



# ANATOMICAL COMPARATIVE STUDY FOR SOME SPECIES OF SOME GENERA OF TRIBE AGROSTIDEAE (POACEAE) DUM IN IRAQ

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## Abstract

The research in this paper includes Anatomical studies for some genera of the tribe Agrostidea in Iraq, which are: *Agrostis olympica* (Boss.) Bor, *Apera intermedia* Hack, *Gastridium ventricosum* (Gouan) Schinz and Thell.

Anatomical characters of leaves epidermis are studied and the importance of their taxonomies are discussed. Different kinds of cells are recognized in the epidermis, which are long-cells, short cells, stomatal complex and epidermal trichomes. The characteristic features of these cells show an important taxonomic value on both generic and species levels such as *Aperaintermedia*.

Transverse sections of the vegetative organs have been studied and their characters are found to have a taxonomic importance especially in the arrangement and distribution of parenchyma and sclerenchyma tissue and the existence of keel or not. The shape of keel in *Agrostis olympica* has V shape in addition the presence of sclerenchyma tissue in the edge of transvers section of the leaf blade.

**Key words** : Agrostidea, anatomical study, *Agrostis olympica*.

## Introduction

### Historical introduction

The Gramineae family is one of the largest families in the world of flora where researchers differed in their number of species and genus (HUBBARD, 1948).

Stebbins (1972) stressed that it is important to study Gramineae family as a taxonomic and life study in order to shed the light on its taxonomic and evolutionary problems that characterize the genera of this family.

In Iraq, the number of species of this family is constantly increasing because of the good progress in taxonomic studies. Al-Rawi (1964) mentioned that there are (95) genera of the Gramineae family which consist of (250) species. Also, AL-Musawi (1987) reported that the family had (101) genera with (270) species.

The Agrostideae tribe is one of the most unstable tribes among the various plant encyclopedias, some researchers have considered it to be an independent tribe in its own right, while others have classified it as a subtribe

within the Avenaeae tribe. As Clayton (1970) pointed out in the "Encyclopedia of Tropical Plants of East Africa", the Agrostideae species are heterogeneous but share one flowered sapling. The anatomical study consisted of the following types: *Agrostis* L., *Adans Apera* and *P. Bauv. Gastridium*.

The study of the epidermis of the leaves in specifying the types of variability between plant species has led to help in knowing and identifying the species according to the study which has been done by Ogie-Odia *et al.* (2010). Also, Stenglein *et al.* (2003) explained that the most important classification tools for many plants families are the anatomical characteristics, which show leaves epidermis such as stomata, long cells and varying shapes and sizes of silica bodies, as they help in the diagnosis of species.

Since the epidermal cells of the Gramineae family have higher variability than the rest of the plant families (Chaudhari *et al.*, 2014), based on Ogie-Odia *et al.* (2010) and Desai and Raole (2013).

The present study aimed at addressing some anatomical aspects of the epidermis of the lower leaves and cross sections of leaves of some species of the tribe Agrostideae. These types are: *Agrostisolympica* (Boss.) *Bor*, *Apera intermedia* Hack, *Gastridiumventricosum* (Gouan) Schinz and Thell.

## Materials and Methods

The anatomical study included some species of the tribe Agrostideae, the Vegetative organs, based on the leaves. It had been studied the lower epidermis and the transversal sections of the leaves.

### Leaf epidermis

The leaf epidermis was prepared by combining the methods of Clark (1960) and Cutler *et al.* (2007) by taking the second leaf before the flag leaf of the plant then put it in a boiling water and using scrape method by sharp blade.

### Leaf Transversal Section

The leaves were soaked in boiling water and raised directly or placed in water until boiling. Then, the sections were made up by hand with a sharp blade.

### Leaves epidermis

The leaves of the Agrostideae species were characterized by four distinct regions: the Costal zone, the Inter costal zone, the Midvien zone and the margin zone. These characteristics are due to the Festucioid pattern developed by Audulov (1931).

