to be preserved for their ecological interest. Each of these three species is essential to maintain balance and protection of desert ecosystems weakened by anthropogenic factors (deforestation and grazing).

In Tunisian Desert, Cauvet (1925) show that *C. comosum* was served to the treatment of the camel scabies. The decoction of roots of this species was vermifuge Le Floc'h (1983). Bhandari (1995) reported that Tunisian *Calligonum* genus is an important source of food for animals and is also appreciated for their wood quality and medicinal purposes. *Calligonum comosum* is used by some healers to treat stomach ailments, the stems and leaves are chewed for curing toothache (Ghazanfar, 1994). The fresh aerial parts of this species show an anti-inflammatory and antiulcer activity (Liu and al., 2001). Some compounds, extracted from *C. comosum* L. present a cytotoxic and antioxidant activity (Badria and al., 2007).

Nevertheless, the valorization of these three bushes requires a good knowledge of their morphological and anatomical traits. These features always remain a key for the taxonomiy of species possessing a doubt. On the 100 past years, a literature skinny content has been published on the *Calligonum* genus, concerning its ecology, its biology and its utilization etc.) (Battandier, 1868; Pervinquière, 1912; Foley, 1925; Le Houérou, 1959; Ozenda, 1977; Pottier-Alapetite, 1979; Greuter and al. 1989; Le Floc'h and Boulos, 2008 ; Neffati, 2008 and Dhief et al. 2009). Well that some authors recognize the majority of existing *Calligonum* shapes and discussed them like separated entities; the *Calligonum* genus of Tunisia understands species again in doubt. It is a little surprising that a genus like *Calligonum* is so spilled in the Tunisian and north-African desert, and possesses an importance in the structure of arid and desert ecosystems zone, which attracted the attention of botanists. As for numbers of species, the Tunisian *Calligonum* genus is not particularly big, but as for its morphological variation we can meet a taxonomic complexity. The *Calligonum* genus of Tunisia characterizes itself by a very important morphological polymorphisme in relation with the local ecological conditions (Le Floc'h and Boulos, 2008), but it has known a difficulty that resides in whether *C. arich* effectively exist as species in the Tunisian desert. Le Floc'h and Boulos (2008) show that *C. arich* as being a species in doubt and this species is just *C. calvescens*. However, Le Houérou (1959) and Dhief and al. (2009), signaled the existence of this species as a separate species. The present study contributes to a survey of interspecific.

To the best of our knowledge, there are no reports in the literature concerning the variability between Tunisian *Calligonum* species.

The present study is an attempt to characterise and compare morphologically and anatomically the three Tunisian *Calligonum* species (*C. arich C. azel* and *C. comosum*) and know their degree of similarity.

MATERIAL AND METHODS

Geographical an natural locations

With collaboration of the Laboratory of Pastoral Ecology of the Arid Regions Institute of Médenine, we studied thirty specimens of the three species of the *Calligonum* genus at the Great Erg Oriental near to the petroleum station of El Borma (31° 63'N and 09° 27' E) (Figures 1a and b).

The meteorological stations of reference are El Borma, which provide reference information for climatic factors. This site has arid-type climate with dry and hot summers and cold winters. The annual rainfall is low, rarely exceeding 100 mm. The rains are infrequent and irregular, sometimes with no rain during long periods of several years. Mean annual rainfall for 10 years were 52.3 mm, and mean annual temperature were 22°C. Annual temperature was 22.6°C and annual precipitation is 65.4 mm (Institut National Météorologique, 1996).

The dates for onset of the rains were variable; March and October were the wettest months and June, July and August are the driest months.

Experimental methods

While using a meter and a digital numeric caliper, we revealed morphometric parameters including: 1) The length, the height and the width of plants, 2) the length and the diameter. Nodes and internodes have been determined while using a digital numeric caliper and graduated ruler. The color and the aspect of flowers, stems, anthers and stigmas have also been identified. The number of anthers and stigmas has been examined by optic microscope. One thousand seed were weighted with a precision balance EW 220 to 3 NM type (1 mg precision). Other biological treats have been also recorded as the date of flowering and the date of fruition (Table 1). The anatomical characters have been determined while using an electronic microscope (MEB/03/Philips XL30) (Table 2).

RESULTS

Morphological variability at the three *Calligonum* species

Table 1 and 2 shows an important variability concerning morphometric treat between the three examined species:

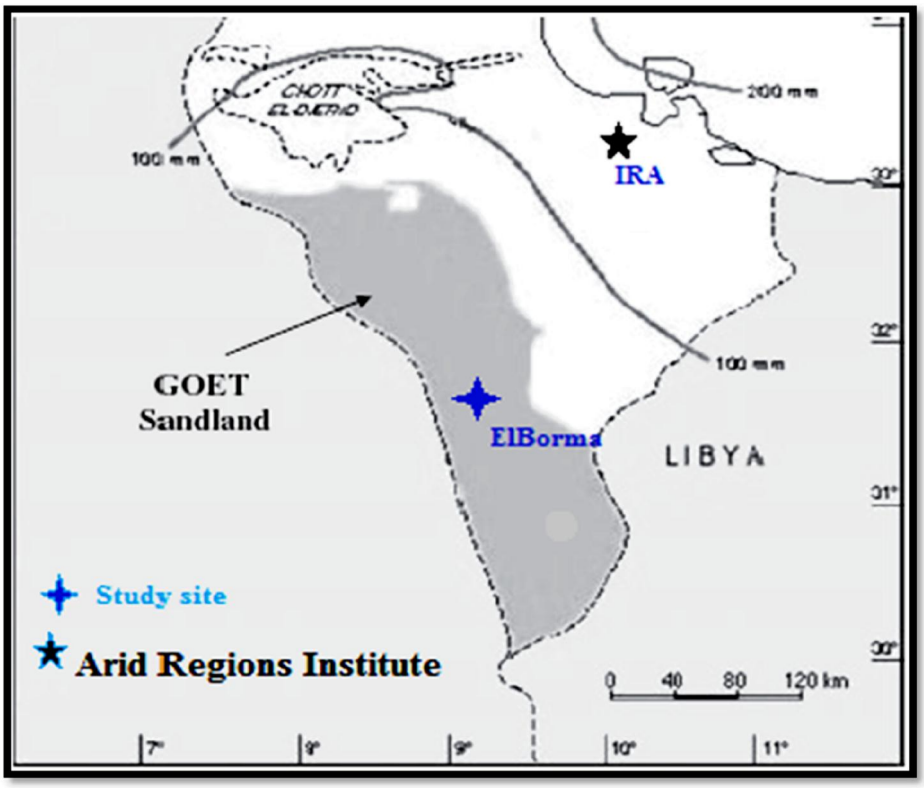

Figure 1a. Geographical location of the study site of the three *Calligonum* species.

