COMPARATIVE VEGETATIVE ANATOMY OF THE TRIBE *TRITICEAE* DUMORTIER (POACEAE) IN TURKEY

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ABSTRACT

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Triticeae Dumort. is a tribe within the Pooideae subfamily of Poaceae. Major crop genera found in this tribe include barley (*Hordeum*), wheat (*Triticum*) and rye (*Secale*) all of which are closely related to each other. In Turkey, with the several subgroups, this tribe is represented by 16 genera and 75 species, many of which have great economic values.

The main objective of this research work is to determine the taxa of this tribe on the basis of their diagnostic anatomical characters of vegetative organs for the recognision of genera, sections, species, and infraspecific categories. By using both fresh and herbarium specimens, transverse sections of vegetative organs were obtained by using two different sectioning methods.

This study covers both qualitative anatomical characters, such as sclerenchyma position, hair density and intercellular cavities of roots, shapes of leaves, presence of midrib, occurrence of leaf hairs, shapes of girders or strands, arrangement of sclerenchyma around vascular bundles and arrangement of epidermal cells, cell wall undulations, appearance of silica bodies, type of bulliform cells, arrangement of culm

vascular bundles and their connections to the epidermis, hollowness of culms, as well as quantitative characters, such as sclerenchyma line number of roots, vascular bundle line numbers and diameters of culms, sclerenchyma line number of leaf margins, line numbers and sizes of all types of costal leaf hairs, stomata and interstomatal cell lines.

In conclusion, all these properties are useful to understand both systematics and evolutionary relationships of the taxa. Moreover, the leaves of the tribe have the most numerous diagnostic characters. The roots do not have central metaxylem. The metaxylem elements are scattered in the vascular cylinder. The internodular parts of the culms may be hollowed or solid. However, the nodular parts of the culms generally have the same structure. Furthermore, there are two species which can be assigned as a subtribe.

Key Words: Vegetative Anatomy, Poaceae, *Triticeae*, Turkey

ÖZ

TÜRKİYE'DE BULUNAN *TRITICEAE* DUMORTIER (POACEAE) OYMAĞININ KARŞILAŞTIRMALI VEJETATİF ANATOMİSİ

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Triticeae Dumort., Poaceae ailesinin, Pooideae alt ailesinde bulunan bir oymaktır. Arpa (*Hordeum*), buğday (*Triticum*) ve çavdar (*Secale*) gibi birbirileriyle yakın akrabalıkları olan başlıca ekin cinsleri bu oymak içindedir. Türkiye'de pekçok altgrupla birlikte 16 cins ve pekçoğu yüksek ekonomik değer içeren 75 türle temsil edilir.

Bu çalışmanın esas amacı, oymak içindeki cinslerin, seksiyonların, türlerin ve türaltı kategorilerin, belirleyici anatomik karakterleri üzerine dayanılarak tanımlanmasıdır. Taze ve kuru bitki örneklerinin vejetatif organlarından iki farklı gömme metodu kullanılarak enine kesitler alınmış ve ayrıntılı olarak çalışılmıştır.

Bu çalışma, köklerdeki sklerenkimanın yeri, kök tüylülüğü ve hücre arası boşluklar, yaprak şekli, stoma tipi, orta damar ve tüylerin olup olmaması, girder ve strand şekilleri, iletim demetleri etrafında sklerenkimanın düzeni, epidermis hücrelerinin dizilimi, hücre duvarı dalgalanmaları, silika hücrelerinin varlığı, bulliform hücrelerinin çeşidi, gövdedeki iletim demetlerinin dizilimi ve epidermise bağlantıları, gövdelerin boşluğu gibi nitel karakterler ile köklerderki sklerenkima sırası, gövdedeki iletim demeti sırası ve gövde çapları, yaprak uçlarındaki sklerenkima

sırası, yapraklardaki tüm tüy tiplerinin sıra sayısı ve uzunlukları, stoma sayısı ve stomalar arasındaki epidermis hücre sayısı gibi nicel karakterleri kapsamaktadır.

Sonuç olarak, bütün bu özelliklerin, taksonların sistematik ve evrimsel ilişkilerini anlamakta yardımcı olduğu gözlenmiştir. Ayrıca, en fazla ayırt edici karakter yaprak anatomisindedir. Kökte merkezi metaksilem bulunmamaktadır. Metaksilem elemanları merkezi silindirde dağınık dizilimlidir. Gövdede nodlar arasında kalan bölge boş ya da dolu olabilir. Fakat, nod kısmının anatomisi genellikle aynı yapıdadır. Ayrıca iki türün subtribe olarak değerlendirilebileceği tespit edilmiştir.

Anahtar Kelimeler: Vejetatif Anatomi, Poaceae, *Triticeae*, Turkiye

To My Father, Ali MAVI
Rest in peace,

and

To my mother, Hikmet MAVI

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EtOH	Ethyl Alcohol
TUBITAK	Scientific and Technical Research Council of Turkey
DPX	(Distrene, Plasticiser, Xylene) mounting medium
Au	Gold
HEMA	Hydroxyethyl methacrylate
J B 4	2-Hydroxyethyl Metaxyethyl Methacrylate
MVSP	MultiVariate Statistical Package
UPGMA	Unweighted Paired Group with Arithmetic Average
µ	micron
µm	micrometer
mm	milimeter
LM	Light Microscope
SEM	Scanning Electron Microscope
sp	species
subsp	subspecies
var	variety

CHAPTER 1

INTRODUCTION

1.1 Family Poaceae

Because of their ecological and economical significance, the evolution and classification of the grasses (Poaceae) have widespread interest. According to the recent studies (Watson and Dallwitz, 1992; Renvoize and Clayton, 1992; Kellogg, 2001) the family has more than 10,000 species within over 700 genera comprising cereals, bamboos, sugarcane, forage and weedy grasses which are all economically important. With their important advantageous features such as adaptation to the environment and the ability for coexisting with grazing herbivores or with humans, grasses are well adapted to open, marginal, and disturbed habitats. They are extremely successful at colonization and cover as onefifth of the Earth's landscape (Shantz, 1954; Clayton & Renvoize 1986; Kellogg, 2001).

Turkey seems to be rich in terms of economically important grasses with 494 species comprised in 134 genera. (Davis 1985; Davis *et al.* 1988; Güner *et al.*, 2000). Moreover, some of the cereals were cultivated as early as 7000 B.C. in the Southeastern (S.E.) and Central Anatolia (Harlan & Zohary, 1966). Ever since that time the general morphological characteristics of the family has been studied and taxonomic relationship of the members has mostly been investigated. In addition to the morphological investigations, other features of Poaceae were previously studied such as characterization of cytotaxonomy (Church, 1940), embryo structures (Yakovlev, 1950; Reeder, 1957) and chromosomes (Tateoka, 1956). Furthermore, Andulow (1931) studied chromosome numbers and size, somatic cell characters when the nuclei of them are at interphase, the starch grain types, morphological characters the first seedling leaf and its anatomy.

It is easy to enlarge the researches, based on the results of the phylogenetic analyses to determine the taxonomic structure of the family in all over the world. For example, Van Tieghem (1897) and Reeder (1957) indicated that there are two or three characters of mature embryos of grass seeds that can be correlated with systematic concepts. Also in the leaf blades, the tissue arrengements and activities were stated as useful taxonomic characters by Schwendener (1890), Lohauss (1905) and Brown (1958). In addition to these studies, there are also researches correlated with the persistence of nucleoli at metaphase of mitosis and taxonomy (Brown and Emery, 1957; Brown, 1958). Finally, Row and Reeder (1957) showed the root epidermal cells and root hairs might be diagnostic characters to decide certain major groups of the grass family.

Because the taxonomist encountered many problems by using the traditional methods based on grass morphology, the features of reproductive organs, floral characters were accepted as the most valuable characters for taxonomic affinities (Nwokeocha, 1996). However, it has been recognized that, amongst all the non reproductive organs, leaf is the most widely used part in plant taxonomy (Stace, 1965, 1984), and leaf epidermis is the second most important character parallel with cytology for solving taxonomic problems.

Grasses are monocots, having leaves that are usually single at each node, flattened or rounded stem called as the culm, roots and inflorescences (Figure 1). Parallel- veined leaves are arranged in two portions; the basal portion, called the leaf sheath around the culm and the upper portion, which is called the leaf blade. Roots are fibrous and adventitious or arising from lower part of the culms. Culms of grasses are usually hollow. However, the nodular parts of the culms are solid. There are also underground stems called the rhizomes or horizontal aboveground brances, which are called the stolons and they both allow vegetative reproduction in perennial grasses. Moreover, the occurance of intercalary meristems allows the grasses to grow below the apex, near the base of the plant. Whenever the culm is removed, it can sprout individually with the help of this meristem (Kallenbach, 2012).

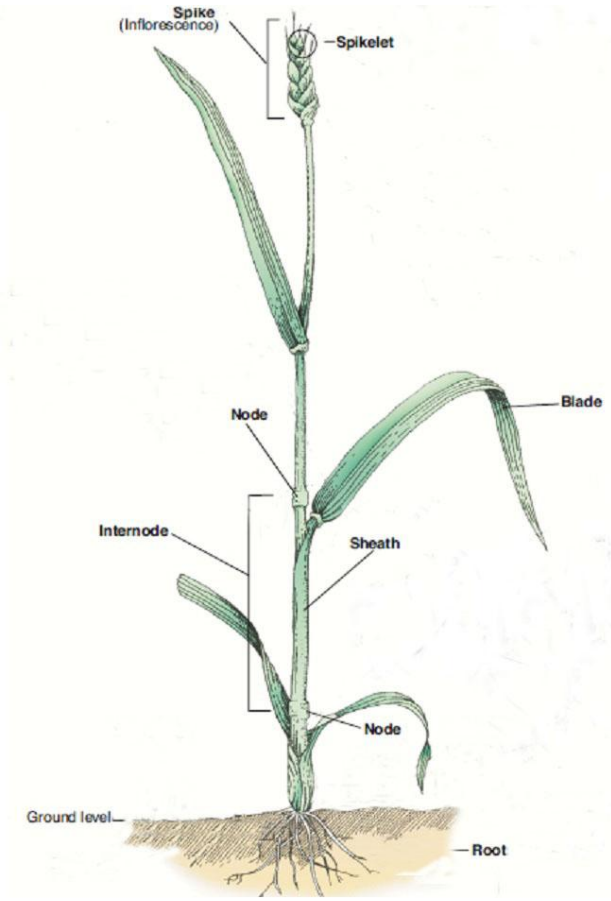

Figure 1. The major parts of a grass plant (Kallenbach, 2012)

Spike is the name of inflorescense of a grass. Small, simple flowers which are called as florets are grouped in inflorescence and called spikelets, subtended by the glumes and lemma. The oneseeded fruit of grasses, known as a caryopsis or grain, is rich in starch. Moreover, it can contain protein and lipid (Peterson & Seberg, 2003).

1.2 Systematics of the tribe *Triticeae*

Brown (1810, 1814) defined the earliest groups as tribes, what now are called subfamilies and divided the grasses into two groups as Paniceae (the modern Panicoideae) and Poaceae (the modern Hitchcock (1951)'s Festucoideae) based on their number of florets, the compression of spikelet and articulation. Bentham (1878) formalized this division and it was sustained by Bentham and Hooker (1883) and Hackel (1887). Afterwards, the number of tribes was enlarged to nine or ten (Gould & Shaw, 1983; Campbell, 1985; Pohl, 1987), some of which contain the same genera

and whereas the others are now seen as artificial.

With its approximately 330 species in the world, the *Triticeae* is one of the smaller, but economically the most important tribe of grasses. Wheat (*Triticum*), rye *(Secale*), barley (*Hordeum*) and forage grasses in *Agropyron* and *Elymus* are some of the important genera which are being found in the tribe. Because of widespread polyploidy and hybridization within the tribe, its taxonomy and phylogeny are still not exactly obvious and it has been subjected more varied classifications than any other tribe of grasses (Stebbins and Walters, 1949). According to their generic status, Löve (1984) diagnosed 37 genera including *Elymus* and *Agropyron* as the largest ones. However, *Agropyron* now centered around *A. cristatum* and *Elymus* has been splitted into several genera (Tzvelev, 1976; Melderis, 1978, 1980; Dewey, 1982, 1983a,b, 1984; Barkworth *et al.*, 1983; Barkworth and Atkins, 1984; Barkworth and Dewey, 1985; Love, 1982, 1984; Baum 1977; Baum *et al.*, 1991).
With 16 genera comprising 75 species (Table 1), the tribe takes a large place in the Flora of Turkey and its' supplements (Davis, 1985; Davis et al 1988; Güner et al 2000). After the description of *Elymus hoffmanni* by Jensen and Asay (1996), the studies have demonstrated that, *Elymus* (incl. spp) is the largest genus of the tribe distributed in Turkey. However, most of the studies have suggested that, the species included in the genus *Elymus* s.l. can be assigned to four or five different genera, namely *Pseudoroegneria*, *Reugneria*, *Thynophyrum*, *Elymus* and *Elytrigia* (Tzvelev, 1976; Melderis, 1978, 1980; Dewey, 1982, 1983a,b, 1984; Barkworth *et al.*, 1983; Barkworth and Atkins, 1984; Barkworth and Dewey, 1985; Love, 1982, 1984; Baum 1977; Baum *et al.*, 1991; Cabi, 2010).

Melderis (1985) recognised the genus *Agropyron* covering *A.cristatum* with two subspecies, subsp. *incanum* and subsp. *pectinatum*. According to this study, the latter has two different varieties according to their texture of spikelets. Moreover, Löve (1984) realized that there is another species named as *A. deweyi*, but Harlan (1948) emphasized this species as a variant of *A. cristatum*. The genus *Leymus* is also represented by two taxa in Turkey, one of which is a Euro-Siberian element, *L. racemosus* (Lam.) Tzvelev subsp. *sabulosus* (Bieb.) Tzvelev, and the other is an

Irano-Turanian element, *L. cappadocicus* (Boiss. & Bal.) Melderis (Mavi *et al.* 2011c).

As seen in Table 1, the tribe has 8 genera comprising only one species in Turkey. For example, *Heteranthelium* Hochst. is represented with *H. piliferum*, native to the middle and west Asia (Watson and Dallwitz 1992); *Amblopyrum* (Jaub. & Spach) Eig, represented with *A. muticum*, known from Central Anatolia of Turkey and Armenia; *Dasypyrum* (Coss. & Durieu) T. Durand distributed in Turkey, Morocco and Greece and represented with *D. villosum* L. for Turkey; *Psathyrostachys* Nevski was revised by Melderis (1985) in Flora of Turkey and the East Aegean Islands, and recognized only one species *P. fragilis* (Boiss.) Nevski.; *Hordelymus* (Jess.) Harz is represented with *H. europaeus* (L.) Harz and it is distributed in Europe and south-western Asia (Bothmer & Jacobsen, 1989); *Taeniatherum* Nevski is native to the Mediterranean Basin, eastern Europe and western Asia (Frederiksen 1986) and represented with *T. caput-medusae*; the genus *Henrardia* C. E. Hubbard is represented with *H. persica* (Boiss.) C.E. Hubbard and the species *Crithopsis delileana* (Schult.) Roshev. is the only member of the genus *Crithopsis* Jaub. & Spach and distributed also in Afghanistan, Iraq, Syria, Greece and Palestina (Frederiksen, 1993).

According to the Flora of Turkey, *Elymus* L. is the largest genus which was first described by Linnaeus in 1753. Because there is a large morphological variation within species, there are lots of taxonomical studies including the relationships of the members of the genus (Bentham, 1882; Löve, 1984; Dewey, 1984; Helfgott & Mason-Gramer, 2004; Cabi, 2010; Cabi & Doğan, 2010).

The second most crowded genus in the tribe in Turkey seems to be *Aegilops* L. with 15 species. In the world, the genus *Aegilops* has more than 20 species within 6 sections (van Slagen, 1994). *Triticum* L., *Hordeum* L. and *Secale* L., which are economically the most important genera in the tribe, respectively follow *Aegilops* considering the number of species distributed in Turkey. The country is one of the important centers of origin and diversity of cultivated wheats (*Triticum*) (Vavilov, 1992). However, the taxonomy of the genus in Turkey has not been studied

adequately. Moreover, there are lots of studies including the taxonomic status of *Hordeum*, but the studies concerning the relationship between the members of these genera in Turkey are limited (Mavi et al, 2011a). The other economically important genus, *Secale* has 5 species in Turkey (Davis 1985; Vol 9), which improves to 14 species by taking into account the Russian herbaria (Roshevitz, 1947). Lastly, in Flora of Turkey, *Eremopyrum* (Ledeb) Jaub & Spach was recognized covering four species, two of which have subspecies (Melderis, 1985).

Table 1. The genera of the tribe Triticeae in Turkey and the species included in each genus

Genus	**Species number**
Agropyron Gaertner	2
Elymus L.	20
Eremopyrum (Ledeb.) Jaub. &Spach	4
Heterantelium Hochst. Hochst	1
Crithopsis Jaub. & Spach	1
Amblyopyrum (Jaub. & Spach) Eig.	1
Aegilops L.	15
Triticum L.	11
Dasypyrum (Coss. & Durieu) T. Durand	1
Secale L.	5
Leymus Hochst.	2
Psathyrostachys	1
Hordeum L.	8
Hordelymus (Jess.) Harz	1
Taeniatherum Nevski	1
Henrardia C. E. Hubbard	1
Totally (16 genera)	**75**

The species number is given according to the Flora of Turkey (Davis 1985; Vol 9), its supplements, Davis *et al.* (1988; Vol 10) and Güner *et al.* (2000; Vol 11) and the research done by Jensen and Asay (1996).