All species under this study share by containing long cells and Stomata, while species varied by containing or not containing short cells, such as silica cells or Cork cells. However, they differed in dimensions, shapes, and numbers of those cells.

### Abaxial epidermis

The study of the lower epidermis of the leaves showed the existence of variations of the long cell dimensions of the species under study, as well as the presence of overlapping in thickness and orientation and pitted. All species shared their long rectangular cells in general and with straight ends.

The study also dealt with the characteristics of the holes in terms of their shape and number and also the lower epidermis of the leaves were characterized by the nature of their surface. On the basis of the taxonomic importance of each of the properties of the lower epidermis, each property will be addressed individually and as follows:

### Long cells

The longitudinal cells were generally rectangle in most of the studied species. The long cells were characterized by fixed straight end of all the long cells in different regions.

The regions of the epidermis were characterized by long cells that had straight and pitted walls for all species under study.

In terms of the quantitative characteristics of the long cells, their lower and upper levels showed heterogeneity and capacity in the rows with and without stomata in general, resulting in isolation at the level of the species under study. Where, they were longer in rows with Stomata in the upper level than the rows with Stomata, especially, in the lower part of the region between the veins.

In the species *Ap. intermedia* and *Ga. ventricosum*, they recorded (500  $\mu\text{m}$ ) and (950  $\mu\text{m}$ ), respectively. For the lower levels, they were recorded as (110  $\mu\text{m}$ ) and (160  $\mu\text{m}$ ), respectively. Whereas in the rows with Stomata, the first one was recorded with (357.5 – 65  $\mu\text{m}$ ) and the second one was recorded with (265 – 22.5  $\mu\text{m}$ ) in their lower and upper bound.

For the *Ag. olympica* species, they were recorded with high range for the long cells in the rows with Stomata in the upper bound, which it was recorded with (777.5  $\mu\text{m}$ ) and (17.5  $\mu\text{m}$ ) for the lower bound. In the rows with Stomata, it was recorded with (287 – 92.5  $\mu\text{m}$ ). It was noted that the type *Ga. ventricosum* recorded high long in the rows with Stomata also in capacity of the levels, so it provided taxonomic important. In the number of rows of the long cells in the region between veins, the type *Ag. olympica* recorded higher number (20-6) class, while *Ga. ventricosum* recorded lower number (7-2) class. The type *Ap. intermea* recorded level between that, where it recorded higher number (20-6). In the case of the number of long cells rows in the middle-vein region, it was (1-2), (1-6) and (1-5) respectively according to the types which mentioned above. In the middle-vein region, the number of long cells rows of the type *Ap. intermedia* was high and recorded (2-7) class as shown in table 1.

### Stomata

The stomata are present in regular or irregular rows distribution. The stomata are arranged sequentially, so that the species under study separate one long cell that varies in length between a gap and a gap. The rows with stomata were significantly different in terms of their quantitative characteristics related to the lengths of the gaps and their number, despite the overlap and

**Table 1<sup>1</sup>** : Quantities of long cells in the lower epidermis of the *Ag.rostideae* species for the tribe under study.

Species	Long cell length in the rows with Stomata (µm)	Long cell length in the rows without Stomata (µm)	The number of long cells in the area between the veins	The number of long cells in the area of veins	The number of long cells in the middle vein region	The number of long cells in the edge region
<i>Ag.olmpicaa</i>	(777.5-17.5)395.5	(387-92.5)189.75	(20-6)13	(6-1)4	(3-2)3	(3-2)3
<i>Ap.intermedia</i>	(357.5-65)164.5	(500-110)224.5	(12-2)6	(5-1)3	(7-2)5	(3-1)2
<i>Ga.ventricosum</i>	(265-22.5)143.75	(950-160)555	(7-2)5	(2-1)2	(3-1)2	(3-1)2

<sup>1</sup>The numbers between the brackets represent the lower and upper bounds and the numbers outside the brackets represent the average.

**Table 2<sup>\*</sup>** : Characteristics of the Stomata in the lower epidermis of the Agrostideae species under the study.