Figure 1b. Natural location of the study site (**d**) of the three *Calligonum* species. (**a**) *C. comosum*, (**b**) *C. arich* and (**c**) *C. azel*.

Table 1. Morphological traits studied of the three *Calligonum* species.

Morphologic traits	Mesures (cm)		
	C. comosum	***C. arich***	***C. azel***
Height of the plant	75-220 (147.5)	430-810 (620)	350-550 (450)
Width of the plant	70-120 (95)	450-650 (550)	260-340 (300)
Old stem color	Grayish	Brown	Grayish
Green stem aspect	Rigueux, articulated and fasciculated	Smooth articulated and fasciculated	Smooth articulated and fasciculated
Length of inter-nodes	2.2-6.8 (4.5)	5.2-12.8 (9)	3.4-9.6 (6.5)
diameter of nodes	0.07-0.2 (0.135)	0.1-0.45 (0.275)	0.1-0.35 (0.225)
Maximal green branch length (cm)	42	76	68
inflorescence type	Simple branched	Simple branched	Simple branched
flower Colour	Whitish	Red	Whitish
flower type	Hermaphrodit	Hermaphrodit	Hermaphrodit
Floral button size average	1.4-2.1 mm	08-1.6 mm	1.6-2.6 mm
Colour of anthers	Red	Whitish	yellow
stigmas Colour	Quick rosy	Whitish	Lucid rosy
Number of stamens	14 (7 inf +7 sup)	14	14(8 inf +6 sup)
Length of nets (mm)	8-10 mm	10-12 mm	8-10 mm

Table 2. Morphological traits studied of the three *Calligonum* species.

Filets types	**Linked at the basis**	**Linked at the basis**	**Linked at the basis**
Style numbers	4	4	4
Style length (mm)	3-4 mm	4 -6 mm	3-4 mm
stigmas numbers	4	4	4
floral Diagram	axial	axial	axial
Type of ovary	tetragone	tetragone	tetragone
Date of flowering	Mid- mars	End April	End mars
Colour of the fruit	brown	Violet	brown
Fruit length	0.7-0.9	1.2-1.4	1.0-1.2
fruit diameter	0.25-0.45	0.2-0.35	0.25-0.5
Shape of the fruit			
Length of the hairs of the fruit	0.15-0.35	0.2-0.4	0.1-0.27
Date of fruition	Beginning April	Mid-mai	Mid- avril
Average mass of 1000 seeds (gr)	30.51g	28.61 g	32.50 g
Aspect of the pollen grain	Ornamental	Ornamental	Ornamental
Shape of the pollen grain	spherical	spherical	spherical
Average length of the pollen grain (µm)	(20-30 µm) 25 µm	(2.6-5.4 µm) 4 µm	(25.2-34.8 µm) 30 µm
Average width of the pollen grain (µm)	(15-25 µm) 20 µm	(8.4-11.6 µm) 10 µm	(24-28 µm) 26 µm
Type of the aperture of pollen grains	Tricolpored (3 sillons + 3 pores)	Tricolpored (3 sillons + 3 pores)	Tricolpored (3 sillons + 3 pores)
Aperture length of the pollen grain (µm)	(18-28 µm) 23 µm	(2.4-3.6 µm) 3 µm	(23.2-32.8 µm) 28 µm

the height and the width of plants, the color and the aspect of branches, length and thickness of between-knots, color of the reproductive device, number of stamens and stigmas, color and length of fruits, length of hairs covering fruits, the date of fruition and the one thousand seed mass (Table 2 and Figure 2 to 11).

Old branch color is whitish at *C. azel* and *C. comosum*, whereas it is reddish at *C. arich* (Figure 2). Measurements of the plant, length, height and width, are different between the three species: *C. arich* is the

Figure 2. Color of the aged branches of the three *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c).

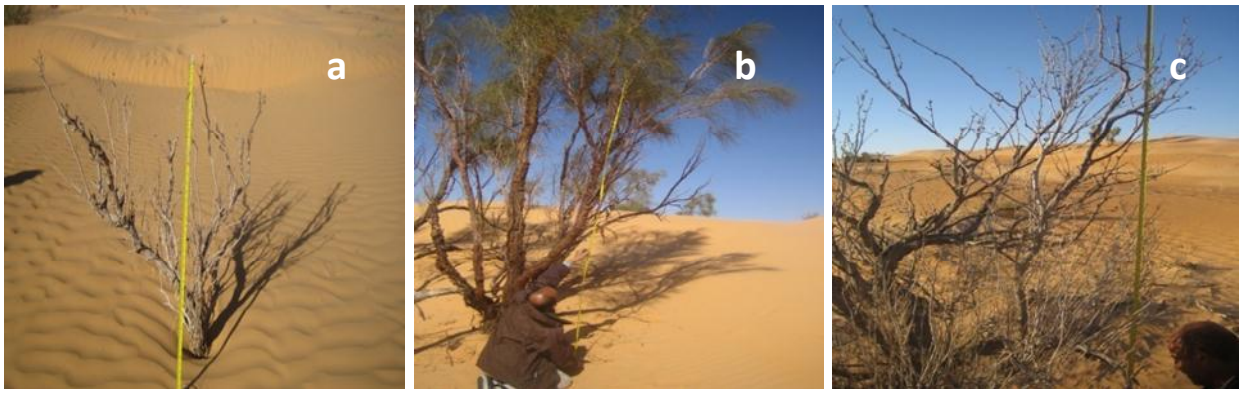

Figure 3. Length and width of the three *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c).