In more recent studies, the morphological, pallynological, moleculer and the anatomical characters of the tribe in Turkey have been investigated (Cabi and Ozler, 2008; Baser *et al.*, 2009; Cabi *et al.*, 2009; Cabi and Dogan, 2009; Ozler *et al.*, 2009; Pehlivan *et al.*, 2009; Cabi *et al.*, 2010a,b,c; Dizkırıcı *et al.*, 2010; Cabi *et al.*, 2011; Mavi *et al.*, 2011a, b, c).

1.3 Literature review

There are some remarkable researches, separating the family into the subgroups as such festucoid, panicoid, bambusoid, chloridoid, arundinoid and aristidoid grasses based on their leaf anatomies (Andulov, 1931; Prat, 1932; Stebbins, 1956). According to this classification, the main characters differ these types are sclerenchymatic and parenchymatic cells of their leaves. For example, festucoid grasses have a well developed inner sclerenchymatic bundle sheath,whereas the outer parenchyma sheath is less developed. Moreover, chloridoid grasses have a well developed sclerenchymatic sheath and parechymatic layer too, but the latter has specialized chloroplasts and chlorenchyma which is composed of a row of long cells in contact with 1 or 2 cells of the parenchymatous sheath. The bambusoid grasses also have the well developed these two layers, but the parenchyma cells have poorly observable chloroplast and the chlorenchyma consists of distinctive cells. However, in arundinoid grasses the inner sclerenchymatic layer is poorly developed, but the outer parenchyma cells are well developed. In the panicoid grasses the inner sclerenchymatic sheath appears only in some of the bundles or lacking, but the parenchyma layer is well developed and has chlorenchyma. Lastly, and the most distinctively in aristidoid grasses there is no sclerenchymatic layer because of two layers of parechymatic cells. According to Brown (1958), the festucoid type is the most primitive type of anatomy of leaf blade and also it is the primary type of all the cereals. Moreover, he studied 101 species in 72 genera of the family and indicated 6 basic types of tissue arrangements, according to the evidence from leaf anatomy and plastid function (Brown, 1958).

There are different kinds of photosynthetic pathways (Crassulacean acid metabolism (CAM), C3, C4) for plants to fix carbon into tissues. Also, there are physiological, phytogeographical, ecological and anatomical differences between C3, C4 and CAM plants (Edwards and Walker, 1983; Hatch, 1987). Most plants use the C3 pathway, in which the Calvin Cycle produces a 3-carbon product in mesophyll cells. Whereas, in certain plant families, that are called C4 plants, the first product of Calvin Cycle is a 4-carbon molecule and the process comprises both mesophyll and bundle sheat cells (Nelson and Langdale, 1992; Ueno, 1998).

In general, plants have the specific characteristics of only one mode of photosynthesis in their leaves, but some plants can alter this mode in response to changes in environmental conditions. For example, some succulent plants have the photosynthetic pathway elasticity from C3 to CAM (Winter, 1985) and in *Hydrilla verticillata* can change the mode from C3 to C4-like without Kranz anatomy, which is known as a specific feature for C4 plants (Bowes and Salvucci, 1989).

Grasses have both C3 and C4 pathways to fix carbon into tissues. Generally, grasses such as bamboos that live at high altitudes where temperature in the growing season is colder, mostly have the C3 photosynthesis. But in warmer habitats at lower altitudes, grasses are better able to fix carbon via C4 metabolism. C4 plants have evolved a different kind of anatomical property, called the Kranz anatomy. This speciality allows grasses to compete with other plants in warm, tropical and subtropical inconvenient habitats by limiting oxidation of photosynthetic products (Ehleringer and Monson 1993).

The structure of Kranz anatomy includes mesophyll cells, surrounding the bundle sheath cells in leaves of C4 plants. These mesophyll cells are located very close to the parenchymatic sheath cells and this arrangement support the transport of C4 cyle metabolites through these two cell types (Peterson & Soreng, 2007). Moreover, the amount of chloroplast around the vascular bundles is highly dense in C4 grasses (Figure 2). However, the leaves of C3 grasses have poorly developed bundle sheaths which contain only a few organels (Brown and Hattersley, 1989).

The basic feature of Kranz anatomy is shown in Figure 2. It can be easily seen that, in *Aristata eludens* (Figure 2a), the bundle sheaths both contain chloroplast and the chlorenchyma sorrounding the outer sheath, consisting of tubuler cells (Cerros-Tlatilpa and Columbus, 2009). Moreover, in Figure 2b another example for Kranz anatomy can be seen. In *Stipagrostis uniplumis*, the outer sheath contains chloroplast and the chlorenchyma is arranged in a radiate fashion around the sheath (Cerros-Tlatilpa and Columbus, 2009).

A picture demonstrating the features of non-Kranz anatomy is shown in Figure 3. As seen in both leaves transverse sections of leaves of *Leymus racemosus* subsp. *sabulosus* (Figure 3a) and *Taeniatherum caput-medusae* (Figure 3b), which were both collected from Turkey and investigated as a part of this thesis study, chloroplasts are few or absent in cells of both sheaths.

Apart from the number of chloroplasts, the other way to identify a grass leaf as C3 or C4 is to count the number of mesophyll cells between the sheaths of adjacent vascular bundles, which is only applicable to grasses (Cerros-Tlatilpa and Columbus, 2009). The maximum lateral cell count in Kranz leaves, always four or less (Figure 2). However, in C3 grasses, the count is always more than four between at least two vascular bundles (Figure 3).

From all these points of view, it is easy to decide that, the members of the tribe *Triticeae* have Non-Kranz anatomy (Clayton and Renvoize, 1986).

The studies including the anatomical properties of grasses are not limited with all the researches about kranz or non-kranz anatomy. The anatomical properties of Poaceae was firstly used for systematic reasons by Duval-Jouve (1875), who stated that the position, presence or absence and type of the bulliform cells in the leaves, could be considered as important diagnostic characters. Then, Schwendener (1890) emphasized the prescence of sclerenchyma between the vascular bundles and the upper or lower epidermises to be of systematic importance. Morover, Vukolov (1929) showed the arrangement of sclerenchyma around the vascular bundles diagrammatically.

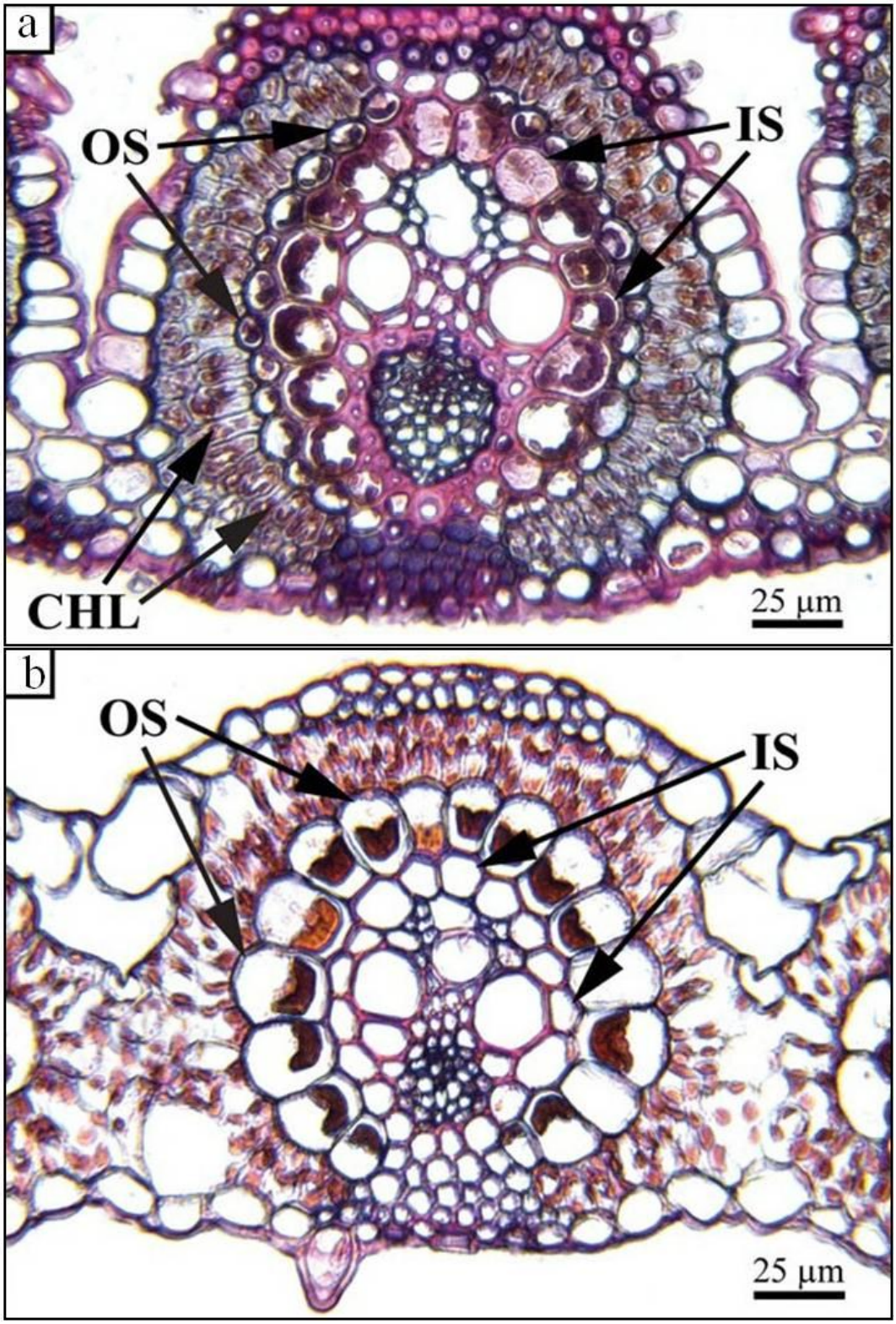

Figure 2. Kranz (C4) anatomy (Cerros-Tlatilpa and Columbus, 2009) **a)** *Aristida eludens* Allred & Vald é s-Reyna **b)** *Stipagrostis uniplumis* (Licht. ex Roem. & Schult.) de Winter. **CHL:** chlorenchyma, **IS:** Inner sheath, **OS:** Outer Sheath

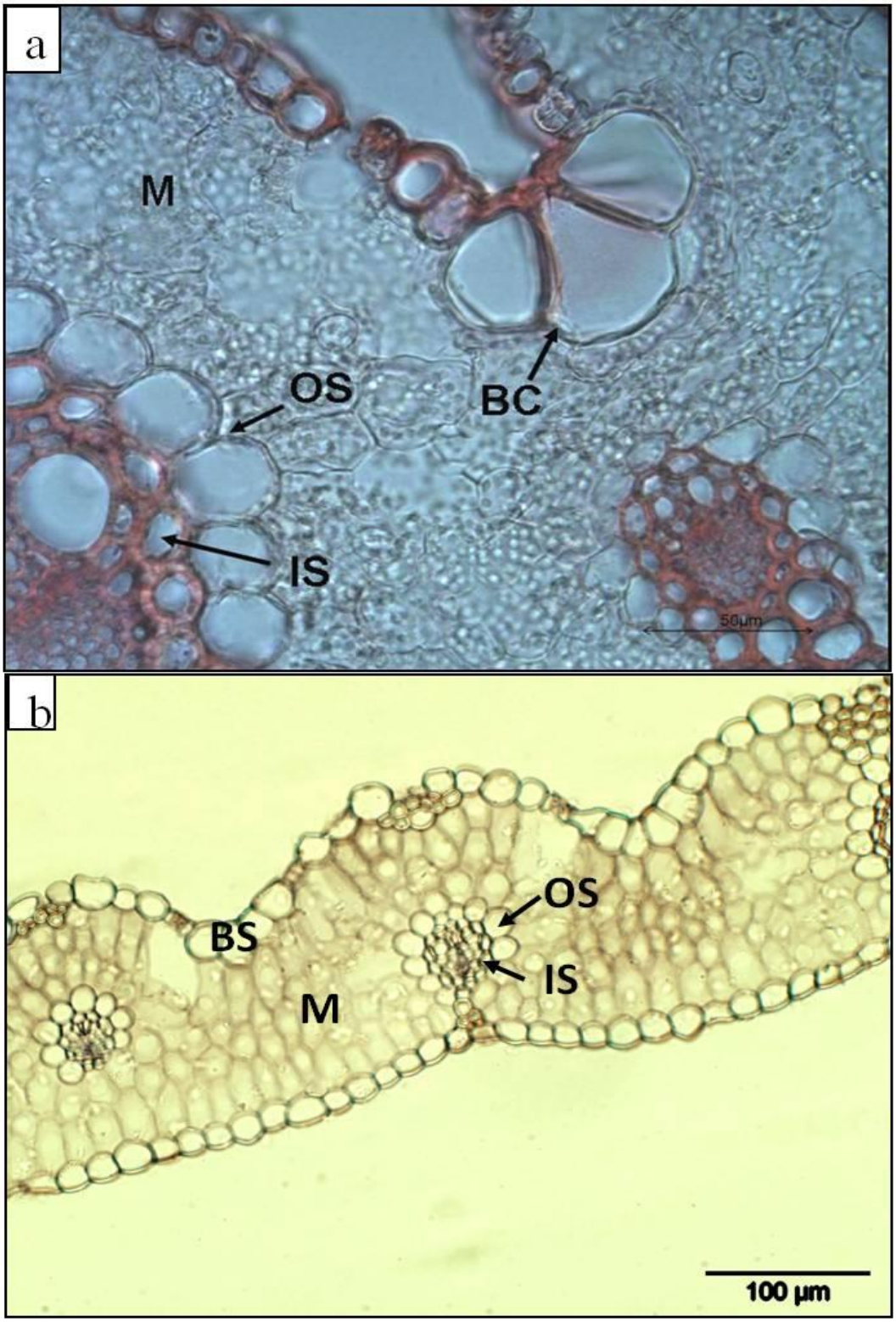

Figure 3. Non- Kranz (C3) anatomy **a)** *Leymus racemosus* (Lam.) Tzvelev subsp. *sabulosus* (Bieb.) Tzvelev (Mavi *et al.*, 2011c) **b)** *Taeniatherum caput-medusae* (L.) Nevski. **BC:** Bulliform Cells, **M:** Mesophyll, **IS:** Inner sheath, **OS:** Outer Sheath

In 1960, Metcalfe examined about 345 genera of Poaceae and found the diagnostic microscopical characters as the shape of girders, the strands and the stoma types based on the subsidiary cells. Girders and strands on the sclerenchymatic cells around the vascular bundles and stomata of the family were also classified according to the shapes of their subsidiary cells (Metcalfe, 1960). This essential study has become a standart reference for grass anatomy and the use of anatomical characters in taxonomy. The importance of grass leaf sections for identification is also recognized after this research (Metcalfe, 1960).

In the light of previous anatomical studies (Duval-Jouve, 1875; Schwendener, 1890; Vukolov, 1929; Metcalfe, 1960), almost all of the anatomy based studies/researches have focused on the correlation between grass anatomy and taxonomy. Khan (1984) indicated that the bulliform cells and sclerenchyma distributions through the leaves of the genus *Brachypodium* have taxonomic importance. Moreover, the relationship among the genera of Poaceae can be identified with the help of some characters such as occurance and the width of sclerenchyma, the hair properties of leaves and the epidermal features (Dube & Morisset, 1987; Jarves & Barkworth, 1992). Also the thickness of the leaves and the arrangement of vascular bundles might be systematically useful above the generic level, but the prickle distribution could change according to the environmental conditions (Ellis, 1976; Ellis, 1986). Furthermore, occurance and the structural details of colourless bulliform cells were also used as a taxonomic character (Metcalfe, 1960; Markgraf-Dannenberg, 1980; Tuan *et al.*, 1965; Jane and Chiang, 1991; Vecchia *et al.*, 1998).

According to the previous studies on stem anatomies of the grasses, most of the hollow stemed grasses were those with only one or two cycles of vascular bundles around large pith (Stover, 1934; Pohl and Lersten, 1975). Stover (1951) classified the members of the family into 3 groups according to the arrangement of their internodular vascular bundles of them. These are; vascular bundles arranged in one layer, in two layers and scattered. Following this study, Esau (1953) discussed the transverse sections of the stems and grouped the family in two types. In one type, there are hollowed stems including two layers of vascular bundles one of which is close to the epidermis. While in the other type, stems are not hollowed and the

vascular bundles are scattered. The hollowness or solidness of internodes seems to be correlated with a wide variety of characters which are important in grass systematics.

Within all the characters, the leaf epidermal anatomy obtains a huge taxonomic data related to grasses. Epidermal cells as stomata and hairs have proved to be an important tool in delimination of taxa in many plant families (Metcalfe & chalk, 1950 – 1989, Uphof *et al.*, 1962, Sinclair & Sharma, 1971; Lackey, 1978, Ditsh et al, 1995; Barthlott *et al.*, 1998; Stenglein *et al.*, 2003). The leaf epidermal features can help to explain taxonomic relationships at different levels and these characters have a great value in grass systematics to distinguish groups, particularly subfamilies and tribes (Prat, 1936; Stebbins 1956; Metcalfe, 1960, Ellis, 1979, Palmer and Tucker, 1981, Palmer *et al.*, 1985, Davila & Clark, 1990, Cai & Wang, 1994; Mejia – Saules & Bisbey, 2003).