Types	Length of the stomata (µm)	Number of rows with stomata between two veins	The number of rows separating the rows with Stomata	Number of stomata in the microscopic field	Shapes of Stomata
<i>Ag.olmpicaa</i>	(42.5-20)34	(4-1)2	(9-1)6	(20-5)13	Paralleled – Low flat
<i>Ap.intermedia</i>	(60-25)46.9	(4-1)2	(6-1)3	(18-2)9	Paralleled – High flat
<i>Ga.ventricosum</i>	(55-35)45	(8-1)5	(3-2)3	(16-1)9	Paralleled – Low flat

convergence in their lower and upper ranges. At the species level, the *Ap. intermedia* recorded the highest length (60-25 µm) while the *Ag. olmpicaa* was less than (20-42.5 µm), while the *Ga. v entricosum* (35-55 im).

As for the number of rows with Stomata between the two veins, the same type recorded the highest number (1 - 8) row, as shown in table 2. While, *Ap. intermedia* and *Ag. olmpicaa* have recorded (1-4) row, as shown in table 2. The number of rows that separating rows with stomata are as follows:

*Ag. olmpicaa* recorded the highest number of (1-9) row and the *Ap. intermedia* type recorded ranges between that (6-1) row between rows that separating the rows with stomata and recorded (18-2) gap in the field of microscopy.

### Indumentum

The superficial clothes of the lower epidermis is represented by prickles only of various types, which usually originate from short cells. The thorns are composed of three types (plate 1). The first known as small hooks. The second type has median thorns with the oval or semi-circular base. The third type has large thorns or bare and characterized by the fact that their base is oval or rectangular long or non-elongated and very thick.

In terms of the dimensions of the structural thorns

and their taxonomic role of isolation and separation at the species level, all the species under study showed a state of overlapping, similarities and convergence in their lower and upper ranges. *Ap. intermedia* and *Ag. olmpicaa* were similar in the upper length of the thorns, where they recorded of (42.5 µm). Whereas, their lower ranges were (10 µm) and (12.5 µm) respectively (table 3).

### Transversal Sections of leaves

The cross sectional of the studied species of the *Agrostideae* tribe are similar in appearance to the epidermis, the mediocre tissue and the vascular tissues but vary in their quantitative and qualitative characteristics. This was evident at the level of species under study.

The cross sectional of the two types *Ap.intermedia* and *Ga.ventricosum* were shown as U (plate 2) and the other was irregular but this did not prevent variation in the general shapes (Fig. 1, plate 2). The anatomical characteristics of cross-sectional papers of taxonomic importance at the species and species level are the forms of Keel. Several cases were observed:

1. The keel is not distinct clearly, where this state was observed in type *Ag. olympica*.

2. The keel had V-shape where this form is widespread in *Ag. olympica*, which shows that this variance is less common in the shape and has a clear

**Table 3<sup>\*</sup>:** The quantitative characteristics of the Indumentum of the lower epidermis of the Agrostideae species that are under study.

Types	Length of prickle (µm)	Number of prickle	Length of edge's prickle (µm)
<i>Ag.olympicaa</i>	(42.5-12.5)30	(12-1)7	(35-5)25
<i>Ap.intermedia</i>	(42.5-10)22.1	(1-15)8	(47.5-10)25
<i>Ga.ventricosum</i>	(30-25)21.25	(18-2)10	(30-7.5)18.75

<sup>1</sup>The numbers between the brackets represent the lower and upper bounds and the numbers outside the brackets represent the average.

**Table 4 :** Quantitative characteristics of transversal sections of the agrostideae species are under the study.

The species	Number of Grooves	The width of the blade in the middle vein area (µm)	Blade thickness in middle vein bundle (µm)	Blade thickness above middle vein bundle (µm)	Blade thickness in the nearest bundle of the vein middle pack (µm)	The width of the Sclerenchyma tissue at the edge (µm)	Number of the motor cells
<i>Ag.olympica</i>	(10-9)10	(275-200)245.8	(212.5-150)193.3	(55-27.5)44	(162.5-125)152	(37.5-25)30	6-35
<i>Ap.intermedia</i>	(28-14)23	(250-225)237.5	(350-225)285.4	(125-75)95.8	(265-125)202.5	(87.5-25)53.8	6-23
<i>Ga.ventricosum</i>	(11-11)11	(222.5-212)217.5	(220-215)217.5	(45-45)45	(137.5-137.5)137.5	(37.5-25)312.5	5-34

taxonomic significance for separation the types at the species level (Table 7 and plate 2).