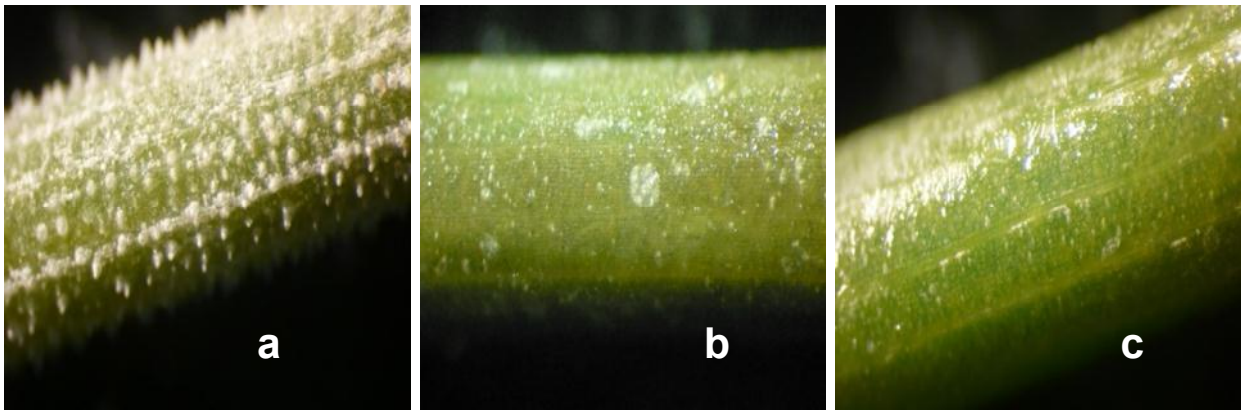

Figure 4. The aspect of the young branches of the three *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c).

biggest and largest while *C. comosum* is the shortest and smallest (Figure 3). The green stems of *C. azel* and *C. arich* are smooth with a length of inter-nodes that reaches 9.6 and 12.8 cm, respectively whereas the green stems of *C. comosum* are rough with a length of inter-nodes that reaches 6.8 cm (Figure 4). Nodes of *C. arich* and *C. azel*

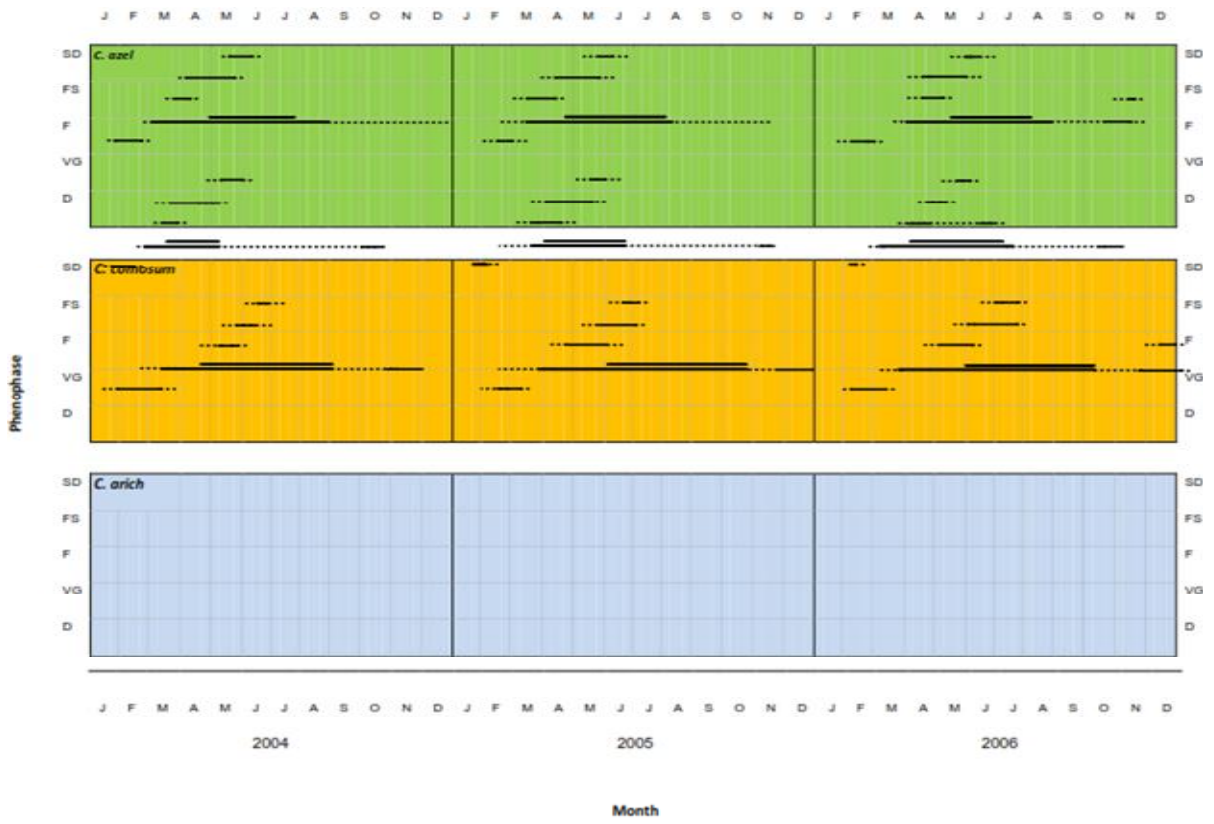

Figure 5. Phenological diagrams of the study species for the study period. Phenophase nomenclature follows (Table 2). The levels of frequency in the population are indicated by double line (level 1); single line (level 2); and broken line (level 3).

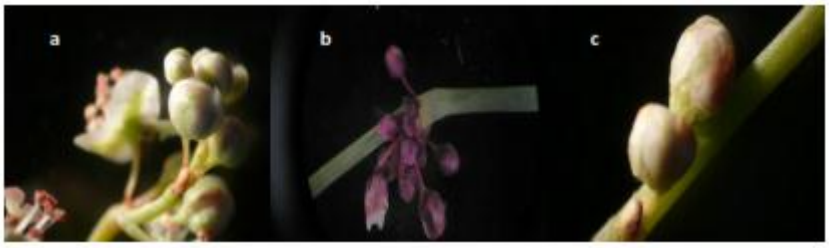

Figure 6. Size and color of the floral buttons of the three *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c).