In more recent studies, shape of leaf blades in cross-sections, epidermal cell types, floral morphologies including glume, awn and caryopsis cross-sections were examined and useful anatomical features in characterizing the major taxa within the family were demonstrated (Doğan, 1985, 1988, 1991a, b, c, 1997, 1999; Doğan and Tosunoğlu, 1992). Ma *et al.* (2005) used two genera of Poaceae and discovered that there are sharp differences on their leaf anatomies in terms of epidermal cells, large vascular bundles, median vascular bundles and the position of them.

1.4 Scope of the study

The main goals of the study are as follow:

- to investigate the anatomical properties of the leaf, culm and root of the tribe,

- to determine the diagnostic anatomical characters, used for understanding the systematics of the tribe

- to emphasize the taxonomic significances of all these anatomical characters in the tribe Triticeae in Turkey,

- to decide how the anatomical characteristics of the taxa in the tribe are correlated with the recent phylogenetic studies,

- to understand the evolution within the tribe.

Moreover, investigation of the diagnostic anatomical characters may provide some help for differentiating the genera and subcategories.

CHAPTER 2

MATERIALS AND METHODS

2.1 Plant materials

The samples used for all anatomical investigations, were collected from their natural habitats during the field trips within the scope of a project supported by TUBİTAK in 2006 and 2009. During these field trips, the freshly obtained samples were placed in 70% EtOH solution for anatomical studies. Moreover, some herbarium materials were also used as well as these fresh samples. The materials used for this thesis are listed with their locations and collectors in Table 2. Moreover, the photographs of the genera is given in Appendix A. The materials and all of the sections mentioned in this study have been deposited in the Plant Systematics and Biodiversity Laboratory in Biological Sciences Department of Middle East Technical University.

At the time of investigation using anatomical studies, the collecting times of the herbarium materials were not more than a year. However, some old samples from the herbarium of KEW and from the herbarium of the University of California Riverside were also used for the determination of leaf anatomy. All these dry herbarium materials had been boiled in distilled water until they were thoroughly rehydrated and then preserved in 70% EtOH.

It is important to select the appropriate region for sectioning within the vegetative organs of the samples. Therefore, the sections used in the anatomical investigations were obtained from the middle part of the mature organs of the plant materials, which were approximately at a similar distance from the both edges of the organ.

In order to example the epidermal architecture, at least 10 slides for each taxa were

prepared and the average numbers were measured for 30 different 234 X 186 μm² regions of 4 or 5 leaves of each taxon.

All the slides were observed using a Euromex FE 2025 light microscope and photographed with a Euromex CMEX DC.1300 camera and Image Focus version 2.0, computer imaging programme, which is also used for all measurements.

Table 2. The list of studied taxa with their locations and collectors.

	Taxa	Collectors	Location
1	*Elymus pycnanthus*	ECabi3209, ECabi2392	A2(E) İstanbul: Silivri to Marmara Ereğlisi, Gümüşyaka coast, 41° 02.827' N 28° 03.180' E, A5 Sinop: W of Sinop, Akliman dune, sands, 42° 01' 34.6" N, 35° 04' 51.2" E
2	*Elymus farctus*	ECabi3202, Ecabi3206, ECabi3600	A2(E) İstanbul: Silivri, İSKİ social facility establishment, sandy soils, 0 m, 41°04.128'N 28°08.468'E, İstanbul: Silivri to Marmara Ereğlisi, Gümüşyaka beach, sea 0 m, 41° 02.827'N 28°03.180'E, C3 Antalya: Manavgat to Alanya, around Alara river, 0 m.
3	*Thinopyrum erosiglumis*	ECabi2438, ECabi1366	B6 Sivas: North of Gürün, corroded calcerous hills, 1492 m, 38° 44' 34.7" N37° 37' 16.4" E, Malatya: Kepez-Akçadağ road, near Develi , 5 km to Akçadağ, 1337 m, 38°22.529' N 37°52.986' E
4	*Thinopyrum intermedium*	ECabi1269, ECabi3767, ECabi3321	A4 Kırıkkale: Balışeyh, Beyobası to Bıyıkaydın,around cemetery, 1038 m, 40°03.084'N 33°43.987'E, A7 Gümüşhane: Bayburt-Gümüşhane, Vauk Mount. Pass, 1875m, 40°22.220'N 39°50.339'E, B3 Ankara: Polatlı to Sivrihisar, 32 km to Sivrihisar, 870 m, 39°33.843' N 31°48.627' E
5	*Thinopyrum elongatum*	H. S. Gentryi-15651, in 1992	KEW material
6	*Elymus transhyrcanus*	ECabi3445	A8 Erzurum: Erzurum to Pazaryolu-İspir, 60 km before Pazaryolu, 40°09.422' N, 41°01.606' E,
7	*Elymus lazicus* subsp *lazicus*	ECabi3791	A7 Trabzon: Zigana silk road, around Zigana pass, 1766 m, 40°37.818' N39°23.699' E,
8	*Elymus longearistatus*	ECabi3795	A7 Trabzon: 2-3 km west of Maçka entrance of Kayadibi Saklıbahçe , 346 m, 40°49.047' N 39°36.670' E,
9	*Elytrigia canina*	ECabi1192, Ecabi2363	A4 Ankara: Kızılcahamam, Soğuksu National Park, to Karacaören, 1265 m, 40°26.769' N 32°36. 499' E, Kastamonu: Ilgaz to Kastamonu road, Ilgaz Pass, Abies forest, 1729 m, 41°03'32.3"N 33°44'23.7" E,
10	*Elytrigia repens*	ECabi3201, ECabi2373	A1 Edirne:Havsa, above Hacıgazi , Hüseyin Cabi wineyard, 98m, 41°33.881'N 26°48.903' E, A4 Kastamonu: Akkirpi Village Exit of İhsangazi , 1047m, 41°15.572' N 33°31.534' E,
11	*Elytrigia sosnowskyi*	ECabi3485, ECabi3496	A8 Erzurum: Oltu, Çamlıdere (Borahan), 1401 m, 40°31.037' N, 41° 59.603' E, A8 Erzurum: Narman to Pasinler, Fairy chimneys place, 1608 m, 40°18.050' N, 41°52.608' E,

Table 2 Continue. The list of studied taxa with their locations and collectors.

	Taxa	Collectors	Location
12	*Pseudoroegneria libanotica*	ECabi3602, ECabi1047	C4 İçel: near Ermenek, limestone slopes, 1304 m, 36°37.697' N 32°54.643' E, Niğde: Çamardı, Emli Boğazı, Sarı Mehmetler mevkii, Çukurbağ köyü üzeri
13	*Pseudoroegneria divaricata*	ECabi2054, ECabi2111	B3 Eskişehir: Sivrihisar to Polatlı, 1km before Oğlakçı, 824 m, 39°32.526' N 31°42.152' E, B4 Ankara Haymana to Polatlı, 35 km to Polatlı, 1004 m, 39°28.205' N, 32°27.888' E
14	*Aegilops kotschyi*	ECabi2700, ECabi1933	C3 Antalya; the entrance of Antalya from Kemer , 0 m, 36°49.553' N, 30°35.897' E, C7 Şanlıurfa: Ceylanpınar State Farmland, Sarnıçtepe distinct, 2 km north of Sarnıçtepe, 450 m, 36°47.652'N 39° 41.378' E
15	*Aegilops markgrafii*	ECabi3190, ECabi1117	A1 Edirne: Keşan, Abdürrahim to Şehitler, 110 m, 40°40.582' N 26°18.299' E, C4 İçel: Silifke to Gülnar, 10 km from Gökbelen village, 1028 m, 36°20.875' N 33°34.563' E
16	*Aegilops speltoides* var. *ligustica*	ECabi955, ECabi1388, ECabi1099	B5 Kahramanmaraş: the east of Göksun Çardak road, 1496 m, 38°01.409' N 36°39.626' E, B8 Tunceli: Kovancılar-Bingöl road, 35 km to Bingöl, 1539 m, road side 38°57.650'N 40°09.869' E, C5 İçel: Aslanköy, the exit of Fındıkpınarı , road sides, 1415 m, 36°59.721' N 34°16.672' E
17	*Aegilops speltoides* var *speltoides*	ECabi1065, ECabi1002	C5 Adana: Pozantı to Gülek Boğazı, around Tekir wold, 1169 m., 37°20.734' N 34°78.747' E, C6 Kahramanmaraş: Ahır Mount., road of Sarıçukur-Kılavuzlu Village, 37°37.693' N 36°48.747' E,
18	*Aegilops umbellata*	ECabi1527, ECabi3106, ECabi968	A5 Çorum,opposite of Çorum city forest , 1062 m, under Pinus forest, 40°34.986' N 35°02.222' E, B3 Eskişehir: 23 km from Polatlı to Sivrihisar, 880m., 39°33.845' N 31°48.664' E, C6 Kahramanmaraş: Elbistan, after passing Tatlar Village, 1288 m, 38°00.716' N 37°32.032' E,
19	*Aegilops biuncialis*	ECabi2126, ECabi3153, ECabi3114	A3 Ankara: Çayırhan to Beypazarı, roadsides, 583 m, 40°06.329' N 31°43.281' E, B1 Çanakkale: Ayvacık to Ezine, 133 m, 39°40.183' N 26°23.488' E, B2 Uşak to Sivaslı, 5 km from Uşak, 910 m, inside the Pinus plantation, 38°37.745' N 29°28.927' E,
20	*Agilops geniculata*	ECabi1204, ECabi3139	A3 Bolu: Abant, Ömerler village, 818 m, 40°41.959' N 31°27.395' E, B1 İzmir: Pınarbaşı, hills above Pınarbaşı, 517 m, 38°24.540' N 27°15.998' E,

Table 2 Continue. The list of studied taxa with their locations and collectors.

	Taxa	Collectors	Location
21	*Aegilops crassa*	ECabi2301, ECabi1923	C8 Mardin: Gercüş to Hasankeyf, 10 km after Gercüşten road sides, 792 m, 37°39'04" N 41°26'25" E, C7 Şanlıurfa: Ceylanpınar, Ceylanpınar State Farm, Şeyh Nasr stream, 454 m, 36°55.741' N 39°38.869' E
22	*Aegilops peregrina*	ECabi3212, ECabi2767	A2(E) İstanbul: Belgrad forest , 90 m, 41°11.522' N 28°57.646' E, C6 Hatay: İskenderun to Antakya, 4-5 km from Belen, 635 m, 36°28.643' N 36°15.455' E,
23	*Aegilops cylindrica*	ECabi1602, ECabi2381, ECabi1432	A3 Ankara: 10 km west of Beypazarı stream, Çayırhande, 40°06.705' N 31°45.943' E, A4 Kastamonu: Kastamonu to Tosya, near Akyaka village, 41°16.478' N 33°56.478' E, B9 Bitlis: Muş to 19 km before Tatvan, step, 38° 31.944' N 42° 07.501' E,
24	*Aegilops columnaris*	ECabi1113, ECabi2829	C4 Mersin: Silifke: Gülnar, 5 km from Gökbelen village, 1028 m, 36°20.774'N 33°37.595' E, C6 Adıyaman: Gölbaşı to Erkenek, above the Karanlıkdere village, 1343 m, 37°54.807' N 37°46.867' E,
25	*Aegilops juvenalis*	ECabi1879	C8 Şanlıurfa: Ceylanpınar, Ceylanpınar State Farm, Güzelyat area, around GSM base station, 437 m
26	*Aegilops tauschii*	ECabi3542, ECabi2235	A9 Artvin: Ardanuç, Gevhernik castle, 582 m, 41°07.605' N 42°03.397' E, C7 Şanlıurfa: Ceylanpınar, Ceylanpınar State Farm, Cevri Anayolu, 478 m, 36°58.254' N 39°38.642' E
27	*Aegilops triuncialis* subsp. *triuncialis*	ECabi789, ECabi1496	A1 Kırklareli: İğneada, on the beach, 41° 53.013' N 27°59.333' E, A8 Erzurum: Erzurum to Oltu, 12 km to Oltu, 1390 m, 40°28.517' N 41°58.697' E
28	*Aegilops triuncialis* subsp. *bozdagensis*	ECabi4050	C2 Denizli: Bozdağ, Geyran yaylası, roadsides, open woody areas
29	*Aegilops triuncialis* subsp *persica*	ECabi2880, ECabi2939	C6 Gaziantep: Nizip to Birecik, 446-450 m, 37°00.720' N 37°51.989' E, C7 Şanlıurfa:Viranşehir, 22 km from Urfa, 484 m, 37°09.635' N 39°00.689' E
30	*Aegilops comosa*	ECabi3820	C2 Denizli: in Üçler Municipalty just before Konutkent 2 housing state, 534 m, 37°47.706' N 29°01.064' E,
31	*Aegilops vavilovii*	ECabi1839, ECabi2322	C7 Şanlıurfa: Ceylanpınar, Ceylanpınar State Farm, Kazıktepe area, 381 m, 36°49.163' N 039°57.238' E, C8 Mardin: Mazıdağı to Derik, 9 km to Derik, 1055m., 37°25'17"N 040°19'30" E
32	*Agropyron incanum*	ECabi2545	A8 Erzurum: Aşkale-Bayburt, Kop Mountain, 2401 m, 40°01´38.0˝N, 40°31´20.4˝E,
33	*Agr. cristatum* subsp. *pectinatum* var. *puberulum*	ECabi2258, ECabi3378b	A9 Kars: Kuyucak village, around Kuyucuk Lake, 1642 m, 40°43´40.9˝N, 43°25´29.9˝E, B6 Sivas: Ulaş to Gürün, the crossroad of Deliilyas and Altınyayla villages, 39°23'47.2" N, 37°04'59.3" E,

Table 2 Continue. The list of studied taxa with their locations and collectors.

	Taxa	Collectors	Location
34	*Agr. cristatum* subsp. *pectinatum* var. *pectinatum*	ECabi2422, ECabi3316	B6 Sivas: Ulaş to Kangal, near Tecel village, roadside, 1405 m, 39°24´25.7˝N, 37°04´50.3˝E, B3 Eskişehir: Polatlı to Sivrihisar, 32 km to Sivrihisar, 38°33.843' N, 31°48.627' E,
35	*Triticum monococcum*	ECabi2374	A4 Kastamonu: İhsangazi, Kuşçalar district, Sekicek street, 41°14'29.8" N, 33°31' 39.1" E,
36	*Triticum dicoccon*	ECabi2375	A4 Kastamonu: 1 km from İhsangazi to Kastamonu, field and sides, 41°12'48.8" N, 33°34'31.8" E,
37	*Triticum boeoticum*	ECabi2411, ECabi3109	A6 Tokat: Tokat to Artova, exit of Boyunpınar village, 1316m, 40°091'46.2" N 36°23'43.1" E, B2 Uşak: Uşak to Sivaslı, 5 km from Uşak, 38°37.745' N 29°28.927' E
38	*Triticum araraticum*	ECabi2159	C6 Gaziantep: Araban to G. Antep, 30 km to G. Antep, 650 m
39	*Eremopyrum confusum* var *sublanuginosum*	ECabi1306, ECabi2508	B6 Sivas: Boğazlıdere to Kutlukaya, 4 km after Kutluköy 39°22.949' N 36°55.726' E, B7 Elazığ; Hazar Lake ANK 28969 B9 Ağrı to Doğubeyazıt 17 km before Doğubeyazıt,
40	*Eremophyrum distans*	ECabi3430	B8 Erzurum: Erzurum to Pazaryolu-İspir.
41	*Eremopyrum triticeum*	ECabi3274	B5 Yozgat: Yozgat to Boğazlıyan, Kaşkışla village, 1682 m.
42	*Eremopyrum orientale*	ECabi3055, ECabi2501	B4 Ankara: 12 km to Koçhisar nr Tuz G., 912 m, B9 Ağrı: Ağrı to Doğubeyazıt, 21km before Diyadin, 1805 m.
43	*Eremopyrum boneopartis* var *sinaicum*	ECabi2254, ECabi3059	C8 Mardin: 10 km from Gercüş to Hasankeyf, 792 m. B4 Ankara: Ankara to Kochisar, 12 km before Koçhisar, shore of Salt Lake 912 m.
44	*Eremopyrum confusum* var *confusum*	ECabi2249, ECabi2245	B5 Kayseri: Sultanhanı to Bünyan 5km from Sultanhanı 1203 m. 38°57'39" N 35°53'19" E, C4 Konya; Cihanbeyli Tuz İşletmesi; near Tuz lake
45	*Eremopyrum confusum* var *glabrum*	ECabi2255	B10 Ağrı: 9.5 km from Doğubeyazıt to Iğdır, , 1545 m, 39° 39' 26.8" N, 44° 02' 34.6" E
46	*Secale sylvestre*	ECabi3169	A1 Çanakkale: Gelibolu to Kesen, 40° 37 002 N 26° 49 968 E,
47	*Secale cereale*	ECabi2244	B2 Uşak: Karahallı to Ulubey, 1 km to Ulubey, 712m. 38°24.304'N 29°17.394'E
48	*Leymus racemosus* subsp *sabulosus*	ECabi767	A1: Tekirdağ to Silivri, 15 km from Tekirdağ, 41°00.269' N 27°41.029' E,
49	*Leymus cappadocicus*	ECabi3313	A6: Ankara Polatlı to Sivrihisar, 32 km to Sivrihisar, 39°33.843' N 31°48.627' E

Table 2 Continue. The list of studied taxa with their locations and collectors.