3. The Keel has U-shape. This is the most studied species, as it is recorded in both *Ap. intermedia* and *Ga.ventricosum*.

As for the forms of the middle rib on the middle vein, the flat shape of *Ap. intermedia* was observed with respect to the quantitative characteristics of the leaf cuttings. As for the number of grooves, it was found that they had characteristics that could be used for species separation. In the case of *Ap. intermedia*, (28) groove while the minimum number type *Ag. olympica* was (9) groove which enhances the taxonomic importance of this characteristic (table 4).

As for the thickness of the blade in the middle sweat area showed variations to be used, *Ap. intermedia* recorded the highest value at the upper limit of (350). Other quantitative characteristics were the blade thickness above the middle vein and other quantitative qualities. *Ap. intermedia* was distinct and isolated in a clear way from the other two types under study. The thickness of the blade at the nearest bundle and the width of the blade at the edge, as well as the width of the sclerenchyma tissue at the edge were (265) and (25-87 µm) (Table 4).

The tissues included in cross-sectional passages include:

#### Epidermis tissue

The epidermis tissue is characterized by heterozygous cells, which are usually individualized. In the case of the number of lower epidermal cells below the middle vein

pack, the differences between the species were observed. That was confirmed the state of isolation between the species under study.

The type of *Ga.ventricosum* was less than (7-7 µm), but *Ap. intermedia* recorded the highest value (6- 20) (7-22 µm) of epidermis cells above the width of the middle vein.

#### Mesophyll

The middle tissue of the *Agrostideae* genus has a homogenous tissue in its components, which consists of the Parenchyma tissue of the photosynthesis. The cells of this region are distributed in two regions:

1. The first area: is an outer encapsulation (chlorenchymatoyus) around the vascular bundles sheath.
2. The second area is represented by the cells that occupy the bulk of the leaf tissue.

As for the quantitative and qualitative characteristics of the Chlorenchyma encapsulation cells around the middle vein pack, *Ap.intermedia* and *Ag. olympica* were observed with the highest number of Chlorenchyma cells around the primary pack for the upper limits which were 14 - 15, respectively.

#### Sclernchyma tissue

The Sclernchyma tissue consists of small cells so thick that the vacuum inside the cell appears very small, circular, angular or irregular, especially in cells far away from the beam and close to epidermis (plate 2). As for the Sclernchyma texture at the edge, it is organized into a fibrous cap as it appeared as follows:

1. Pointed cap and noted in type *Ap.intermedia*.

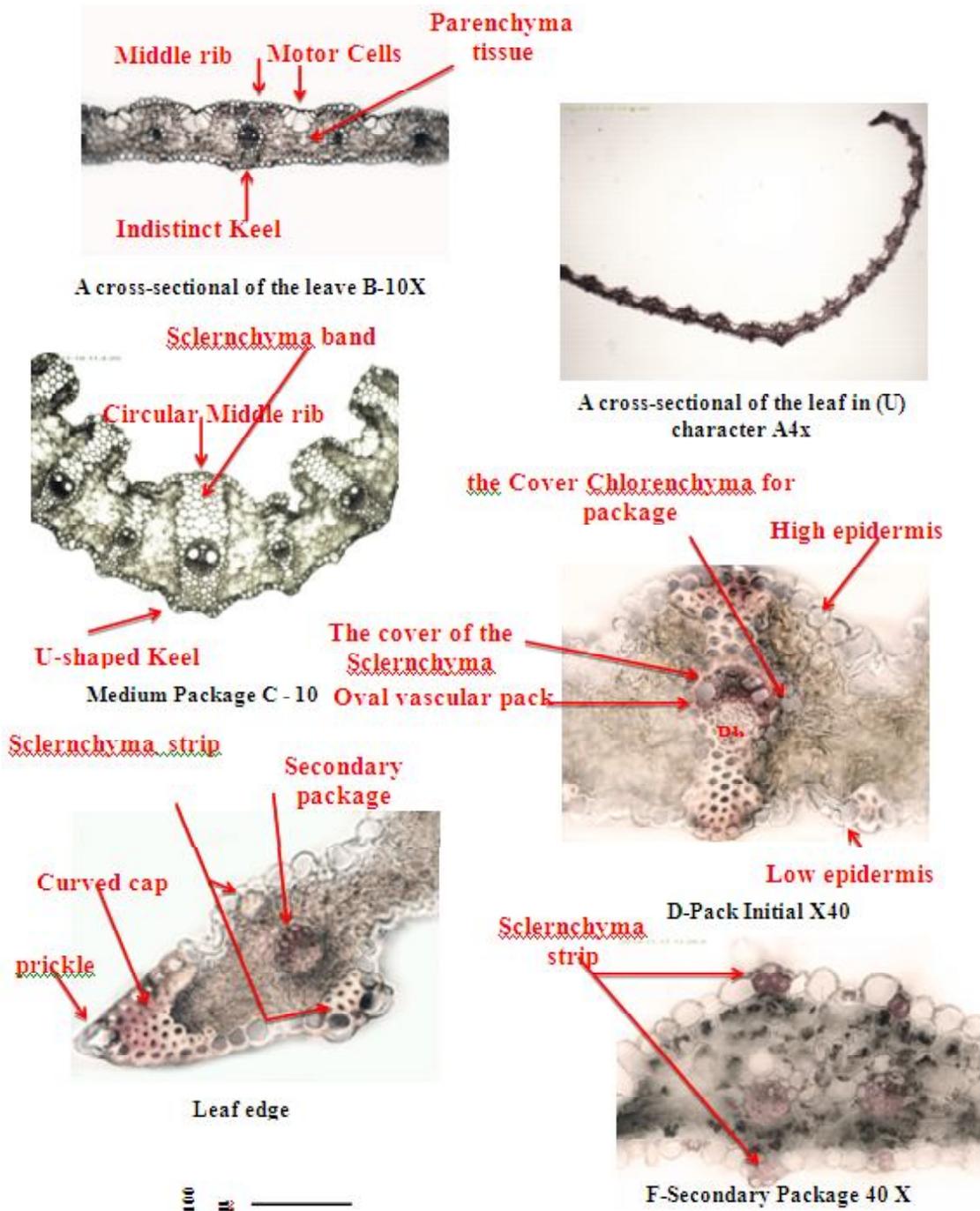


Fig. 1 : Part of the cross section is the leaf, the vascular bundles, and the tissues that make up it.

2. Rounded cap and observed in the three species.

With regard to the quantitative characteristics of the Sclernchyma tissue, it was observed that the number of cells of the Sclernchyma tissue below the median bundle was clearly different. At the edges, the type *Ap. intermedia* was isolated and scored the highest limit (125) (plate 2).