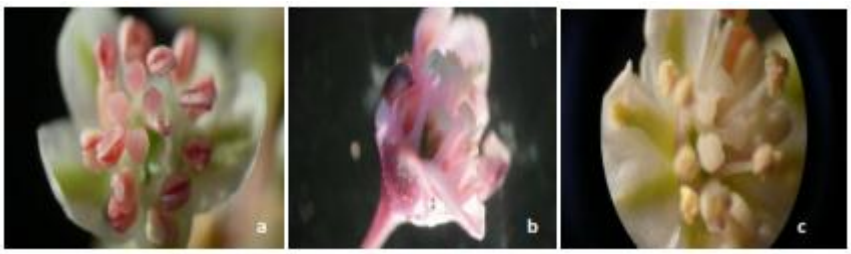

Figure 7. The color of flowers of the three *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c).

Figure 8. Stigmats* and anthers**of the three *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c).

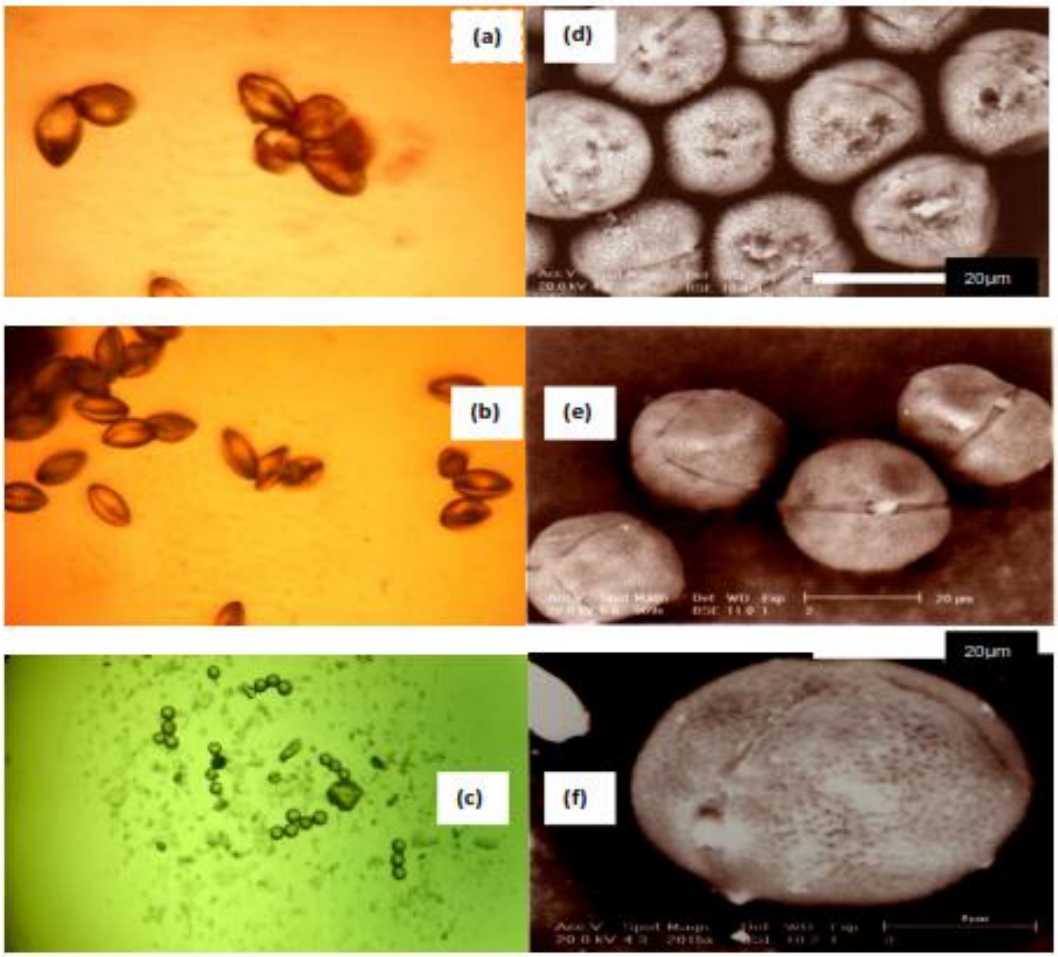

Figure 9. Types of grains of pollen of the three *Calligonum* species observed to microscope photonique(a,b and c) and the electronic microscope to sweep (d,e and f): *C. comosum* (a,d), *C. arich* (b,e), *C. azel* (c,f).

Figure 10. Length and color of the seeds of the three *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c).

Figure 11. Length of hairs regaining the seeds of the three *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c).

are generally bulged (0.1 to 0.45 cm of width) while knots of *C. comosum* are slightly big. Generally, for the three *Calligonum* species, fruition generally follows flowering two weeks later. The date of flowering for *C. azel* and *C. comosum* is recorded in March and is generally baffled for one month for *C. arich* (Figure 5 and Tables 3 and 4).

- Flowrey button diameter varies between a minimum of 0.8 mm at *C. arich* and a maximum of 2.6mm at *C. azel* (Figure 6).
- Color of the flower is white for *C. azel* and *C. comosum* and red for *C. arich* (Figure 7).
- Flower of the three species possesses the same number of stigmas (4) but the number of stamens is different: (12 for *C. comosum*, 16 for *C. azel* and 14 for *C. arich*) (Figure 8).
- Stigmas and the anthers of *C. arich* and *C. azel* are white while *C. comosum* possesses red anthers and pink stigmas (Figure 8).
- Grains of pollen are spherical, symmetrical and isopolar at *C. azel* and *C. comosum*, whereas they are oval, unipolar at *C. arich*. They have three furrows that lie down toward poles at *C. azel* and *C. comosum*, whereas they are only one aperture at *C. arich*. The surface of the pollen grain presents ornaments (sculpturate) at *C. azel* and *C. comosum*, whereas it is smooth at *C. arich* (fig.9). The length and the most elevated middle width of pollen grains exist at *C. azel* (30 and 26 µms) respectively, whereas those weakest exist at *C. arich* (15 and 10 µms) respectively.
- Length and the width of the grain of the pollen in the same way the length of the furrow doesn't defer a lot between *C. azel* and *C. comosum* (Figure 9). *C. comosum* and *C. azel* possess tricolporate pollen (three furrows and three pores), rough of length that varies between (20 and 35µm) and possess three symmetric apertures. *C. arich* possesses tricolporate pollen and length that varies between (2 and 6 µm) (Figure 9).
- Length of fruits of all species is roughly a centimeter with a brown color for *C. azel* and *C. comosum* and a purplish color for *C. arich* (Figure 10). *C. arich* presents the middle mass of the weakest 1000 seeds (28.61 g), whereas *C. azel* presents the most elevated mass (32.50 g). All fruits are rectangular with the longitudinal lines of