	Taxa	Collectors	Location
50	*Hordeum brevisubulatum* subsp *violaceum*	ECabi2437	B6 Sivas: Kangal to Gürün, crossroad of Konakpınar, roadside and slopes, 1793 m
51	*H. geniculatum*	ECabi2781	C6 Hatay: İskenderun to Arsuz, around Madenli district, 67 m,
52	*H. bulbosum*	ECabi3703, ECabi2750	B5 Yozgat: Yozgat to Sivas, road sides , 1064 m, 39°44.952' N 35°16.586' E, C4 Mersin: Silifke, 11 m, 36° 20.357' N , 33° 54.852' E
53	*H. distichon*	ECabi2584	Afyon: Dazkırı to Çardak, close to Çardak, roadside, 844 m,
54	*H. spontaneum* var *anatolicum*	ECabi1932, ECabi2818	C7 Şanlıurfa: Ceylanpınar State Farm, Sarnıçtepe bölgesi, 450 m., 36°47.652' N 39°41.378' E, C6 K. Maraş, City center to Sarıçukur village, road sides , 682 m, 37° 36' 506" N 36° 49' 631" E
55	*H. vulgare*	ECabi2655	Antalya: Kalkan to Elmalı, junction of Bezirgan village, 740 m,
56	*H. murinum* subsp *glaucum*	ECabi3215, ECabi3544	A3 Bolu: Düzce, 27 km from Hendek, 241 m, 40°49.307' N 30°48.563' E, A9 Artvin: Ardanuç, Gevhernik kalesi, 582 m, 41°07.625' K, 42° 03.397' E,
57	*H. marinum* var *pubescens*	ECabi3199b, ECabi3165	A1 (E) Edirne: Keşan, Enez to İpsala 2 km N of Enez, on the road of border, 40°44.033' N 26°07.435' E, Çanakkale: 8 km from Gelibolu to Keşan, roadsides, 76 m, 40°29.104' N 26°42.666' E
58	*Henrardia persica* var *persica*	Baboü10038E in 1965, F.Funse8439 in 1966	KEW materials
59	*Heteranthelium piliferum*	ECabi2181, ECabi2940	C6 Adıyaman: Besni to Keysun, 918 m, 37°41'05" N 37°52'38" E, C7 Şanlıurfa: Şanlıurfa to Viranşehir, 22 km from Urfa, 484 m, 37°09.635' N 39°00.689' E,
60	*Taeniatherum caput-medusae*	ECabi645	C4 Konya: Küçükköy, roadsides, 1015 m, 37°40.285'N 32 49.390' E
61	*Ambylopyrum muticum*	ECabi3816, Davis19088 in 1952	B2 Denizli: Sarıgöl to Buldan, 35 km to Buldan, 38°14.627' N 28°46.120' E, KEW material from Turkey,

Table 2 Continue. The list of studied taxa with their locations and collectors.

	Taxa	Collectors	Location
62	*Crithopsis delileana*	ECabi2232, ECabi2289	C7 Urfa: Ceylanpınar State Farm, Çevri mainroad, 478 m, 36°58.254' N 39°38.642' E, C8 Mardin: around train station, 666 m, 37°17'10" N 40°44'06" E
63	*Psathyrostachys fragilis*	ECabi2424	B6 Sivas, 18 km from Kangal to Gürün, near crossroad of Kuşyakası village, 39°07'45.1" N 37°14'32.8"E
64	*Dasypyrum villosum*	Davis40811 in 1965	KEW material
65	*Hordelymus europaeus*	ECabi2558	A4 Kastamonu: Kastamonu to Ilgaz Mountain, Abies forest, 1854m, 41°04' 0.08" N 33°45' 01.4" E,

2.2 Preparation of Solutions

The chemicals and materials with their suppliers are listed in Appendix B.

2.2.1 Alcohol series

Absolute EtOH (ethyl alcohol) was used for all preparation of alcohol solutions. In order to prepare a certain % of alcohol solution, the required amount of alcohol per 100 ml alcohol solution was measured and the solution was completed to 100 ml by addition of distilled water. The prepared solutions were kept in sealed bottles in flammables cabinet. For the experiments 50%, 70%, 80%, 90% v/v solutions of EtOH in distilled water and absolute (100%) EtOH were used.

2.2.2 Alcohol-Xylene series

For dehydration and clearing steps of paraffin embedding method (Johansen, 1940) mixtures of pure EtOH and xylene were used. These mixtures can be listed as:

a) alcohol / xylene, 1:2,
b) alcohol / xylene, 1:1,
c) alcohol / xylene, 2:1

2.2.3 Dyes

To decide the best staining procedure for the sections, different staining methods by the use of different dyes were applied. Moreover, in order to set an appropriate secondary staining, procedures by modifying Johansen (1940)'s both Safranin with Fast Green and Sass (1958)'s Safranin with Alcian Blue protocols were applied. However, the best visualization of the cellular differences within plant sections was achieved by staining the sections only with safranin.

2.2.3.1 Safranin

According to Johansen (1940), safranin is the most important and commonly used dye to stain lignified structures. While preparing the solution of the dye, 10 g safranin powder was weighted and put into a 1 litre bottle. Then 1 litre, 50% ethanol added into the same bottle. The bottle was gently shaked by hand until safranin thoroughly dissolved. When this dye is used to stain the tissues, the lignified cell walls are observed in brilliant red.

2.2.3.2 Fast Green

Fast green, soluble both in alcohol and water, stains plant tissues intensely in a very short time (Gurr, 1965). The solution of the dye was prepared by adding enough dye powder to equal parts of absolute alcohol and two or three drops of clove oil. Then this mixture was agitated until the dark greenish color was obtained. After staining of the plant cells with this dye, cytoplasm appears in brilliant green (Johansen, 1940).

2.2.3.3 Alcian Blue

This dye can be an alternative to Fast Green (Johansen, 1940). Alcian blue solution was prepared by adding 1 litre distilled water to 10 g Alcian blue powder. Then the bottle, in which they were mixed, was agitated gently by hand. According to Akinloye *et al.* (2010), alcian blue has affinity for thin walled cells of plant tissues.

2.2.3.4 Toluidine Blue

Toluidine blue is commonly used as an effective stain for sections obtained after embedding into a resin (Johansen, 1940). To prepare 100 ml solution of the dye, 1 g of Toluidine blue powder was mixed with 5 g of Sodium borate and diluted in 100 ml distilled water. After standing overnight, the mixture was filtered before use. According to Parker *et al.* (1982), with toluidine blue, sclerenchyma and xylem are stained in blue-green, whereas parenchyma is stained in red-purple.

2.2.4 Adhesive solution

To stick the sections onto the slides, a modified version of Mayer's Adhesive solution was used. Mayer's Adhesive solution is prepared by adding equal amount of glycerin to white part of a fresh egg and 1 g of sodium salicylate (Johansen, 1940).

According to Johansen (1940), Mayer's Adhesive is easier to prepare and apply than the other several mounting media such as Haupt's adhesive. However, Mayer's adhesive possesses less holding quality when compared with Haupt's adhesive (Johansen, 1940). In the experiments of the thesis, equal amounts of egg albumin and glycerin were mixed and applied to the slides right before the sections were placed onto the slides.

2.3 Preparation of permanent microscope slides

In order to make a permanent slide, very thin sections must be taken from the tissues. There were sections prepared by peeling from both surfaces of leaves as well as transverse sections of leaves, stems and roots. The terminology used from Metcalfe (1960), Doğan (1992) and Ellis (1979) for all structural characters of leaf surfaces and anatomical features of transverse sections of roots, stems and leaves.

For preparing permanent slides, there are 2 different sectioning methods used to cut thin transverse sections from the materials in 70% EtOH:

1. Paraffin sectioning method,
2. Resin embedding method.

Moreover, there are permanent slides prepared from both upper and lower surfaces of leaves.

2.3.1 Paraffin sectioning method

For uniformity and thinness of transverse sections for anatomical studies, the rotary microtome offers the best alternative (Metcalfe, 1960; Johansen, 1940; Marson, 1983). Moreover, by hardening the tissue in paraffin, very thin sections can be obtained easily. With the paraffin sectioning method, not only samples are embedded into paraffin wax, but also paraffin wax is infiltrated in to the tissues (Johansen, 1940). There are necessary steps for the preparation of a specimen to be examined under microscope on a permanent slide. However, if the pieces of the tissues are not thin enough, the fluids do not penetrate to the middle of the pieces and the wax blocks will be soft. So, it is important to dissect the tissues into small pieces before applying the solutions in the steps of the embedding method.

2.3.1.1 Fixation

Fixed tissues can be embedded and sectioned easily for microscobic studies and Formalin- Acetic acid- Alcohol (FAA) solution is the most commonly used plant fixative (Metcalfe, 1960; Johansen, 1940). However, by using only 70% EtOH itself, the stabilization of the important constituents of the tissue can be provided (Marson, 1983). Moreover, macromolecules as proteins, carbohydrates and lipids are preserved within fixed tissues (Johansen, 1940), which can cause unclear observations after staining. Since the materials were kept in 70% EtOH right after they were collected, FAA solution was not applied, and fixation was done with 70% EtOH.

2.3.1.2 Dehydration

Since paraffin wax is immiscible with water, it is important to remove water of the tissues before replacing the tissue in wax. Dehydration is a well established method to use a compound which is miscible with water and capable of replacing it. The most commonly used dehydrating agent for this purpose is ethyl alcohol (Johansen, 1940). Because it is necessary to make the tissues completely rigid, both the freshly

obtained samples and the softened herbarium samples, being preserved in 70% EtOH, were dehydrated with ethyl alcohol solutions of increasing strength.

In this step, both the concentrations of the alcohol solutions and the exposure time of them are essential. Short times of the exposures may cause the fluids not to penetrate the middle of the pieces. On the other hand, long exposures to high concentrations can cause brittles. Also, long exposures to low concentrations of dehydrating agent make the tissue soft and promote disorganization.

For dehydrating the pieces of the tissues, the alcohol compounds listed below were orderly applied:

- Fresh 70% EtOH, 30 min
- 80% EtOH, 30 min
- 90% EtOH, 30 min
- Absolute EtOH, 30 min

2.3.1.3 Clearing

Since paraffin is not miscible with the dehydrating media, alcohol must be removed from the tissues for the paraffin infiltration. For this purpose, Benzol, Chloroform, Terpineol, Trichloroethylene and Xylene are commonly used antimedia, which are both miscible with dehydrating media and paraffin wax (Johansen, 1940). In this thesis, xylene was used to provide a good medium for removing the alcohol as well as paraffin penetration.

Leaf specimens seem to be sensitive to sudden changes of xylene, therefore the solvent changes were made less abrupt by introducing a mixture of increasing concentrations of xylene in alcohol.

The dehydrated tissue pieces were respectively placed in to mixtures concentrated as:

- 75% EtOH in xylene, 30 min

- 50% EtOH in xylene, 30 min
- 25% EtOH in xylene, 30 min
- Pure xylene, 30 min.

2.3.1.4 Paraffin infiltration

Xylene was removed from the tissues, after clearing. As in the clearing step, infiltration was done by increasing the amount of paraffin. Infiltration is best accomplished in 60°C heated oven (Johansen, 1940). This degree is suitable for both melting paraffin and evaporation of xylene at the same time. For taking smooth sections; the time of the infiltration is important. If paraffin is not wholly infiltrated in to the tissues, it causes the tissue and paraffin to separate while sectioning.

For infiltration of paraffin, some paraffin was added in to the cups which already had both the pieces of the tissues and xylene. These cups were kept in the 60°C oven for two days with their lids on them. After these two days, the lids of the cups were taken off and the evaporation of xylene was provided for one or two weeks, while some more paraffin was also added in to the cups at the same time. This step can be summarized as:

- adding some solid paraffin, cups with their lids, 2 days, 60°C,
- adding more solid paraffin, cups without their lids, 1 or 2 weeks, 60°C.

2.3.1.5 Embedding and sectioning

In this step, the tissues were placed in paraffin wax and when the wax became solid, the properly oriented tissues were ready to be sectioned. For embedding the grass samples, after the thorough impregnation, the paraffin infiltrated tissues were placed and encased in melted paraffin. When the paraffin was solidified, the paraffin blocks which had tissues were attached to the microtome for taking thin sections from them. By using a 'Leica 2 RM2125RT' model of Rotary Microtom, 10-20 microns (μ) thickness slides can be taken easily. The angle and the sharpness of the knife are necessary for sectioning stage (Johansen, 1940; Ruzin, 1999). After sectioning, the

pieces were sticked to the slides by using an adhesive such as Mayer's Adhesive Solution (Johansen, 1940). While applying, a very thin layer of this adhesive solution was brushed on the slides and one or two drops of distilled water was added before placing the sections on these slides.

The sections on the slides were observed by using a 'Euromex FE 2025' light microscope and photographed by using a 'Euromex CMEX 5 DC.1300' camera.

2.3.1.6 Hydration and Staining

Before staining the slices, it was necessary to remove the paraffin which had already been impregnated to the tissues. After paraffin was removed from the slices, they were hydrated by passing through a series of alcohol of decreasing strength.

The most commonly used dye for staining the plant tissues is safranin (Johansen, 1940; Sass, 1958; Gurr, 1965; Clark, 1981). Safranin solution was used to stain the lignified tissues such as sclerenchyma and xylem. Because safranin solution was prepared by using 50% EtOH, after staining with safranin for a minute, the slides were put under running tap water then they put in to 50% EtOH to remove excess dye.

All the applications to the slices in these steps can be summarized as:

- slides were kept in 60ºC oven for 24 hours to liquidize the wax
- slides were kept in pure xylene for 24 hours to remove the wax.
- 25% EtOH in xylene, 5 min
- 50% EtOH in xylene, 5 min
- 75% EtOH in xylene, 5 min
- Absolute EtOH, 5 min
- 90% EtOH, 5 min
- 80% EtOH, 50 min
- 70% EtOH, 5 min
- 50% EtOH, 5 min

- Safranine solution, 1 min
- slides were kept under running tap water and 50% EtOH to remove excess dye.

In secondary staining, each dye is used to stain a different parts of the cell. According to Johansen (1940), the safranin stained tissues are counterstained with Fast Green or Alcian Blue. In the secondary staining procedures, while safranin stains thick cell walls red, fast green or alcian blue gives bluish-green colour to cytoplasm and thin cell walls.

To apply secondary staining Sass (1958)'s safranin-fast green and safranin-alcian blue procedures were modified. After deparaffinization in pure xylene and hydration, slices were kept in safranin solution for an hour and washed in distilled water until the excess dye was removed. The slices were dehydrated in 50%, 70% and 90% EtOH for 2 min in each and fast green solution was applied them for 10 sec. To remove the excess dye, the slices were kept in 100% EtOH for 10 min and moved in to pure xylene before mounting. Alcian blue was also used instead of fast green. However, the slices were kept in alcian blue for 30 min.

2.3.1.7 Dehydration and Mounting

The slides are run through increasing strength of alcohols in order to prevent distortion of the slices due to shrinkage. The stained slices, which were in 50% EtOH, were put in to 70%, 80%, 90%, 100% EtOH and than 75%, 50%, 25% alcohol in xylene solutions and finally in pure xylene.

The mounting media are mostly dissolved in organic solvents such as xylene and are not miscible with water or alcohol (Johansen, 1940). Here, Canada balsam and Entellan were used as mounting media. One or two drops of mounting media were dropped on the slices then covered with coverslip. Because the drying period of Entellan is shorter than Canada balsam and also it seems to be more colourless, Entellan was generally used to coverslip the slides.

2.3.2 Resin embedding

This method was used only for very old leaf samples from the herbariums of KEW, Royal Botanical Gardens and University of California, Riverside. First these herbarium samples were softened by boiling water. Then they were moved in 50% EtOH for an hour and kept in 70% EtOH for a day to be fixed.
There are two different resin materials used to embed the leaf samples. One of which was Technovit 7100 liquid and the other one was JB-4. Both these procedures were modified from previous studies (Aparichio and Marsden, 1969; Popp and Zwick, 1987; Mason and Mackie, 1985).

2.3.2.1 Dehydration

For embedding samples into Technovit 7100 and hardeners; the leaves were softened in boiling water and fixed in 70% EtOH. After that, they were dehydrated by increasing strength of EtOH solutions as fresh 70%, 95%, 100% and fresh 100% for 1 hour per each. However, before using the other resin embedding medium, JB-4, dehydration was done to 95% EtOH and the samples in 95% EtOH solution were kept in refrigerator for one day.

2.3.2.2 Pre-infiltration

The pre-infiltration solution was prepared by mixing equal parts of absolute alcohol and Technovit 7100 liquid, HEMA-based resin embedding solution. The samples were transferred into this pre-infiltration solution and slowly shaked with the help of a simple tube-rotator for 2 days. This step was only carried out while using Technovit liquid as an embedding medium.

2.3.2.3 Infiltration

For preparing the infiltration or preparation solution, hardener 1 was used. 1 g powdered hardener 1 was dissolved in 100 ml of Technovit 7100 liquid. This

solution remains stable in 4ºC for a month. The samples were also shaked by using the rotator while kept in this solution for 2 days.

With JB-4, infiltration was done within two steps. In the first step, 0.22 g catalystC was dissolved in 25 ml JB-4 for 20 min. After that, 25 ml 95% EtOH was added to this mixture. The samples in this solution were shaked slightly by using tube-rotator for a day and then kept in refrigerator over night. In the second step, 0.135 g catalystC was dissolved in 15 ml JB-4 and then mixed with 10 ml 95% EtOH. The samples applied with this second solution were shaked and stored as in the same way of the first step.

2.3.2.4 Polymerization

While the resin material was Technovit 7100, polymerization or embedding solution was prepared by using another hardener, which was called hardener 2. One ml of hardener 2 was mixed with 15 ml of infiltration or preparation solution. After filling the moulds with the embedding solution, samples transferred in to the moulds and kept there until the embedding solution was solidified.