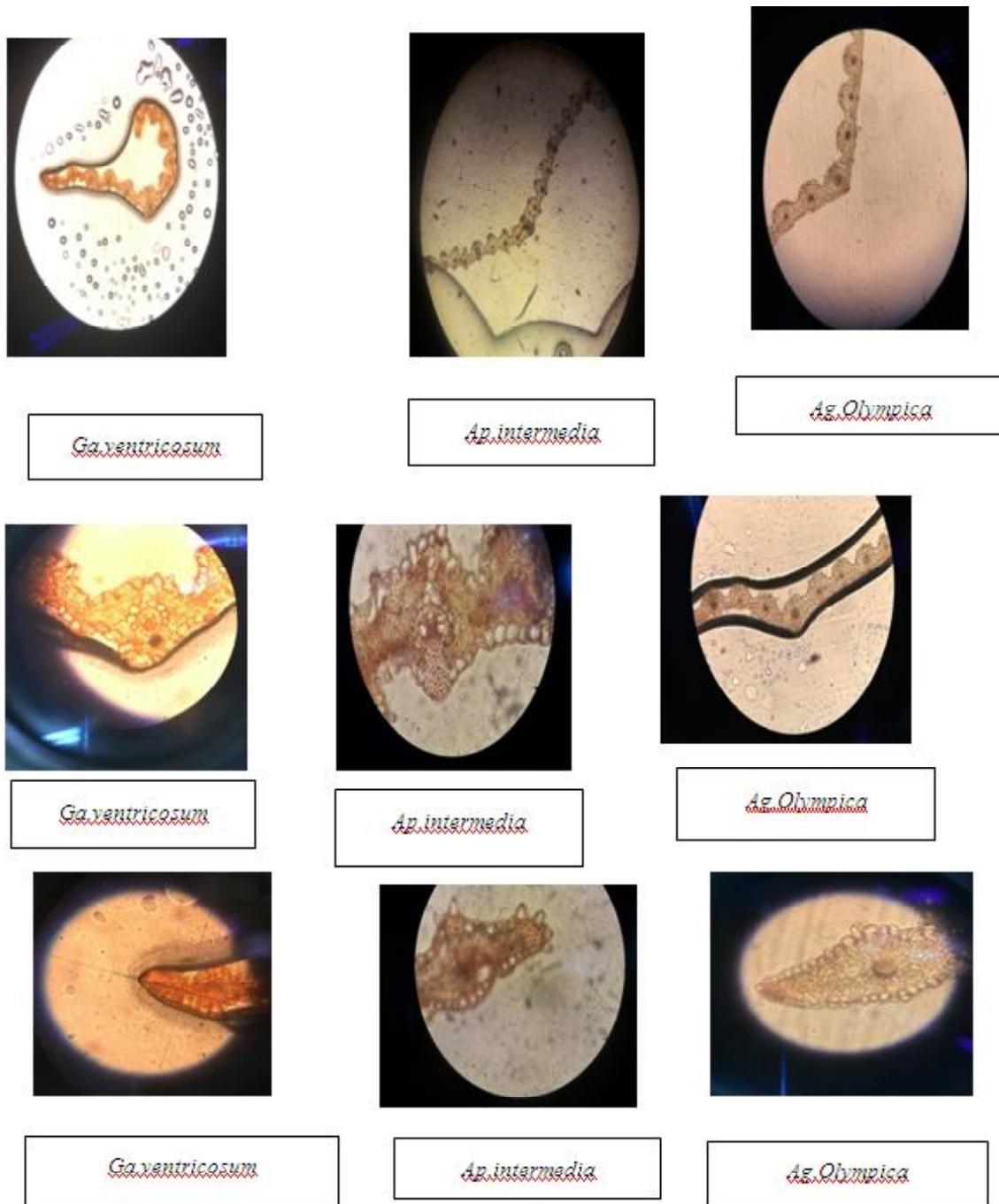
**Vascular bundles**

The cross sectional of the leaves of the Agrostideae

species were characterized by two types of vascular bundles, which differed in size and varied in quantitative and qualitative characteristics, as follows:

1. First-order vascular bundles: Vascular beams are larger in size and more numerous. They are characterized by the distinction of their wood to the first wood and the next wood, as well as the existence of the Sclernchyma beams above or below it.

Second – order vascular bundles: These packs are



**Fig. 2 :** Cross-sections of the leaves under study (X40 and X10).

made up of two easy-to-observe areas; wood and bark, and it is often difficult to distinguish them from the third beam. The oval forms of *Ap. intermedia* and the circular shape of all species (Table 6).

### Discussion

The anatomical study, which included some species of the family Agrostideae recorded many quantitative and qualitative characteristics of the taxonomic importance at the level of species, including the study of the epidermis

of the vegetative parts and the area between the veins were also divided on the basis of the presence of gaps in rows or not.