Table 3. Anatomical traits studied of the three *Calligonum* species.

Species / Anatomical traits	*C. comosum*	*C. arich*	*C. azel*
Thickness of the cuticule	thick	Thin	Thicker
Nature of the cuticule	No streaky	streaked	No streaky
Shape of wax	In shape of needles	Absent	In shape of needles
Nature of secretions	Mealy secretions	Mealy secretions	Mealy secretions
Texture of stings	Cylindrical	Cylindrical	Cylindrical
secretriced pockets	Present (in relief)	absent	absent
stomata types	Anomocytic Stomata, long and driven	Anomocytic Stomata, long and driven	Anomocytic Stomata, long and driven
External aspect of cells of the anther	Thumbed smooth	Bent streaky	Thumbed smooth
Presence of wax	present	absent	present
Shape of the epidermal cells	cubic	cubic	cubic
stomata types	anomocytic	anomocytic	anomocytic
wood vessels (xylem)	spiraled vessels	spiraled vessels	spiraled vessels

Table 4. Phenological events considered.

Phenophase	Description
D	Disbursement
VG	Vegetative growth: expanding leaves and/or internodes
FS	Fruit setting: green fruits visible to the naked eye.
F	Flowering: appearance of open flowers
SD	Seed dispersal: dispersal of fruits, seeds or parts of infrutescences

Figure 12. Transverse cuts at the level of the seeds of the three *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel*(c).

hairs that have a length roughly 3 mm (Figure 10). The fruit of *C. comosum* is a brown color achene and enveloped of a very hard tegument, of 1à 2 cm, having in transverse cut the form of a cross of which every top carries two rows of hairs. The achene of *C. azel* possesses flattened 4 large coasts, not twisted in spiral, carrying 12-16 sets of hairs (bristles), and separated by 4 narrow, barefaced and smooth, visible vallecula only after the abduction of silks (Figures 11 and 12). The achene of *C. arich* carries 4 large coasts not twisted, carrying on their margins 8 sets of hairs and separated by the narrow vallecula (Figures 11 and 12).

The degree of similarity between the three species also studied through the morphological traits. The gotten dendrogramm showed two groups (Figure 13). The first is

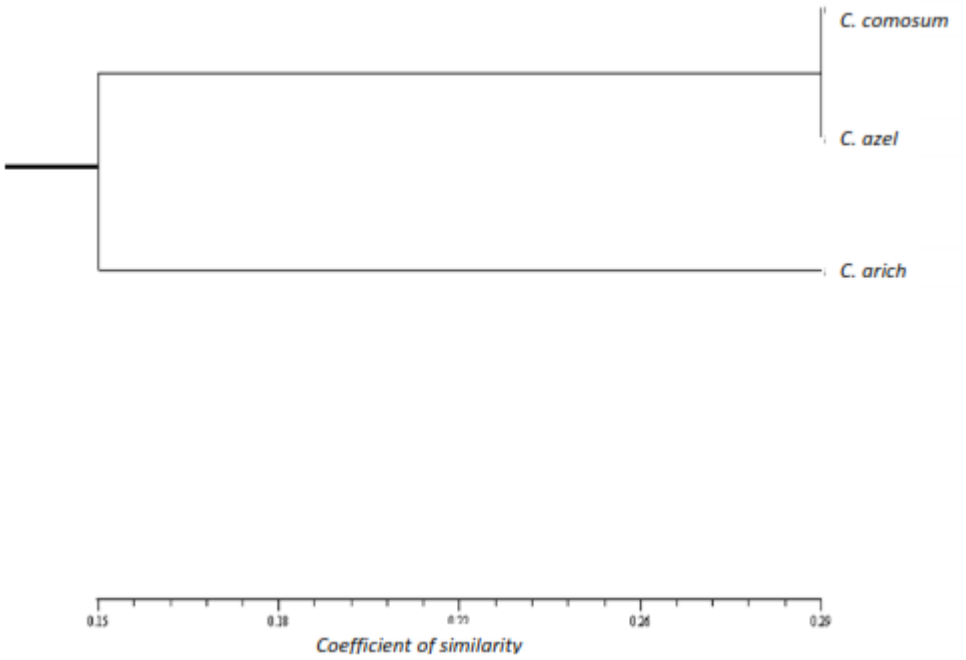

Figure 13. Cluster analysis of the three *Calligonum* species: dendrogram generated by the cluster analysis of morphological identified descriptors usingñWPGMAòmethod.

composed by *C. Comosum* and *C. azel* with a similarity of 29% in the morphological traits. The second is formed by C. arich with a likeness of 15% with the first group.

Anatomical variability at the three *Calligonum* species

The anatomical characters of the three *Calligonum* species have been recorded in (Figures 14 to 17).

The main stomatal types observed using an optic microscope at the three species of *Calligonum* are anomocytic (Figure 14). The epidermal cells of *C. comosum* and *C. azel*, present a film of no streaky and thick cutine or cuticle. This cuticle papers the external face of the partition that is in contact with the outside middle and that is doubled of waxy deposits (Figure 15). The epidermal cells are diametric with the whitish secretions (Figure 15).

The observation to the electronic microscope to sweep showed that anthers of the three species are connected to filaments of different positions (Figure 16). The observation to the electronic microscope to sweep showed that the surface of anthers is bent (formed of several curlings) at *C. arich*, whereas it is thumbed at the two other species (Figure 16).