The polymerization solution was prepared by dissolving 0.45 g catalystC in 50 ml JB-4 and adding 2 ml Solution B, if JB-4 was used as the resin embedding solution.

2.3.2.5 Sectioning, Staining and Mounting

After the embedding solutions hardened with the samples in it, max. 6 µ thickness sections were taken by using a special microtome blade. The slides were stained by pulling them in a container including Toluidine blue (O'brien *et al.*, 1964). Then they were pulled out quickly and washed three times with distilled water. After they stained and got dry, they were mounted by using DPX and Entellan.

2.3.3 Leaf surface Micromorphology

Leaf samples, which were softened by boiling water and kept in 70% EtOH, were used for peeling. Samples were placed on a slide with the side of interest for observation facing downwards. Then the cells and tissues of the other side were gradually scraped away with a sharp blade. This was applied to both adaxial and abaxial epidermis. If the image was clear enough without staining, photographs were taken with a drop of distilled water. However, for some samples of surfaces, staining was accomplished using safranin. If staining was necessary to obtain clear observations, after peeling, following steps were carried out orderly:

- 50% EtOH, 1 min
- Safranin solution, 30 sec
- Distilled water to remove excess dye, 1 min
- 50% EtOH, 1 min
- 70% EtOH, 1 min
- 80% EtOH, 1 min
- 90% EtOH, 1 min
- Absolute EtOH, 1 min

Because xylene can cause shrinkage of the samples, the slides were embedded in Entellan with the outer side of epidermis and covered with coverslips on the heels of dehydration.

2.4 Scanning Electron Microscopy (SEM) Studies

Nature of the specimen is one of the important parameters for the quality and resolution of SEM images. The SEM studies were carried out for the surface view of the leaf samples. Because the adaxial epidermis seemed to be more hairy than the abaxial one, which means it was difficult to peel the adaxial epidermis by hand, the upper sides of the leaves were mostly imaged by SEM technique.

Dry leaf samples were mounted on SEM stubs with double sided adhesive band, both of which were approximately 12 mm in diameter. After the samples were adhered to the stubs, they were coated with gold by sputtering. They were examined by using a JEOL JSM-6060LV (JEOL Technics Ltd., Tokyo, Japan) Scanning Electron Microscope (Doğan, 1988).

2.5 Numerical Taxonomic Method

To determine the phonetic similarity among the species and the genera of the tribe, cluster analyses were carried out. The data were obtained from the characters of both the transverse sections and the abaxial and adaxial surfaces of the leaf samples. The characters and the character states used in the method are given in Table 3. The data formed by both the qualitative and quantitative values. With the help of Principle Component Analyses, the overlapping characteristics were eliminated (Doğan 1992, 1997). The best cluster representing the natural grouping was obtained from the mixed data, was found to express the natural grouping best. This mixed data was analyzed based on Gower's Coefficient of Smilarity (Friedman & Meulman, 2004). For the constitution of the phenograms, the MVSP (MultiVariate Statistical Package) program was used. In order to group the taxa based on their anatomical similarities of leaves, UPGMA (Unweighted Paired Group with Arithmetic Average) clustering method was performed (Sneath & Sokal, 1973).

Table 3. List of characters and character states used in both analysis.

1) Shape of leaf blades in cross section
Flat (0)
V- shaped (1)
U- shaped (2)
Convolute (3)
Rolled (4)

2) Adaxial furrows
less than shallow (0)
shallow (2)
deep (3)

3) Abaxial furrows
less than shallow (0)
shallow (2)
deep (3)

4) Midvein appearance
uncertain (0)
certain without rib (1)
certain with rib (2)

5) Chloroplast of Outer Sheath
absent (0)
present(1)

6) Sclerenchyma around VB
without sclerenchyma (0)
with abaxial girder (1)
with both abaxial and adaxial scl (2)

7) Continuous Sclerenchyma
absent (0)
present (1)

8) Sclerenchyma on Margins
Line numbers

9) Epidermis in cross section
irregular (0)
regular (1)

10) Types of Bulliform cells
uncertain (0)
regular and same sizes (1)
regular and different sizes (2)
irregular and different sizes (3)
fan shaped (4)

11) Adaxial Sclerenchyma
I- shaped (0)
T- shaped (1)

12) Cell wall undulation
absent (0)
present (1)

13) Hooks on adaxial surface
absent (0)
costal (1)
both costal and intercostal (2)

14) Hooks on abaxial surface
absent (0)
costal (1)
both costal and intercostal (2)

15) Macrohairs on adaxial surface
absent (0)
costal (1)
both costal and intercostal (2)

16) Macrohairs on abaxial surface
absent (0)
costal (1)
both costal and intercostal (2)

17)Costal Mh Line Number on adaxial

18)Costal Mh Line number on abaxial

Table 3 Continue. List of characters and character states used in both analysis.

19) Costal prickle line number on adaxial	34) Scl line number inner of the root cortex
20) Costal prickle line number on abaxial	35) Scl line number outer of the root cortex
21) Adaxial silica bodies Costal (0) Intercostal (1)	36) Hollowness of culms Hollowed (0) Solid/with parenchyma (1) Solid/with VB (2)
22) Abaxial silica bodies Costal (0) Intercostal (1)	37) Outer frame of culms smooth (0) waved (1)
23) Stomata line numbers on abaxial	38) VB line number of culms
24) Max interstomatal cell line	39) Connection of all VB
25) Average density of stomata	negative (0) positive (1)
26) Average length of stomata (μm)	40) Connection of small VB to epidermis
27) Average width of stomata (μm)	negative (0) positive (1)
28) Length/Width ratio of stomata	41) Connection of large VB to epidermis
29) Average prickle length (μm)	negative (0) positive (1)
30) Average hook length (μm)	42) Average Diameter of culm (μm)
31) Average Mh length (μm)	43) Culm Assimilatory tissue
32) Root hair density Rare (0) Dense (1)	narrow (0) wide (1)
33) Root Sclerenchyma absent (0) inner of the cortex (1) outer of the cortex (2) both inner and outer of the cortex (3)	44) Average length of culm cellular part (μm)

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Root anatomy

Metcalfe (1960) studied anatomy of 345 genera from the family Poaceae and indicated that even though the roots of the family were in different sizes, they had the same general anatomical structure. In this structure, both the hairs and the thick walled cells of outermost layer were presented. The roots of grasses are generally fibrous and adventitious or arising from lower part of the culms (Enstone *et al.*, 2002).

Within the studied species, the root of *Aegilops speltoides* has been found to fit to Metcalfe (1960)'s general root anatomical structure (Figure 4). According to this structure, under the epidermal layer, which is called exodermis, there is a narrow ring of sclerenchyma. It covers cortex, irregularly arranged, thin walled, with wide parenchymatic cells. Cortex covers endodermis, periclycle and vascular cylinder. Same root anatomy is found in the following taxa: *Ae. tauchi*, *Ae. peregrina*, *Ae. triuncialis* subsp *persica* and *bozgadensis*, *Ae. markgrafii, Thinopyrum intermedium*, *Eremopyrum confusum* subsp. *sublanuginosum*, *Eremopyrum orientale*, *Eremopyrum boneopartis*, *Eremopyrum confusum*, *Hordeum distichon* and *H. marinum* var *pubescens* as well as the genera *Triticum, Secale* and *Henrardia*.

According to Arber (1965) roots of many grasses have variation between long unbranched and shorter branched roots. The lateral branch roots, arising from the pericyle, anatomically resemble that of the main root. Moreover, there is no record of existence of contractile roots among the Poaceae. Although most of the root anatomical characters seem to support to the previous studies. General anatomical

root characteristics of the studied taxa are given in Table 4.

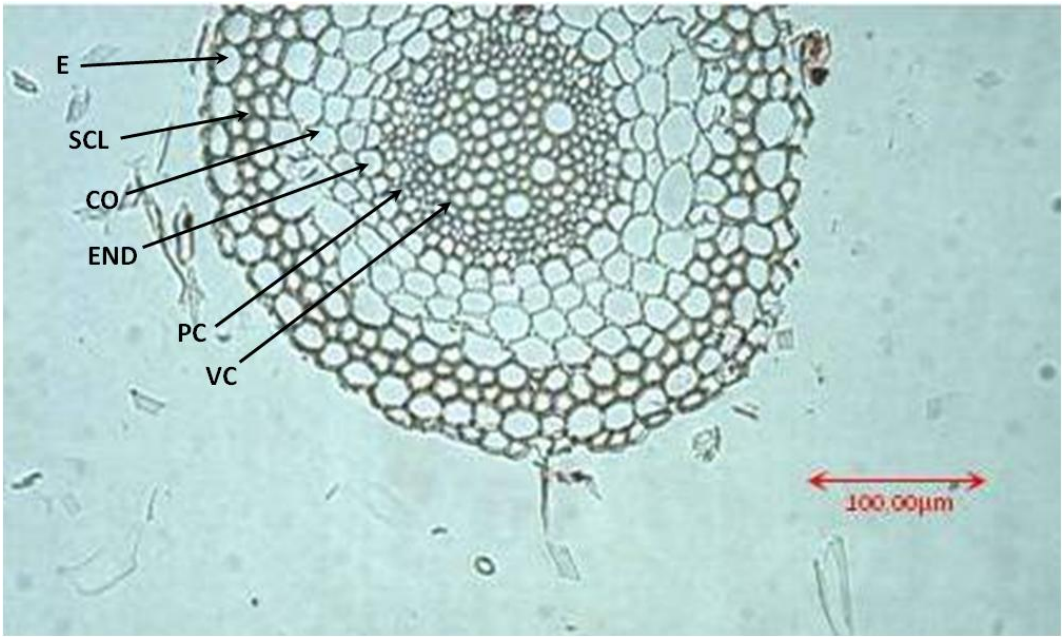

Figure 4. Root transverse section of *Aegilops speltoides* (10X). **CO**: Cortex; **END**: Endodermis; **E**: Epidermis; **PC**: Pericyle; **SCL**: Sclerenchyma; **VC**: Vascular cylinder

3.1.1 Sclerenchyma

By contrast to the general root structure of Metcalfe (1960), within the studied taxa, the position and the number of the sclerenchymatic layer show variation. In some of the taxa, the sclerenchymatic cells of roots are located at the inner part of the cortex, which includes parenchymatic cells. Moreover, there are also roots having sclerenchyma in both inner and outer part of the cortex. Even in some taxa, roots do not seem to have sclerenchyma.

Including sclerencyhma located both at the inner and the outer parts of the cortex, is a character seen in the genus *Heteranthelium* and all the taxa of the genus *Agropyron*. Moreover, it is seen in the roots of *Ae. triuncialis, Aegilops columnaris*, *Elytrigia sosnowskyi*, *Eremopyrum triticeum*, *H. brevisubulatum subsp violaceum*, *H. geniculatum* and *H. murinum* subp. *glaucum*. However, the roots of *Aegilops geniculata*, *Ae. umbellata, Ae. juvenalis*, *H. spontaneum* var *anatolicum*, *H.*

vulgare, Pseudoroegneria libanotica and the genus *Ambylopyrum* do not seem to have any sclerenchymatic layer in their outer part of the endodermis.

The differences of the sclerenchymatic cell locations in the roots of the tribe Triticeae can be listed as;

a) sclerenchyma located at the inner part of the cortex (Figure 5)
b) sclerenchyma located at the outer part of the cortex (Figure 4 and Figure 6)
c) sclerenchyma located both at the inner and outer parts of the cortex (Figure 7)
d) no sclerenchyma (Figure 8)

Number of sclerenchymatic cell layers in the roots within the tribe vary between 1 to 3. There is only one species, *Elymus pycnanthus*, having 3 layers of sclerenchyma in their roots, found in the inner part of the cortex (Figure 9). Moreover, *Secale cereale*, *Eremopyrum confusum* var *glabrum* with var *confusum* and the genus *Henrardia* have 3 layers of sclerenchyma in the outer part of the cortex in their roots and they do not have any sclerenchyma at the inner parts of cortex. However, *Eremopyrum triticeum* and *Elytrigia sosnowskyi* have one layer of sclerenchyma in the inner cortex and 3 layers located in the outer cortex.

According to Esau (1953), sclerenchyma cells are dead at maturity and have thicker secondary walls due to having lignin. Although there is not enough study including the diagnostic property of root sclerenchyma cells; it is obvious that, they have support function in structure (Foster, 1944; Esau, 1953). Moreover, close to the soil surface, it seems that, the outer part of the cortex develops a sclerenchymatic tissue beneath the epidermis, which gives the root mechanical strength (Ennos; 1991). Because of these reasons, the location and the number of the sclerenchymatic layers are not included in the diagnostic character list.

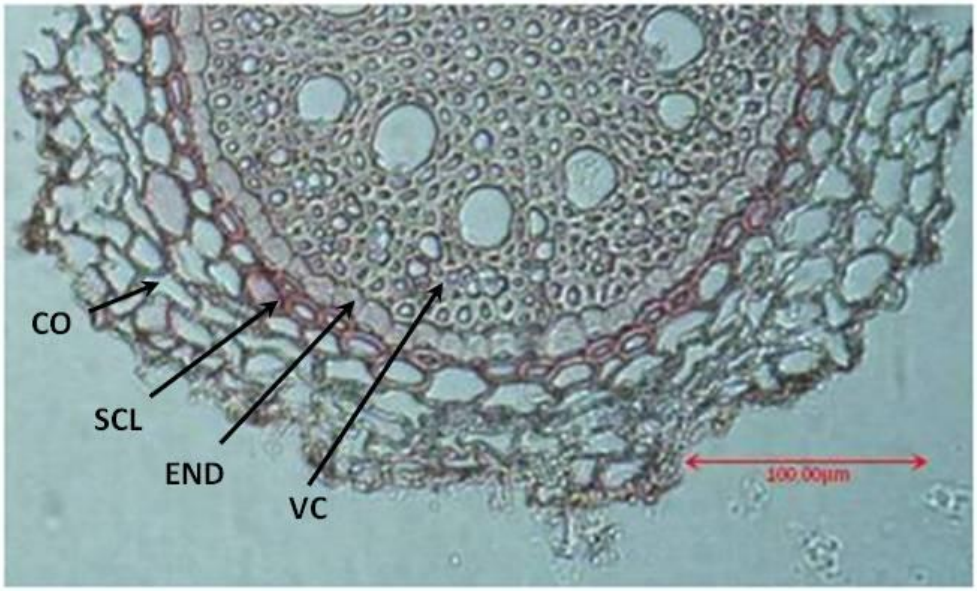

Figure 5. Root transverse section of *Aegilops biuncialis* (10X). **CO**: Cortex; **END**: Endodermis; **SCL**: Sclerenchyma; **VC**: Vascular cylinder

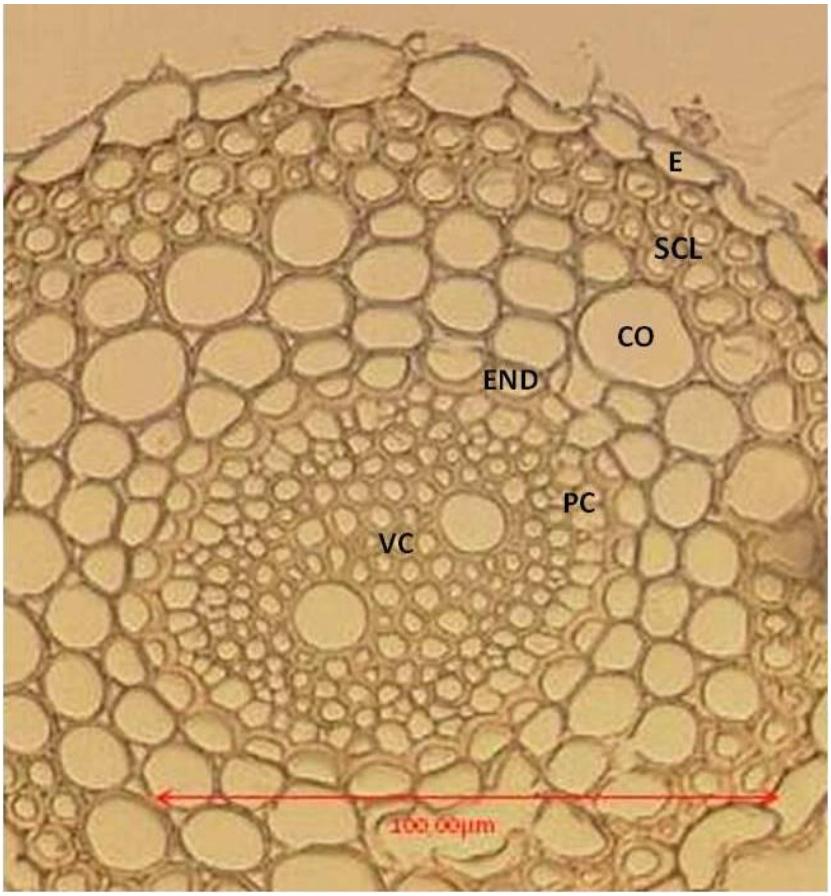

Figure 6. Root transverse section of *Eremopyrum orientale* (40X). **CO**: Cortex; **END**: Endodermis; **PC**: Pericyle; **SCL**: Sclerenchyma; **VC**: Vascular cylinder