This was explained by Cutler *et al.* (2007) and (AL – Na'amani, 2012). The arrangement of the stomata complexes in parallel with the longitudinal axis of the leg in stomata rows.

### Epidermis leaf

The leaves have a significant variation based on their internal structure of their cell types, whether long or short,

**Table 5\*:** Quantitative characteristics of the middle vein bands in cross-sectional leaves of the Agrostideae species are under study.

Types	Diameter of middle bundle (µm)	Diameter of the meta xylem vessels in the middle vein (µm)	The number abaxial cells below the middle vein	The number abaxial cells below the middle vein	Number of Chlorenchyma envelope cells around the middle vein
<i>Ag.olmpica</i>	(87.5-57.5)66.6	(17.5-7.5)13.2	(12-10)11	(8-5)6	(16-10)12
<i>Ap.intermedia</i>	(125-75)97.5	(25-15)20.4	(20-6)12	(22-7)12	(16-10)13
<i>Ga.ventricosum</i>	(57.5-35)46.25	(12.5-7.5)10.8	(7.7)7	(8-6)7	(14-9)11

**Table 6 :** Qualitative characteristics of vascular bundles and Sclernchyma tissue at the edge of cross-sectional leaves of the Agrostideae species under study.

Types	Shapes of the middle vein packages			Primary bundle forms			Secondary bundle forms		Forms of Sclernchyma tissue at the edge	
	Oval	Circular	Elliptical	Oval	Circular	Elliptical	Oval	Circular	Rounded cap	Pointed cap
<i>Ag.olmpica</i>	+			+				+	+	
<i>Ap.intermedia</i>	+	+	+	+	+	+	+	+	+	+
<i>Ga.ventricosum</i>	+				+			+	+	

and stomata complexities of their helper cells as well as the human plaques.

Therefore, the study of the Epidermis of the leaves helps to identify patterns of variability in plant species, which helps to identify or identify species, according to Ogie-Odia *et al.* (2010). In spite of the study, Cutler *et al.* (2007), the epidermis leaf distinct for two types of cells, they are long cells and short cells. It is not possible to confuse between them.

In terms of quantitative characteristics of the longitudinal cells their upper extremities showed heterogeneity in the rows with and without stomata in general. That is resulting in isolation at the level of the species under study. They are longer in rows without stomata in the upper limits than in the lower limits of the rows with stomata, as in the type *Ap. intermedia* and *Ga.ventricosum* types where each recorded (500) µm and (950) µm, respectively. For the minimum limits were (110 µm) (160 µm) respectively. In the rows with stomata, the first recorded (35-357.5) and the second (265-22.5) in their upper and lower limits (table 1).

In regarding stomata, many studies (Baser *et al.*, 2010) and Al-Na'amani (2009) have emphasized the adoption of stomata in the epidermis of leaves as an important classification key, leading to accurate diagnosis.

Many researchers, AL-Bermani (1991), AL- Husaini (1999) and Stenglein *et al.* (2003) depended on what (Metcalf, 1960) had noted that the classification of species of stomata has a taxonomic significance in the

diagnosis and isolation at the species or species level in the Gramineae family.

This study showed that the stomata of the leaves of the species of Amphistomatic leaf, which related to the long cell rows in the area between the veins in the lower epidermis was observed overlap between the dimensions of this stomata between the studied species. The areas of the epidermis were characterized by long cells with straight and pitted walls of all species under study.

#### Cross-sectional of the leaves

Many researchers have relied on these cross-sectional passages to separate the types of species they studied, according to Ellis (1976). Most of the transverse sections of most of the sections were U-shaped in line with Abbasi *et al.* (2010). During his study of the stomata of Puccinelliadolicholeps, the transverse sections of the two types A intermedia and *Ga.ventricosum* were shown as U (plate 2). And the other was irregular but this did not prevent the existence of variation in the general shapes (plate 2).

As for the number of grooves, it was found that they had characteristics that could be used in species separation. In the case of *Ap. intermedia*, it was distinguished from the rest of the species with a maximum of 28 grooves, while *Ag.olympica* had a minimum of 9 grooves.