The electronic microscope showed that stomata are long, driven and surrounded by edges.

The observation to the electronic microscope to sweep (MEB) showed that the external surface of the green branches of the three species is covered by the whitish and mealy secretions. These secretions are very dense at *C. comosum* what gives it the whitish aspect, but weakly dense at the two other species of *Calligonum.* The excretion of these whitish substances has been done by the excretory pockets at *C. comosum* and by stomata at *C. azel* and *C. arich* (Figure 17).

The observation to the electronic microscope to sweep showed that wood vessels (xylem) of the three *Calligonum* species are spirally. Diameters of these vessels are (38 µm at *C. arich*, 39 µm at *C. azel* and 28 µm at *C. comosum*.

DISCUSSION

The morphological and anatomical differences observed at the three species of *Calligonum* can be due to the ecological conditions of the origin site as well as to the adaptive characters acquired by every species facing the stressful conditions of the life area. The effect of the ecological environment on phenotypic characters of spontaneous plants was, since long times, a primordial objective. These adaptative characters are due to the plant phenotypic plasticity which is the essential response against environmental changes. Measurements of the plant, length, height and width, are different between the three species. *C. azel* presents the most elevated mass of the weakest 1000 seed+s. Urs and al. (2012) show that the relationship between seed size and fitness in

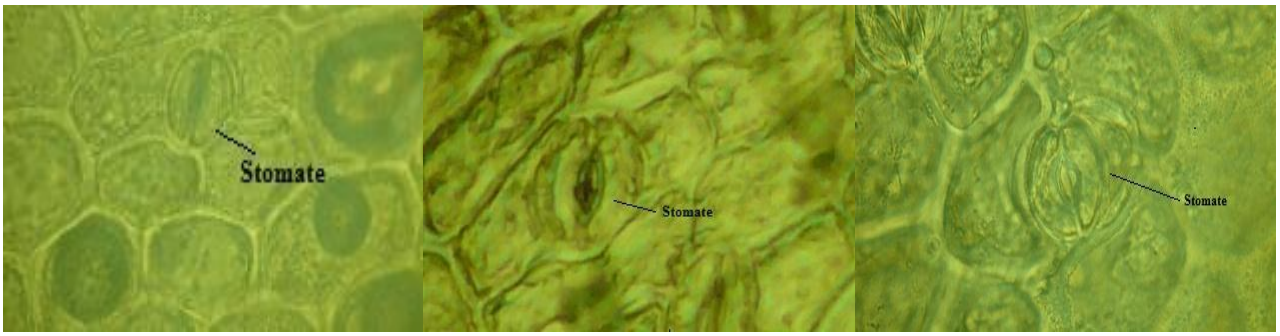

Figure 14. Stomatal types of the green branches of the three *Calliogonum* species observed to the optic microscope (Gx400) : *C. comosum* (a), *C. arich* (b), *C. azel*(c).

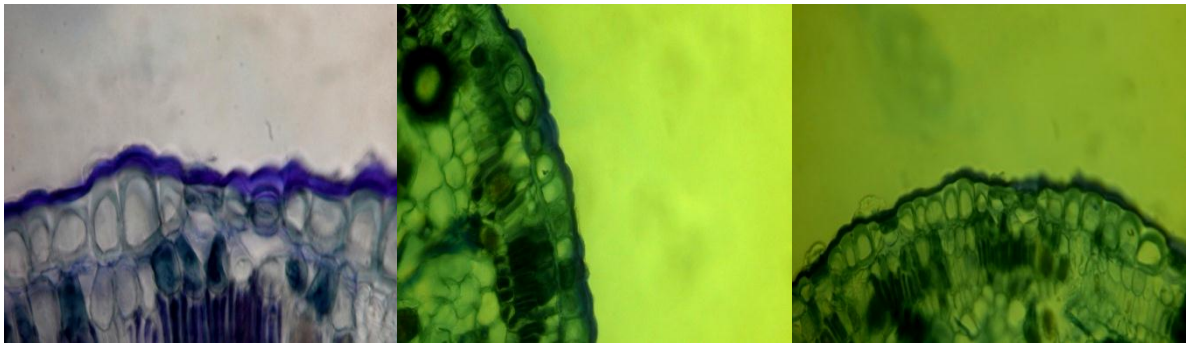

Figure 15. Transversal section of the green branches of the three *Calligonum* species : *C.comosum* (a), *C.arich* (b) and *C.azel* (c).

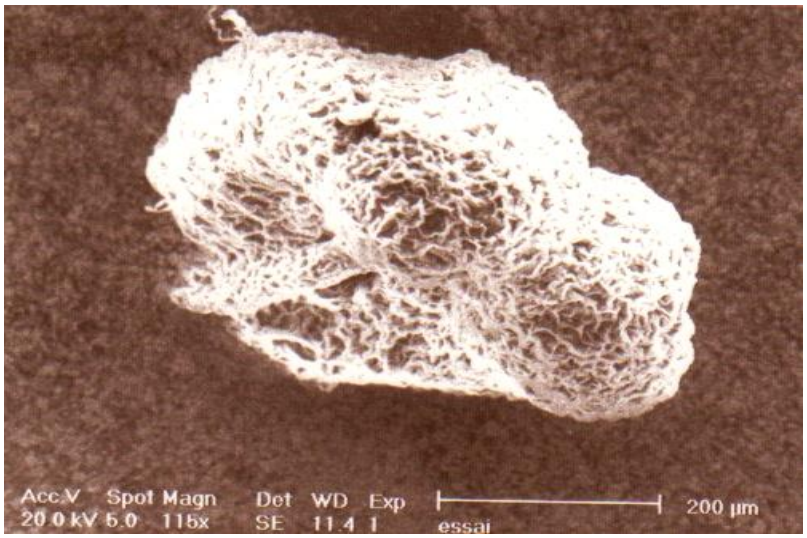

Figure 16. Anther of *Calligonum arich* observed at MEB.

plants may depend on offspring density, especially in cases where seed size affects the outcome of competition. The same authors show that there is relationship between seed mass, germination, intraspecific competition length, height and width on three European white oak species (*Quercus robur*, *Quercus petraea* and *Quercus pubescens*). Shaltout and al. (1989) show that the morphological differences characters,