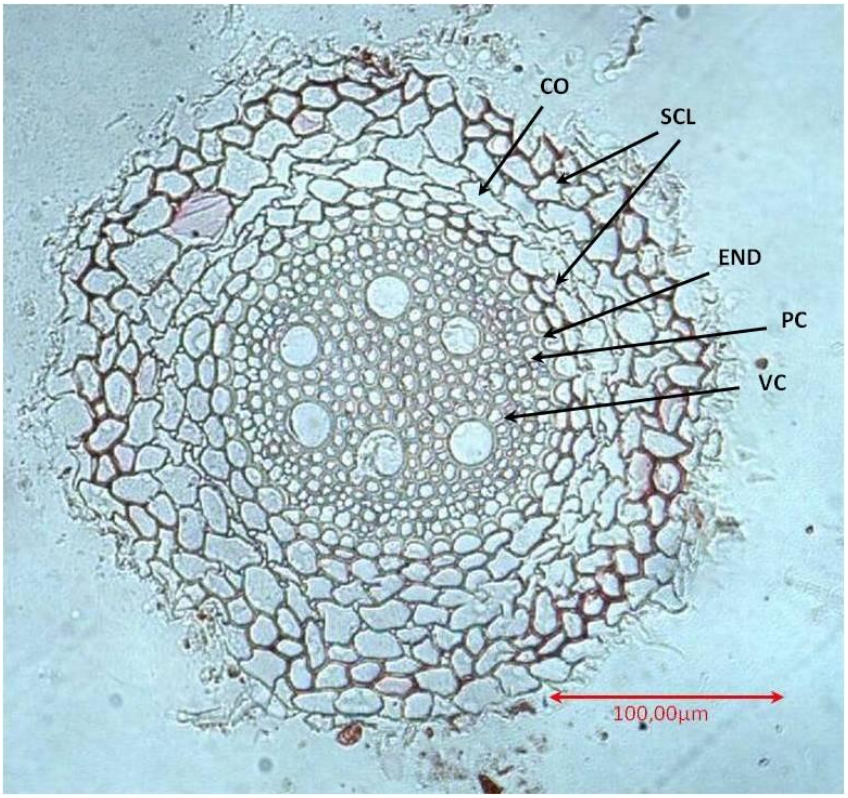

Figure 7. Root transverse section of *Aegilops triuncialis* subsp *triuncialis* (10X). **CO**: Cortex; **END**: Endodermis; **PC**: Pericyle; **SCL**: Sclerenchyma; **VC**: Vascular cylinder

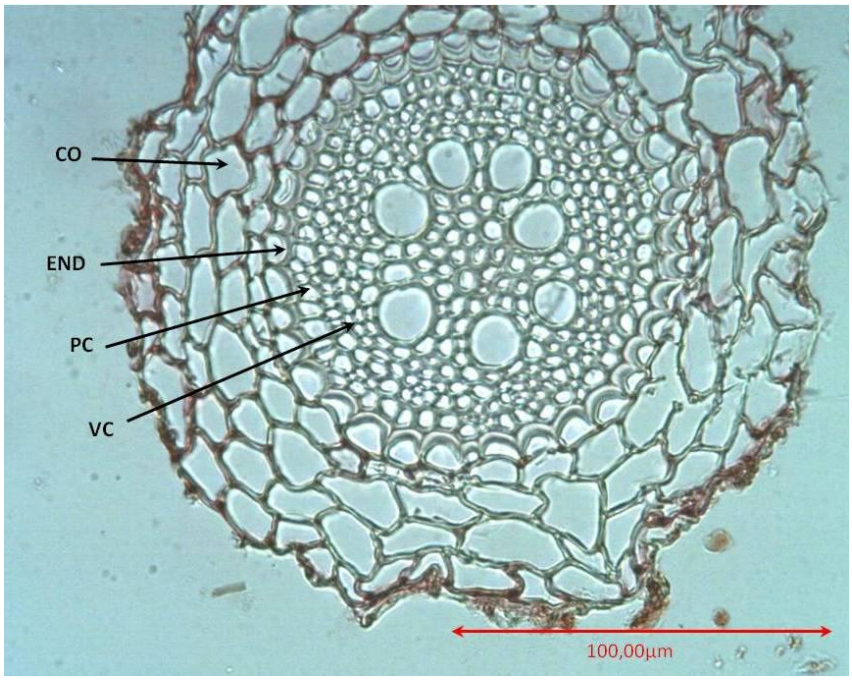

Figure 8. Root transverse section of *Aegilops geniculata* (40X). **CO**: Cortex; **END**: Endodermis; **PC**: Pericyle; **VC**: Vascular cylinder

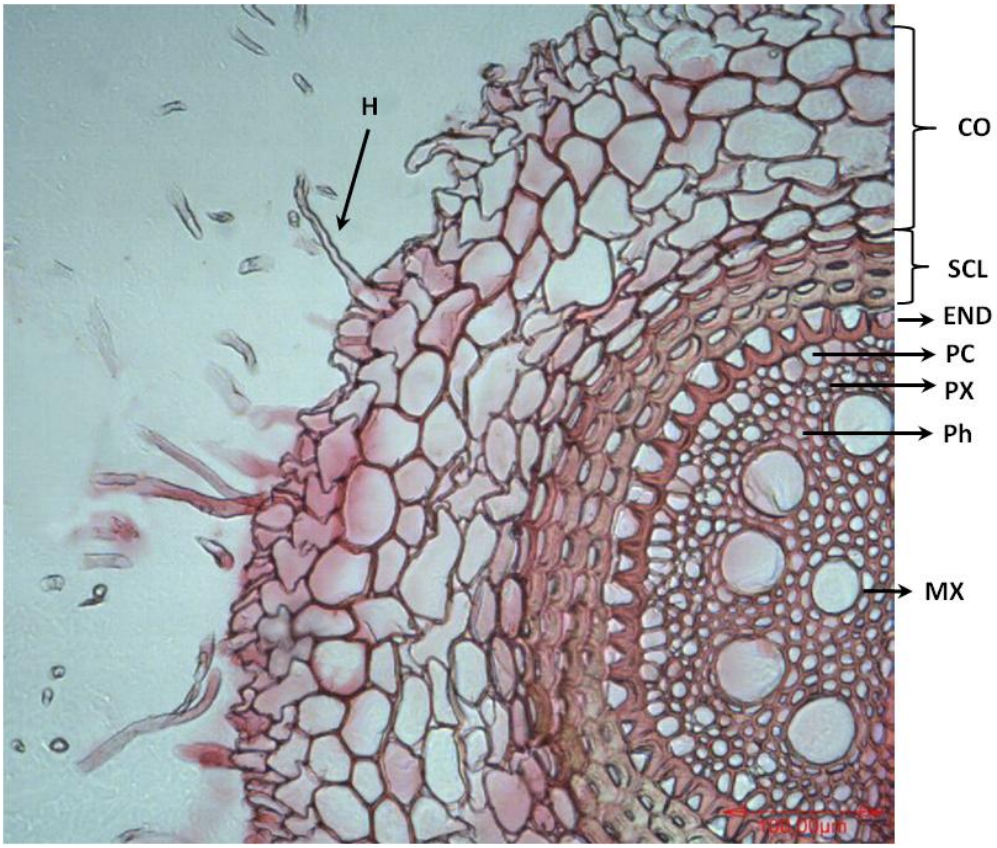

Figure 9. Root transverse section of *Elymus pycnanthus* (10X). **CO**: Cortex; **END**: Endodermis; **H**: Hair; **MX**: Metaxylem; **PC**: Pericyle; **Ph**: Phloem; **PX**: Protoxylem; **SCL**: Sclerenchyma

3.1.2 Cortex

The cortex of the roots has radiating plates of cortical parenchyma (CP) cells with radiating intercellular cavities (IC) and the thickness of the cortex shows variation considering the size of the root (Metcalfe, 1960). In some of the roots within the tribe *Triticeae*, the cortex cavities are not distinguable (Figure 5, 6, 7, 8, 9). However, some other roots have air spaces. In addition to these, there are roots with wide intercellular spaces between the parenchymatic cells of the cortex (Figure 10).

The large air spaces in the cortex of the roots are generally called as aerenchyma (Esau, 1953). Producing root aerenchyma is a kind of adaptation seen in metabolically cheaper roots to explore the soil better. That means; with the help of these cavities, roots reduce the metabolic costs of soil exploration.

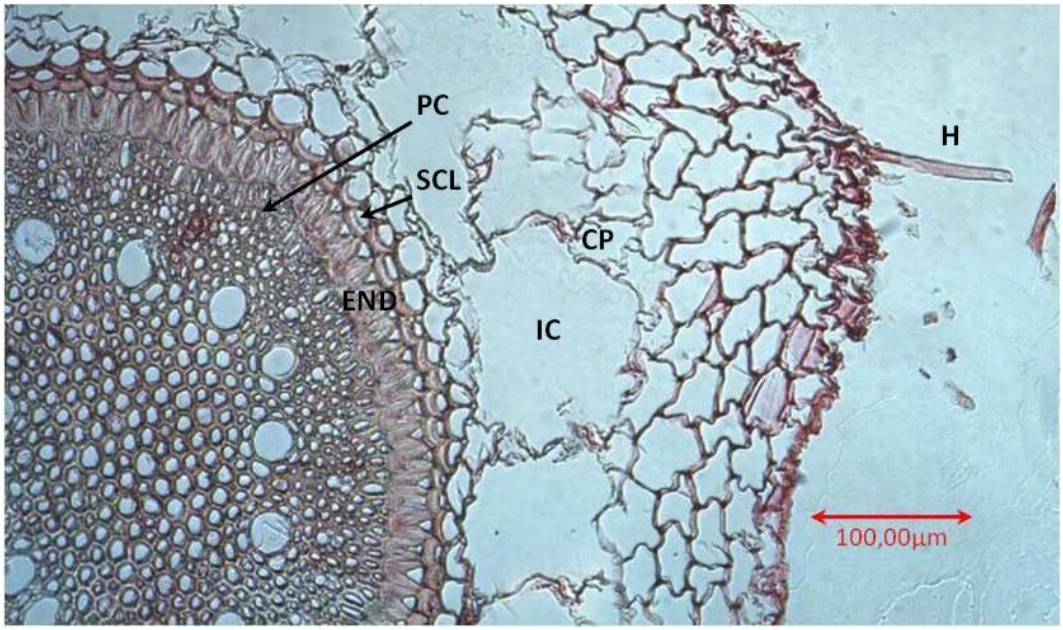

Figure 10. Root transverse section of *Thinopyrum erosiglumis* (10X) **CP**: Cortical Parenchyma cell; **END**: Endodermis; **H**: Hair; **IC**: Intercellular Cavity; **PC**: Pericyle; **SCL**: Sclerenchyma

Table 4. General root anatomical characters

Taxa	**Hair density**	**Sclerenchyma position**	**Sclerenchyma line number**	**Intercellular cavities in cortex**
Aegilops triuncialis subsp. *triuncialis*	dense	both sides of cortex	at inner: 1, at outer:2	some
Aegilops kotschyi	dense	inside of cortex	1	narrow
Aegilops markgrafii	rare	inside of cortex	1	narrow
Ae. speltoides var. *ligustica*	dense	outsideof cortex	2	wide
Ae. speltoides var. *speltoides*	dense	outside of cortex	2	wide
Ae. tauchi	dense	outside of cortex	1	wide
Ae. umbellata	rare	No sclerenchyma	0	some
Ae. biuncialis	rare	inside of cortex	1	narrow
Ae. geniculata	rare	No sclerenchyma	0	narrow
Ae. crassa	rare	inside of cortex	2	some
Ae. peregrina	dense	outside of cortex	2	wide
Ae. cylindrica	rare	outside of cortex	2	some
Ae. columnaris	rare	both sides of cortex	at inner: 2, at outer: 2	wide
Ae. juvenalis	rare	No sclerenchyma	0	wide
Ae. triuncialis subsp. *bozdagensis*	dense	outside of cortex	2	wide
Ae. triuncialis subsp. *persica*	dense	outside of cortex	2	wide
Ae. vavilovi	rare	inside of cortex	1	some
Agropyron incanum	dense	both sides of cortex	at inner: 2, at outer:1	wide
Agr. cristatum subsp. *pectinatum* var. *puberulum*	dense	both sides of cortex	at inner: 2, at outer:1	wide

Table 4 Continue. General root anatomical characters.

Taxa	**Hair density**	**Sclerenchyma position**	**Sclerenchyma line number**	**Intercellular cavities in cortex**
Agr. cristatum subsp. *pectinatum* var. *pectinatum*	dense	both sides of cortex	at inner: 2, at outer:1	wide
Ambylopyrum muticum	dense	No sclerenchyma	0	wide
Crithopsis delileana	dense	inside of cortex	1	narrow
Dasypyrum villosum	dense	inside of cortex	1	wide
Elymus pycnanthus	dense	inside of cortex	3	narrow
Thinopyrum erosiglumis	dense	inside of cortex	1	wide
Elytrigia sosnowskyi	dense	both sides of cortex	at inner: 1, at outer:3	wide
Elymus transhyrcanus	dense	inside of cortex	2	some
Elymus lazicus subsp *lazicus*	dense	inside of cortex	1	wide
Elymus longearistatus	dense	inside of cortex	1	some
Thinopyrum intermedium	dense	outside of cortex	2	wide
Elytrigia repens	dense	inside of cortex	2	wide
Thinopyrum elongatum	rare	inside of cortex	2	wide
Elymus farctus	dense	inside of cortex	3	narrow
Pseudoroegneria libanotica	rare	No sclerenchyma	0	wide
Pseudoroegneria divaricata	dense	inside of cortex	1	wide
Eremopyrum confusum subsp *sublanuginosum*	rare	outside of cortex	2	some
Eremopyrum distans	dense	inside of cortex	1	wide
Eremopyrum triticeum	rare	both sides of cortex	at inner: 1, at outer:3	wide
Eremopyrum orientale	rare	outside of cortex	2	some

Table 4 Continue. General root anatomical characters.

Taxa	**Hair density**	**Sclerenchyma position**	**Sclerenchyma line number**	**Intercellular cavities in cortex**
Eremopyrum boneopartis var *sinaicum*	dense	outside of cortex	2	narrow
Eremopyrum confusum var *confusum*	dense	outside of cortex	3	wide
Eremopyrum confusum var *glabrum*	dense	outside of cortex	3	wide
Henrardia persica var *persica*	rare	outside of cortex	3	wide
Heteranthelium piliferum	dense	both sides of cortex	at inner: 1, at outer:2	wide
Hordelymus europeus	rare	inside of cortex	2	some
H. brevisubulatum subsp violaceum	dense	both sides of cortex	at inner: 2, at outer:2	wide
Hordeum geniculatum	dense	both sides of cortex	at inner: 1, at outer:2	wide
Hordeum distichon	dense	outside of cortex	2	narrow
H. spontaneum var *anatolicum*	dense	No sclerenchyma	0	wide
Hordeum vulgare	dense	No sclerenchyma	0	wide
Hordeum murinum subsp *glaucum*	rare	both sides of cortex	at inner: 1, at outer:2	wide
H. marinum var *pubescens*	rare	outside of cortex	1	wide
Psathyrostachys fragilis	dense	inside of cortex	2	some
Secale sylvestre	dense	outside of cortex	2	some
Secale cereale	dense	outside of cortex	3	some
Triticum monococcum	dense	outside of cortex	2	some
Triticum dicoccon	dense	outside of cortex	2	some
Triticum boeticum	dense	outside of cortex	2	some
Triticum araraticum	dense	outside of cortex	2	some

It can be seen from Table 4 that, wide intercellular cavities are found in the genera *Agropyron, Ambylopyrum, Dasypyrum, Henrardia* and *Heteranthelium*. Moreover, the genus *Hordeum* seems to have wide cavities, except *Hordeum distichon* with its narrow cavities. However, in *Psathyrostachys, Secale* and *Triticum* the intercellular cavities are not as wide as these genera. Also in the genus *Crytopsis*, the cavities are narrow.

3.1.3 Root hairs

A young root is bounded externally by a piliferous layer bearing root hairs. Rosene (1943) pointed out the presence of glabrous root surfaces and the water absorption ability of the cells on these surfaces. Also, according to Metcalfe (1960) species growing in sandy regions have roots with long and persistent hairs.

Transverse sections of the roots show that some epidermal cells have become long unicellular hairs. It is clearly observed that the root epidermal cells of some of the studied taxa are differentiated as unicellular macro-hairs (Figure 7, 9, 10, 11), whereas in some of the other taxa, the hairs do not seem to differ very much (Figure 6, 8). The hair density of the roots can be classified as dense and rare according to the taxa in the tribe. It is clearly seen from the Table 4 that, most of the the genera as *Agropyron, Ambylopyrum, Crithopsis, Dasypyrum, Heteranthelium, Psathyrostachys, Secale* and *Triticum* have dense macrohairs. In the roots of *Hordelymus europeous* hairs are rarely observed. Moreover, in the other genera having more species, the density of hairs in the roots seems to vary from one species to another. The root transverse section of *Psathyrostachys fragilis* is shown in Figure 11 as an example for the roots having dense hairs.