The U-shaped of the keel appeared to be more common among the species under study, consistent with the results of a study (Bell *et al.*, 2012) for type

Eragrostis obtusa flora, so this diversity in the forms of keels among the species under study strengthened the taxonomic value of this status as an important taxonomic and separator between species. The following forms were observed :

1. Keel not really distinct which was observed in *Ag. olympica*
2. V-shape keel: this form was widespread in *Ag. olympica*, which shows that this variance is less widespread in shape and has a clear taxonomic significance for separation at species and species level (plate 2).
3. U-shaped keel: This is the most studied species since it was recorded in both *Ap. intermedia* and *Ga. ventricosum*.

The width and thickness of the central vein region showed a marked variation between species, *Ap. intermedia* recorded the highest value in the upper limit of 350  $\mu\text{m}$  (table 4).

Other quantitative traits were the thickness of the blade above the middle vein and other quantitative traits. *Ap. intermedia* was distinct and distinctly isolated from the two species under study.

The highest values at the upper limit of the traits were recorded at the nearest bundle and width of the blade at the edge, as well as the number of motor cells at 265 and (5-87  $\mu\text{m}$ ) in line with the results of AL- Bermani (1991), Rafash (2006), AL – Na'amani (2012) and AL-Khafaji (2015), who emphasized the taxonomic importance of some quantitative characteristics including the thickness of the blade above and below the package of the middle race and the thickness of the blade at the nearest package.

In the case of the number of lower epidermal cells below the middle vein. *Ga. ventricosum* recorded a minimum value of (7-7  $\mu\text{m}$ ), but *Ap. intermedia* recorded the highest value (6-20) (7-22  $\mu\text{m}$ ). The number of epidermal cells at the top of the central vein package, while the other two types of scales were introduced (table 5).

The average texture of the Agrostideae genus is homogenous in its components, consisting of a Parenchyma tissue. The green tissue of the leaf is the main component of the tissue, which processes photosynthesis. The cells of this region are divided into two regions:

1. The first region / is encapsulated (Chlorenchymatoyus) around the outer bundle sheath, which is complete in most of the secondary beams

and some of the initial beams of the species under study and incomplete from the upper and lower epidermis, or only the lower epidermis, where it is cut with a Sclernchymabelts (2).

2. The second region is represented by the cells that occupy the bulk of the paper tissue, filling all the distances between the vascular bundles and surrounding the Chlorenchyma shell in a completely irregularly arranged way around them.

The average tissue of the species appeared to be homogenous in its components and cells divided into two regions, according to the Ahmad *et al.* (2011) study of six species, including eight plant species belonging to the Eragrostideae.

The first of which is in the form of an outer envelope (Chlorenchyma) around the vascular bundles, which is integrated in most of the secondary beams and some of the initial beams of the species or not integrated on both sides of the human beings or on the lower epidermis only may be Sclernchyma belts and agree with AL – Na'amani (2012) about vascular belts. This qualitative characteristic did not have a distinct classification role for the involvement of all species as such, *i.e.*, their radial arrangement around the vascular bundle.

The second area, which occupies the bulk of the paper tissue and surrounding the chlorine membrane in general and arranged irregularly around it and in a compact and lightly characterized the walls and irregular shape of all species under study.

## Conclusions and Recommendations

### Conclusion

1. The study showed the importance of taxonomic study as a tool for the separation of species of the tribe Agrostideae.

2. The three types varied very accurately in the anatomical study, despite the similarity between them.

3. *Ap. intermedia* was clearly isolated from the anatomical study of some quantitative and qualitative characteristics as well as some cross-sectional characteristics.

### Recommendations

1. Conducting comprehensive taxonomic studies for the other species of the Agrostideae.

2. Extending aspects of the study at the level of chemical studies.

3. Conduct a molecular study of the ITS region and more genes to reverse the genetic relationships between

the plant species of the studied population.

4. Use of numerical classification to find relationships and the extent of convergence between species under.

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