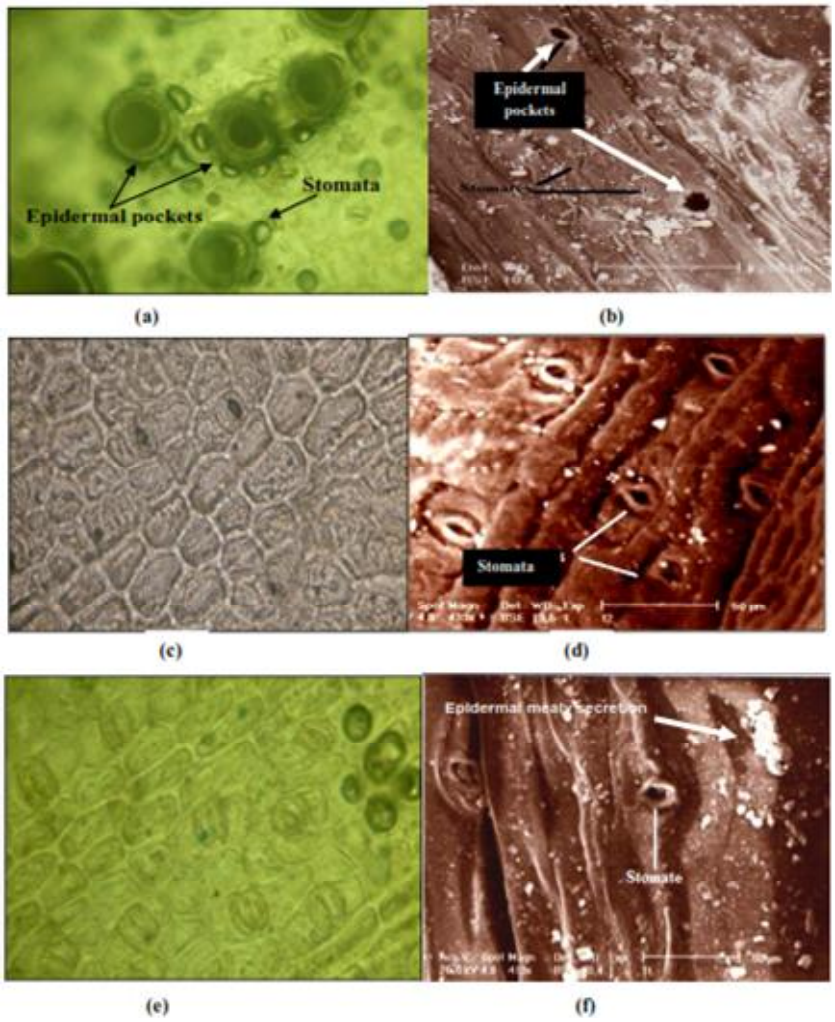

Figure 17. Epidermal secretions of the three *Calligonum* species: *C. comosum* (a,b), *C. arich* (c,d), *C. azel*(e,f).

especially those of leaf structures, at *Thymelaea hirsute* (L.) Endl., species occupying different environmental surroundings in many regions of Egypt are related to the aridity degree and the CaCO3 soil content. Svetlana and al. (2010) show that All recorded differences in morphological and anatomical structure of the Tansy (*Tanacetum vulgare* L.) from two specific locations, were caused by differences of cumulative environmental conditions with dominant effects of the contamination degree of the location, soil type and microclimate.

De Wet and Van Wyk (2013) show that leaf morphological characters are required to identify the *A. delagoensis* endemic species. These include the petiole length and venation pattern, lamina shape (including the base and apex), lamina dimensions and pubescence.

Taia and El-Olayan (2003) show that the humidity content, the leaf structural traits as well as the plant size and their vital state can be affected by the different ecological conditions of life surroundings. Taia and El-Ghanem (2004) show that at *Calligonum* comosum, there are no changes at the level of leaves (green branches) especially in the beginning of the growth phase of the plant. It is in agreement with our results, which showed that most affected morphological characters by the change of the ecological areas are the length, the width, the colors and the aspect of the organ. The color of the flower and the fruit can also be affected. It indicates that the sexual reproductive characters (floral) are steadier than the vegetative characters. Lucía and al. (2012) show that in the evolution towards unisexuality, the developmental differences of anther wall tissues and pollen grains between pistillate and staminate flowers might become more pronounced in a derived condition, such as dioecy. Stigmas and the anthers of *C. arich, C. azel* and *C. comosum* are different. Jia and Tao (2012) show that variation in style length and antherï stigma

distance (ASD) can be investigated for identify 18 populations of *Ixiolirion songaricum*. Thomlinson (1984) show that the use of vegetative and morphological characters in the taxonomy of the superior plants is limited to the reproductive morphological characters because of an important innate conservation of these reproductive traits. White (1979) show that the plant structure is formed by an indefinite number of repeated units that named modules. These modules have a very big functional diversity and for this reason the vegetative characters have a big capacity of change. On the other hand the floral reproductive traits have a lot of invariable functions. Taia and El-Ghanem (2004) show that leaf characters can be used in the taxonomy but they always remain of very variable characters. Results from White (1979) and Barthlott (1984) show that the microstructural and morphological characters of seeds provide important and precious taxonomic information. In our survey the morphological and structural characters of the fruit (color, size, shape and disposition of hairs) provide also very important taxonomic information at *Calligonum* genus. The arrangements of the plant epidermal cells are also steady. The only variable thing is the wax deposition that can be caused by the climatic and ecological factors. The wax density that is absent at *Calligonum arich*, can be due to the root system that is able to exploit water that is in the most humid and deep layers and to an important photosynthetic activity explained by elevated stomata density for this species (Dhief and al., 2009). The differences in the pollen size show that the pollination regime at these three *Calligonum* species is the autogamy. However, the allogamy regime is very rare and accidental. Joana and Juan (2012) show that *Ranunculus weyleri* (Ranunculaceae) is predominantly allogamous. Combinations of both insects (belonging to *Coleoptera*, *Diptera*, *Lepidoptera* and *Hymenoptera*) and wind (ambophily) constitute the main pollination vectors, and pollen limitation was observed. Interpopulation crossing was effective, and exogamic depression was not present, as evidenced by the fecundity levels. Bao and Li (1993) proposed a tribal classification system and postulated an evolutionary framework for Atraphaxideae. Based on the evolution of morphological characters as well as pollen and embryo.