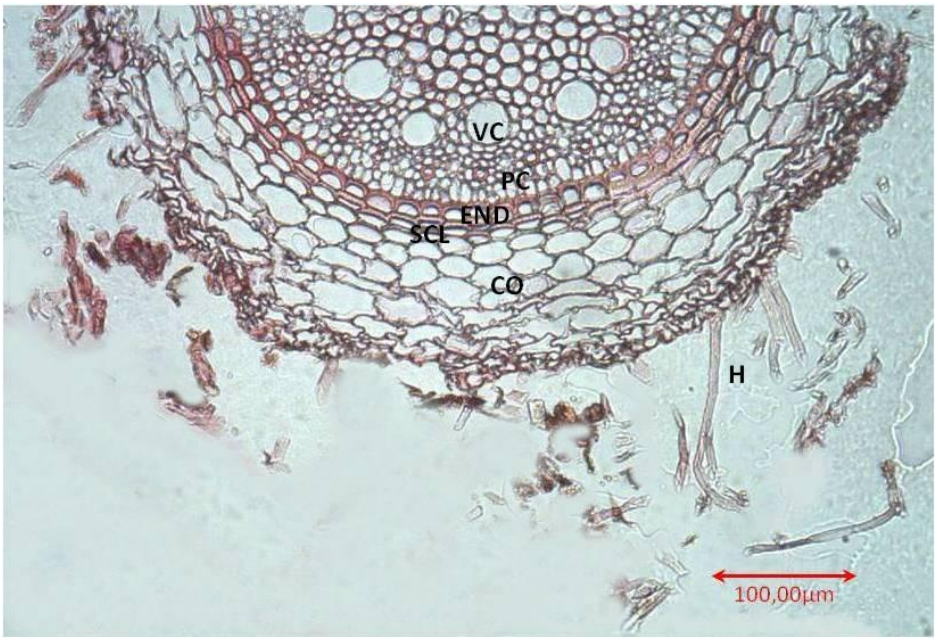

Figure 11. Root transverse section of *Psathyrostachys fragilis* (10X). **CO**: Cortex; **END**: Endodermis; **H**: Hair; **PC**: Pericyle; **SCL**: Sclerenchyma; **VC**: Vascular cylinder

3.1.4 Endodermis and Vascular Cylinder

Jirasek (1964) and Chrtek & Jrasek (1965) established in their works that, there are two types of cells in the endodermis of the root; cells of O-type and those of U-type. It is conclusively seen that, all the studied taxa of the tribe *Triticeae* have U-type endodermis cells in their roots. This U- shaped endodermis covers the metaxylem elements. The number of metaxylem vessels varies in diameter of the roots. There are also protoxylem elements which are much smaller in diameter then metaxylem vessels and small floem elements found in the vascular cylinder (Figure 12). Generally, there is not a central large metaxylem in the middle of the vascular cylinder and all protoxylem elements are separated from endondermis by a pericyle.

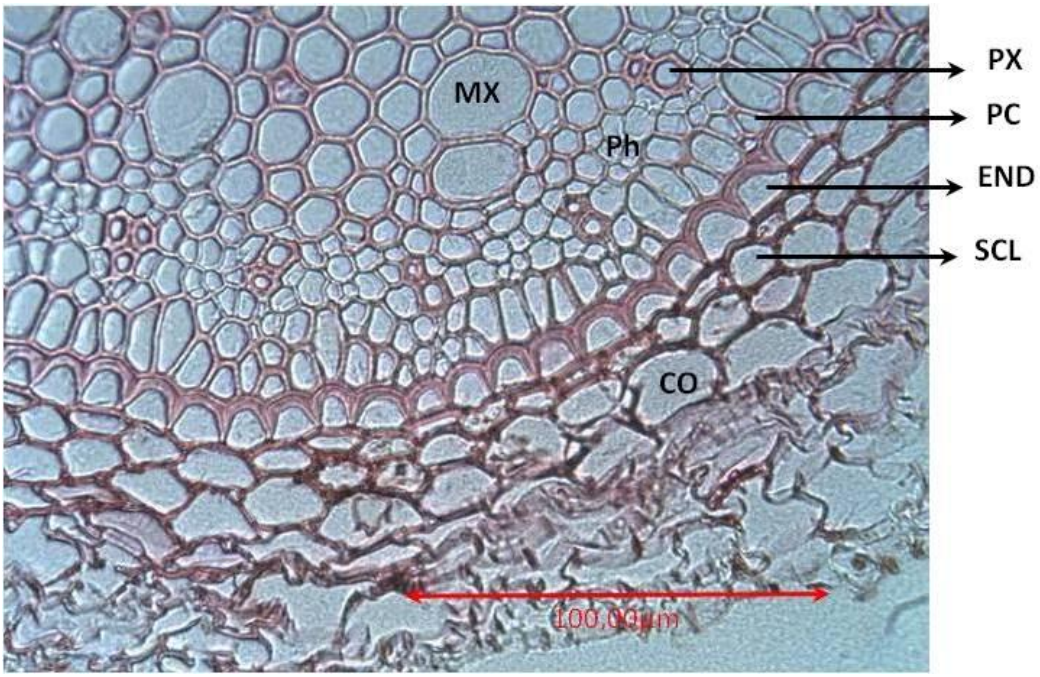

Figure 12. Root transverse section of *Elymus longearistatus* (40X) **CO**: Cortex; **END**: Endodermis; **MX**: Metaxylem; **PC**: Pericyle; **Ph**: Phloem; **PX**: Protoxylem; **SCL**: Sclerenchyma

3.2 Culm anatomy

Generally, the culm tissue is the central axis of a mature shoot, which composed of nodes and internodes. According to Cabi (2010), the length of the culms within the tribe shows a huge variation even in different samples of the same species. For example, the culm length of *Secale cereale* and *Elytrigia canina* changes from 50 cm to 120 cm. Likewise in *Leymus racemosus* and *Hordelymus europeaus*, the length of the culms varies between 50 cm to 100 cm. However, in some of the taxa, as *Eremopyrum boneopartis*, *Hordeum geniculatum* and *Chritopsis delileana* the shortest culms are measured as 1.5, 7, 6.5 cm and the longest culms were measured as 32.5, 32 and 49 cm respectively. Considering the results of the Cabi's (2010) study, the shortest culm was measured in *Eremopyrum boneopartis* as 1.5 cm and the longest culm was observed in *Thinopyrum elongatum* samples as 150 cm. Because of this variation, the length of the culms within the tribe could not be used as a diagnostic character.

According to Waller *et al.* (1985) the differentiation of nodes and internodes was a typical character for many of grasses. The vascular bundles, which connect the horizontal strands at the level of the nodes, grow vertically in culms. The vertical vascular bundles of the culms anastomose into an elaborate plexus or the horizontal strands link up the bud bundles with the vertical system (Arber; 1965).

3.2.1 Nodes

Nodes are the regions clearly observed by the presence of a distinct thickening, the pulvinus, above the nodular part of a culm. According to Arber (1965), in many grasses a curvature occurs with changes in the turgour of pulvini whose colour always associates with the colour of the leaf sheath. Also, if there is a pulvinus, the formation of it may be from the axis, the leaf base or both (Arber, 1965).

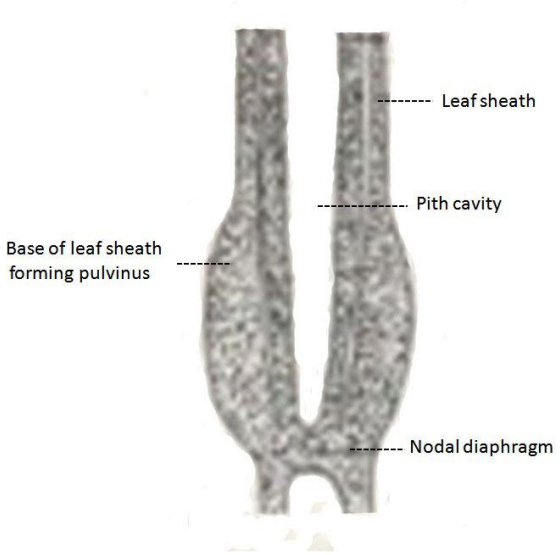

Figure 13. Diagrammatic longitudinal section of node of *Agropyron repens* (Leaf pulvinus) (Arber, 1965).

In the tribe, the pulvinus is formed from the leaf base as shown in Figure 13. It is obviously observed from the transverse sections of the nodes that the pulvini of the culms have no intercellular cavities. The total node numbers of the culms of the tribe were calculated as 2 or 3 (Cabi, 2010).

Majumdar and Saha (1956) studied the nodular parts of the culms of the family and indicated that the nodes of the culms are included in 3 regions as the lower, middle and the upper. In the light of this study, nodular parts of the culms were also sectioned and the results support this study (Figure 14). Moreover, the rhizome and the region between the beginning of the culm at the soil surface and the upper side of the root under the soil surface, same anatomical structure was found (Arber, 1965).

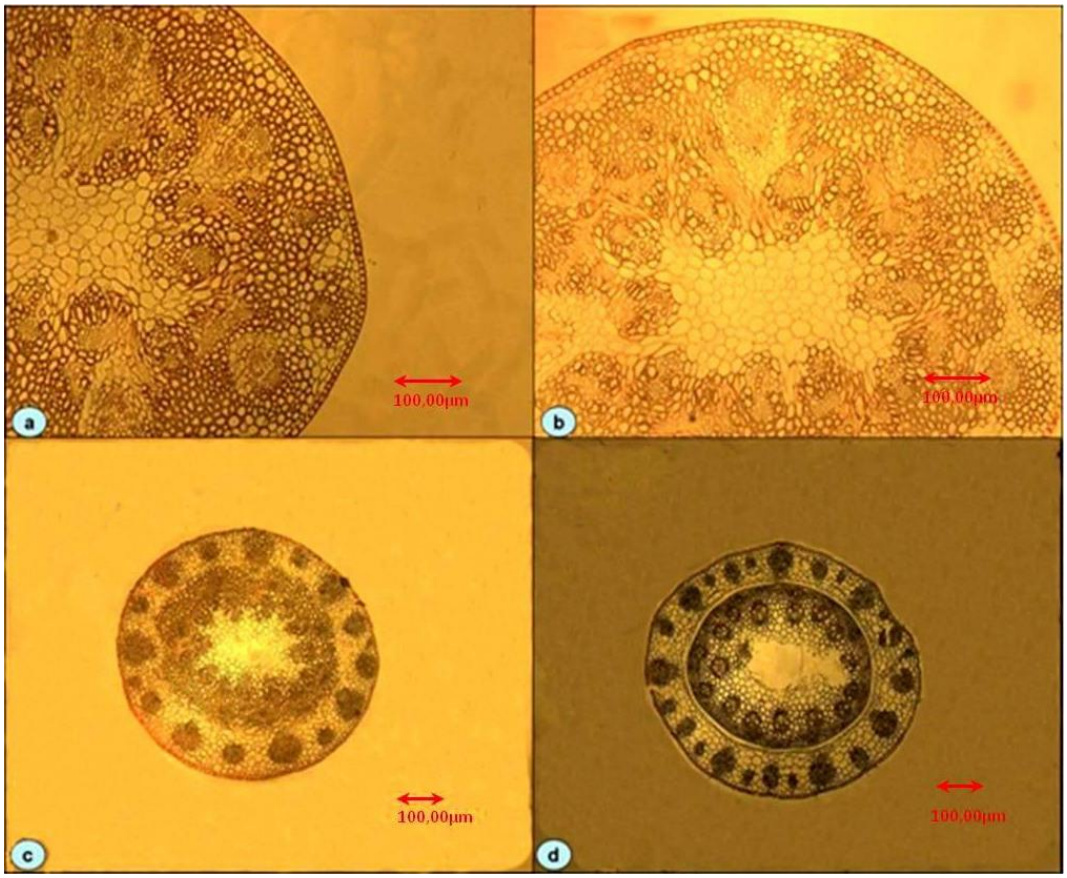

Figure 14. Nodular anatomy of *Hordeum geniculatum*. **(a)** X40 **(b)** X40 **(c)** X10 **(d)** X10

Within the tribe *Triticeae*, at the level of the nodes there are horizontal vascular strands connecting the vertical ones. This network was also observed in the young nodes of a mature plant (Arber, 1965). The nodular 3 regions of the culms can be summarized as given below;

- In the lower region, the vascular bundles begin to appear and the bundles of the sheath leave the inner ring (Figure 14a and Figure 14b).

- In the middle region, the vascular bundles of the culm structure and the bundles of the sheath can be easily separated from each other, but there is still a connection between them (Figure 14c).

- In the upper region, the sheath gets separated from the vascular tissue of the culm (Figure 14d).

3.2.2 Internodes

The culm tranverse sections of the tribe *Triticeae* show that, epidermis is the outermost layer and there are 2 or 3 layers of vascular bundles under the epidermis. The vascular bundles generally have the same structure, closed collateral, but in different sizes for different layers. They are getting larger towards the pith and the number of them shows variation depending on the width of the culms. Moreover, the vascular bundles may connect to each other or to the epidermis with sclerenchymatic cells. The larger vascular bundles of a culm are also embedded in a region comprised of wide parechymatic cells having small triangular intercellular spaces. These parenchyma cells and the spaces between them are getting larger towards to the pith, which can be solid or hollow according to the species.

Figure 15 shows the transverse section of the hollow internode of *Aegilops markgrafii*. From the figure, the layers of the tissue can be easily observed in an order that epidermis covers the 2 layers of vascular bundles, which are connected to eachother with sclerenchymatic cells and the hollow pith is surrounded by parenchymatic cells. This structure is common in most of the studied taxa with little variations.

The anatomical characters showing variation in culm structure of the tribe *Triticeae* can be listed as:

- The number of vascular bundle lines and the connection of vascular bundles to the epidermis or to each other with sclerenchymatic cells,

- The hollowness of the pith,

- The appearance of the outer frame,

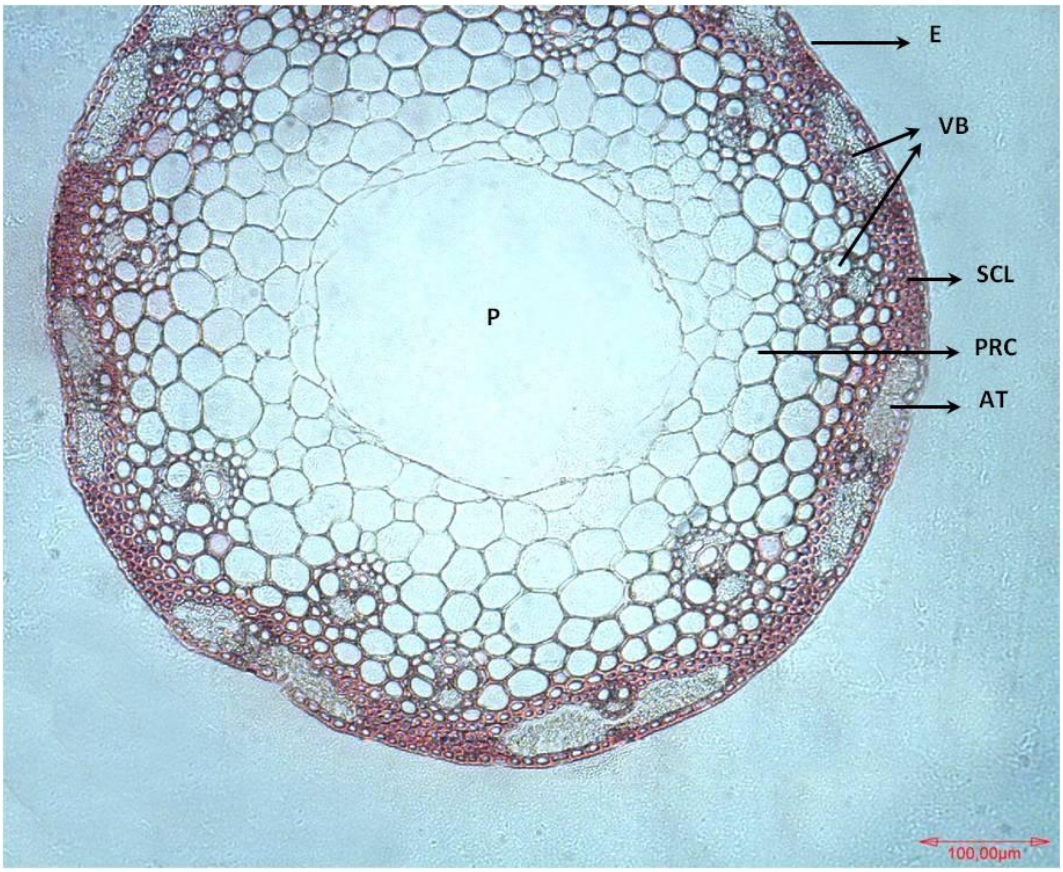

Figure 15. Transverse section of internode from *Aegilops markgrafii*. **AT**: Assimilatory Tissue; **E**: Epidermis; **P**: Pith; **PRC**: Parenchyma; **SCL**: Sclerenchyma; **VB**: Vascular bundle

3.2.2.1 Sclerenchyma and Vascular Bundles

Culm transverse sections of the tribe demonstrate that, the vascular bundles are arranged in 2 or 3 circular rings, composed of circular small bundles and mostly elliptical larger bundles with or without connection between each other. The connection is provided with sclerenchymatic cells.

Figure 16 and Figure 17 are examples for culms including 3 layers of vascular bundles. In Figure 16, the first two layers are connected to the epidermis, but the

third layer is not. On the contrary, in Figure 17 the first layer is the closest to epidermis that has small bundles connected both to the epidermis and to each other, the larger bundles of the rest lines are connected neither to each other nor epidermis.

The sclerenchymatic cells of the culms in the tribe arrange under the epidermis and provide the connection of vascular bundles to the epidermis. There are three kinds of sclerenchymatic cell arrangements in the mature culms of the tribe. These can be listed as:

- All the vascular bundles attach to the epidermis and to each other with the sclerenchyma cells (Figure 15). This structure is the most common one for the tribe.

- Small vascular bundles attach to the epidermis and to each other vith sclerenchymetic cells. However, larger bundles are covered with large parenchymatic cells towards to the pith (Figure 16 and Figure 17).

- Small vascular bundles which are closer to the epidermis are connected to the larger vascular bundles, but they are not connected to the epidermis (Figure 18). In this structure, the larger vascular bundles bind to the epidermis with sclerenchyma.