The predation of the floral stems by herbivores (mainly goats) severely limits the reproductive success of this endemic species in wild populations. Li et al. (1998) show that the four genera of Atraphaxideae (*Atraphaxis*, *Calligonum*, *Pteropyrum*, and *Parapteropyrum*) are all shrubs, usually have five petals and a 3-coporate aperture. Most species of the tribe occur over an area including Central and Western Asia, westward to North Africa.

The main stomatal types observed using an optic microscope at the three species of *Calligonum* are anomocytical. Ayodele and Olowokudejo (2006) show that there is a relationship between different stomata types may occur on the same leaf surface and the taxonomy of the polygonaeae family in West Africa. All species of the Polygonaeae family in West Africa, except *Afrobrunnichia erecta* and *H. snowdenii*, are amphistomatic and the family is characterized by a wide range of stomata types such as the anisocytic in *P. plebeium*, cyclocytic in *S. paniculata* and a few *Persicaria,* anomocytic, diacytic, parallelocytic and paracytic which is regarded as the basic type for the family based on its widest occurrence among the species.

There are differences between the three *Calligonum* on the fruit structure. Louis and al. (2000) show that the fruits of Polygonaceae have a basically similar construction of indchiscent nuts or achenes. Sections of fruits, coupled with surface patterns were studied with SEM and LM in all genera of the tribes Persicarieae and Polygoneae (Polygonoidcae-Polvgonaceae). The outer layer of the pericarp is usually thickened and its anatomy can be used consistently to delimit genera more than any other character of the fruit. Fruits of Polygonaceae are invariably indehiscent achenes or nuts formed by three carpels (pyramidal or triangular) or two carpels (lenticular fruits): Rarely, there are four carpels, which is a constant feature for the genus *Calligonum*. The number of styles or stigmatic lobes corresponds with the number of carpels. The fruit is enclosed by the persistent perianth, which may play an important role in the dispersal mechanisms. For example, the tepal lobes can become accrescent and covered with hooks in *Rumex* and *Emex* (Brandbyge, 1993).

According to fruit morphology *Calligonum* has been divided into three sections: *Calligonum* (with bristled fruit), *Pterococcus* (with winged fruit) and *Calliphysa* (with membranous saccate fruit) (Rechinger and Schiman-Czaika 1968).

Recently, Tavakkoli et al. (2008) presented a cladistic analysis of 18 species using 30 morphological characters. The results revealed that *Calligonum* was monophyletic and composed of two clades: one including the winged fruit species (section *Pterococcus*), and the other the bristled fruit taxa (section *Calligonum*).

Ying and al. (2010) show that by examination of *C. pumilum* A. Los. and *C. ruoqiangense* Liou f., collected from natural habitats, and the analysis of their variance, this paper revealed that the morphological characters of these specimens are quite different from each other, especially in the twist direction of ribs on fruits, rows of bristles along each rib, rigidity, interweaving degree of bristles, and their geographical distribution. Therefore, it is suggested that *C. pumilum* and *C. ruoqiangense* should be considered as two independent species.

The anatomical desert plant structures always give an idea concerning their adaptation to meet environmental stresses. In *Calligonum*, in terms of anatomy of young

branches, Mao et al. (1983) considered section *Calliphysa*, identifying *C. junceum* as the most primitive, and section *Medusa* as the most advanced. Lyshede (1977) studied the epidermal and sub-epidermal cellular structure of *Anabasis articulata* and *Calligonum comosum* and found that the partitions of the epidermal cells of the green stem, at the two studied species, inflate themselves quickly during the absorption of water and lose this water slowly in case of dehydration. Slatyer (1967) found that this ecological criteria is especially for the pinaceae family of that has needle laves. According to our observations, the green stem, at *C. comosum* and *C. azel*, is covered with a wax deposit, in addition to an epidermal cuticle that can protect these cells from the dehydration.

The only places that permit the water exchange of stomata and lenticels. Jonsson (1902) show the presence of a great deal of mucilage in the external partitions of *Calligonum sp.* this can be another reason to avoid the water loss because mucilage is able to absorb and to stock water.

All these anatomical characters; the wax disposition, the epidermal cuticle and mucilage in addition to other morphological characters indicate the important adaptation of these species against the aridity of sites where they exist. The whitish epidermal secretions are very raised at *C. comosum*, whereas they are weak at the two other species. Heinricher (1884) and Nobel (1991) show that the long leaf is an advantage for the plant of bright and arid environments. This length permits the scattering luminous radiation that is going to reach all parts of the leaf in the desert environments and on beaches.

Sharaf et al. (1998) found that *C. comosum* has the lowest contents of N, PS, KS and Na but of important contents of Ca, Mg and Mn. The same author noted that contents in Mg, P, S, Cl varies according to the ecological area. It seemed that the three Calligonum species reject content raised of Ca under the shape of Calcium oxalate through the stomatas and the excretory pockets.

The observation to the electronic microscope to sweep showed that wood vessels (xylem) of the three *Calligonum* species have different diameters.

Al-Khalifah and al. (2006) show that extremely narrow vessels arranged in radial files in latewood were recognized having 40% increased volume fraction in stressed plants. This adaptation is believed to play an important role in the species survival during hot summer months.

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Conclusion

From this study, we can say that the three *Calligonum* species are very morphologically and anatomically adapted with the Tunisian arid zones. The morphological characters can be changed with environmental change, but the floral reproductive characters are very steady. However colors are the characters that can be affected with the mineral composition of soils. The anatomical characters that are in relation with the protection against the dessiccation change with stress and especially facing the climatic stress. The degree of similarity among the three species showed two groups: The first is composed by *C. Comosum* and *C. azel* with a similarity of 29% in the morphological an anatomical trait. The second is formed by *C. arich* with a likeness with the first group of 15%.

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