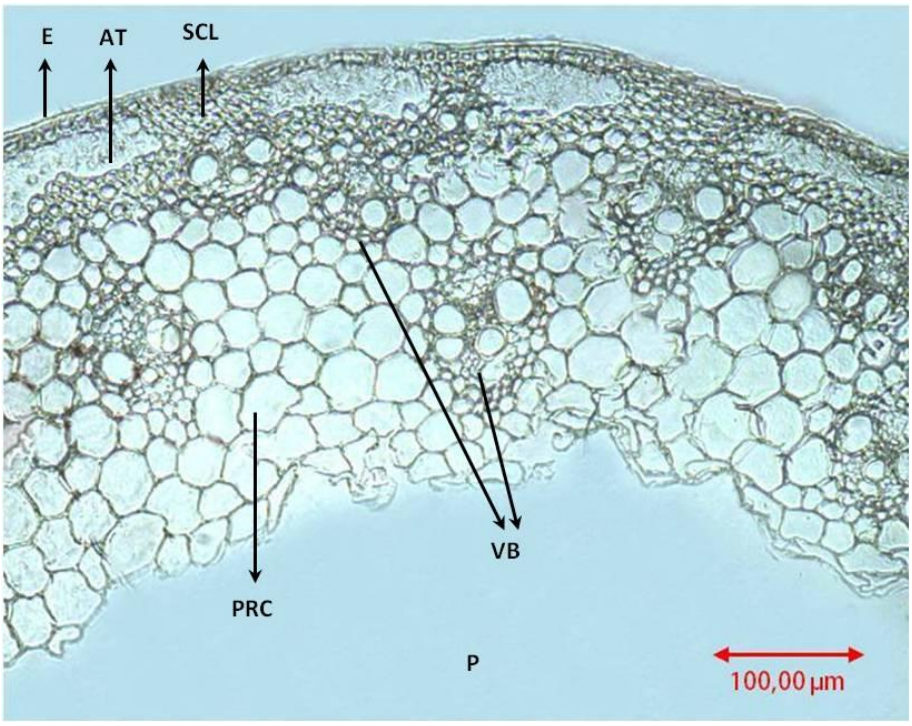

Figure 16. Transverse section of internode from *Hordeum bulbosum*. **AT**: Assimilatory Tissue; **E**: Epidermis; **P**: Pith; **PRC**: Parenchyma; **SCL**: Sclerenchyma; **VB**: Vascular bundle

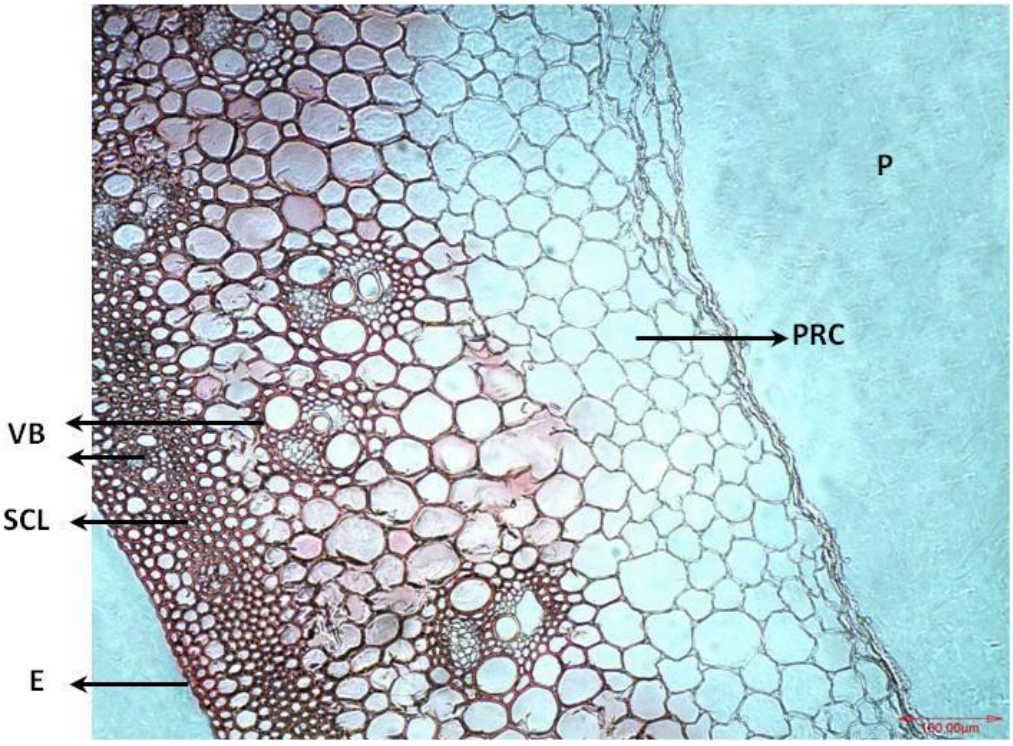

Figure 17. Transverse section of internode from *Secale cereale*. **E**: Epidermis; **P**: Pith; **PRC**: Parenchyma; **SCL**: Sclerenchyma; **VB**: Vascular bundle.

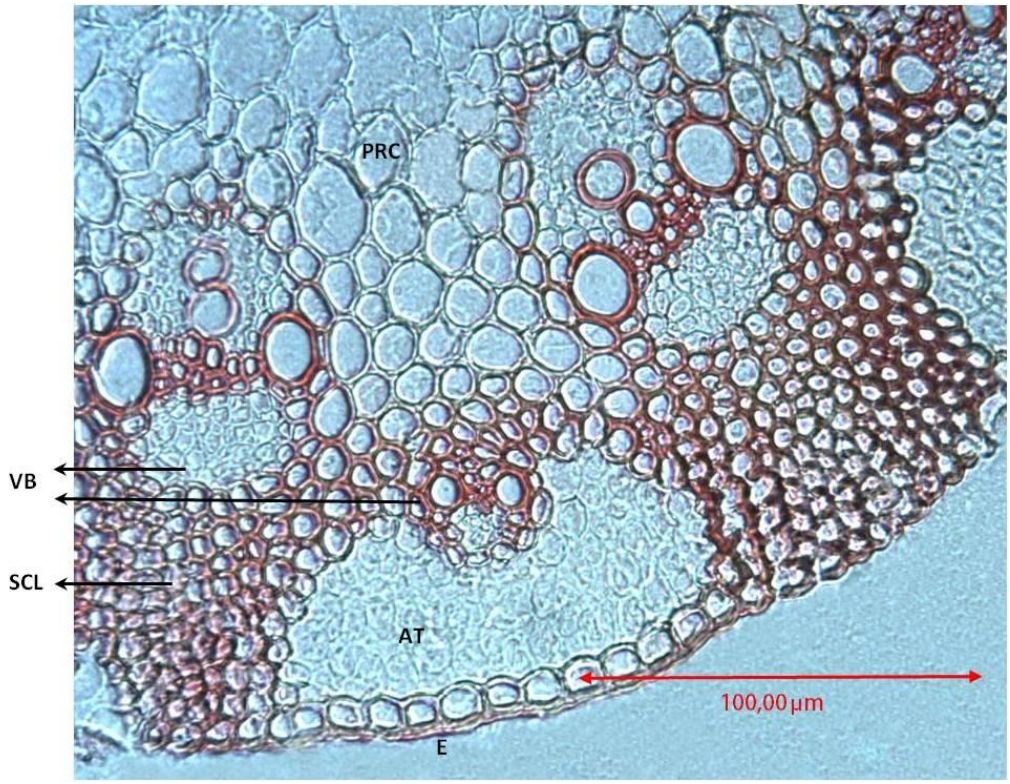

Figure 18. Transverse section of internode from *A. cristatum* subsp. *pectinatum* var. *pectinatum*. **AT**: Assimilatory Tissue; **E**: Epidermis; **PRC**: Parenchyma; **SCL**: Sclerenchyma; **VB**: Vascular bundle.

Within the studied taxa it can be generalized that, if all the vascular bundles connect to each other, the large vascular bundles connect to the epidermis. However, small vascular bundles may not be connected. For example in *Elymus transhyrcanus, Pseudoroegneria libanotica, Hordelymus europeus, Hordeum bulbosum, H. spontaneum* var *anatolicum, H. vulgare, H. murinum* subsp. *glaucum, H. marinum* var *pubescens, Leymus racemosus* subsp. *sabulosus, Secale cereale* and *Triticum monococcum* the small and large vascular bundles are not connected to each other. Moreover, in these taxa the large vascular bundles are not connected to the epidermis. However, in *Ae. speltoides* var. *ligustica, Ae. comosa, Agropyron cristatum* subsp *pectinatum* var *pectiatum, Crithopsis delileana* and *Triticum dicoccon* small vascular bundles are connected to the larger bundles but they are not connected to the epidermis. The genera *Ambylopyrum, Eremopyrum, Henrardia, Heteranthelium, Psathyrostachys* and *Taeniathyrum* have vascular bundles in their culms are both connected to each other and to the epidermis.

With the help of these arrangements, also the assimilatory tissue is formed under the epidermis. The small or the larger bundles connect to the glabrous epidermis by sclerenchymatic girders and these girders separate the near columns of assimilatory tissue, called as chlorenchyma. The width of the assimilatory tissue varies depending on the sclerenchymatic arrangement. In the genera *Agropyron, Crithopsis, Dasypyrum, Heteranthelium, Thinopyrum* and *Leymus* assimilatory tissue appears widely. However, in the genera *Ambylopyrum, Henrardia, Hordelymus* and *Taeniatherum* assimilatory tissue can not be distinguishable.

Generally, in culms having two layered vascular bundles, the bundles are arrangened as a large bundle follows a small bundle and all the bundles are connected to each other with 2-3 layered sclerenchymatic cells. In some stems within this type, all the vascular bundles are also connected to the epidermis with sclerenchymatic cells. While in the other culms, small vascular bundles do not connect to the epidermis.
Moreover, in culms having three layered vascular bundles, the central part of the stem is always hollowed. The stems including 3 layered vascular bundles have small bundles which are close to the epidermis, meaning that vascular bundles are getting larger towards to the pith. Generally, the numbers of vascular bundle show variation depending on width of the stems.

3.2.2.2 The pith

Most of the studies based on the anatomy of grasses suggested that, the central part of the culm transverse sections of the internodes were found to be hollow in many of the taxa of the family (Hackel, 1890; Metcalfe, 1960; Arber, 1965). However, some research findings supported the presence of solid internodes of the culms (Canfield, 1934; Brown *et al.*, 1959). According to Canfield (1934) there was a relation between the solid culm structure and the environment. He also added that having solid culm was an adaptation to arid regions in grasses which were originated in cool temperate regions.

As it was mentioned in previous studies based on the family (Stover, 1934; Pohl and Lersten, 1975), the transverse sections of the internodes of the tribe *Triticeae* show

that, the central parts of the culms are generally hollowed. However, there are also variations. The pith properties of the culms can be categorized as below:

- hollowed culms (Figure 15, 16, 17 and 18),

- solid culms, having a pith coated with parechymatic cells (Figure 19),

- solid culms, having a pith coated with scattered vascular bundles that are surrounded by large parechymatic cells (Figure 20).

As seen in Figure 19, *Crithopsis delileana* has a solid pith covered with large parenchymatic cells (Cabi *et al.* 2010b). The same structure is also seen in *Aegilops comosa*, *Thinopyrum intermedium, Pseudoroegneria libanotica* and *Hordeum murinum* subsp *glaucum*. In *Crithopsis delileana*, vascular bundles arise in a continuous band of circle, including the smaller and the larger bundles connected to each other with sclerenchyma. However, in the others, the large vascular bundles are surrounded with parenchymatic cells and not connected to the small ones or the epidermis.

The second type of solid piths is observed only in *Elymus farctus* (Figure 20) and *Elymus pycnanthus*. In both culms of these species, there is one layer of vascular bundles connected to the epidermis and each other with sclerenchymatic cells. The pith is formed by large parenchyma cells and scattered vascular bundles. The vascular bundles of the pith are rather larger then the others closer to the epidermis.

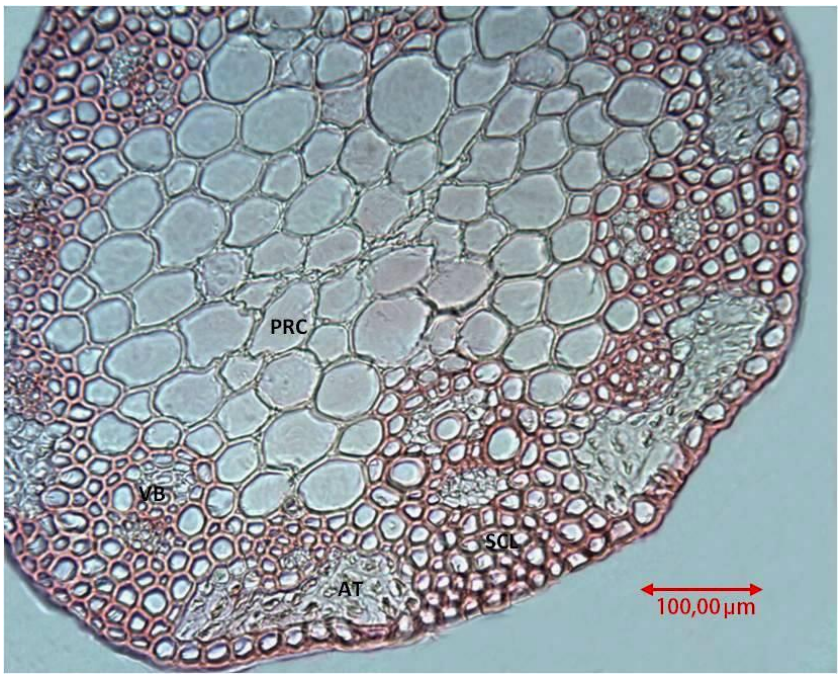

Figure 19. Transverse section of internode from *Crithopsis delileana.* (Cabi *et al.* 2010b). **AT**: Assimilatory Tissue; **PRC**: Parenchyma; **SCL**: Sclerenchyma; **VB**: Vascular bundle.

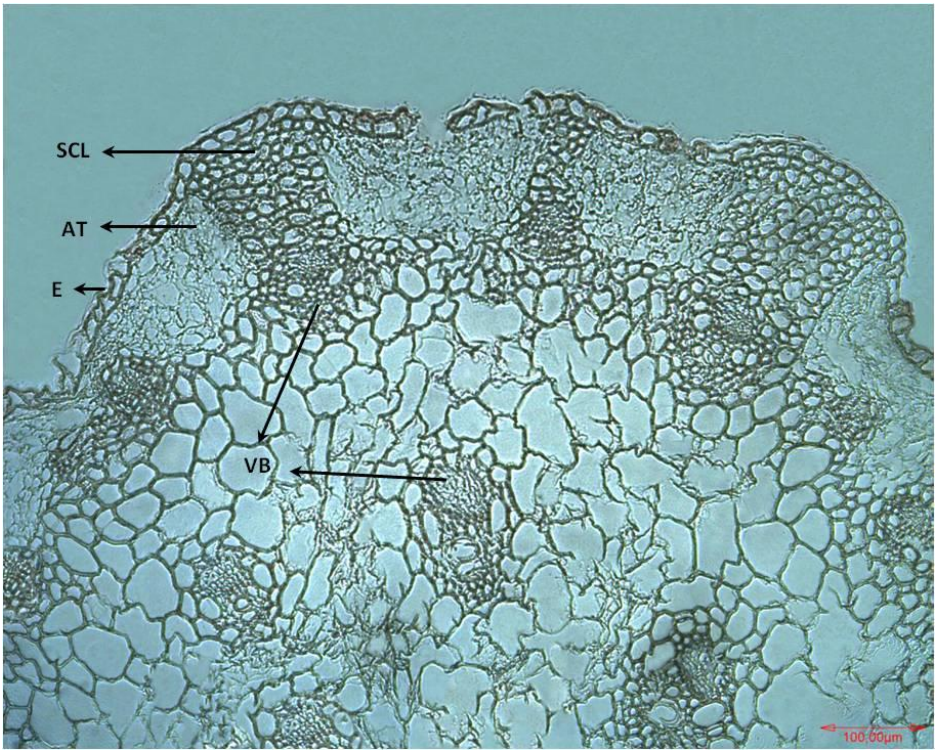

Figure 20. Transverse section of internode from *Elymus farctus*. **AT**: Assimilatory Tissue; **E**: Epidermis; **PRC**: Parenchyma; **SCL**: Sclerenchyma; **VB**: Vascular bundle.

3.2.2.3 Outer frame

The transverse sections from the culms of the tribe demonstrate that, the shape of the culms can be wavy or smooth. The culm section of *Leymus racemosus* subsp. *sabulosus* (Figure 21) is a good example for the culms having wavy outer frame (Mavi *et al.* 2011c). As seen in Figure 21, the vascular bundles are arranged in two circular rings, composed of the circular small bundles and the larger bundles with no connection between each other. The small vascular bundles, connecting each others with sclerenchyma, are also attached to the epidermis with 4-5 layers of sclerenchymatic cells, which seem to make epidermis to form domes outwardly. Moreover, at this attachment point the epidermal cells tend to get larger. Moreover, the assimilatory tissue, forming about 3-4 cells wide, thin and flattened layers, is covered by sclerenchyma, not only subtending the epidermis but also surrounding the small bundles. Irregularly arranged large bundles of the inner ground tissue have no relation with sclerenchyma. Each large bundle has protoxylem vessels between large metaxylem vessels. The ground tissue is made of parenchymatic cells. The central part of this tissue seems to be hollowed.

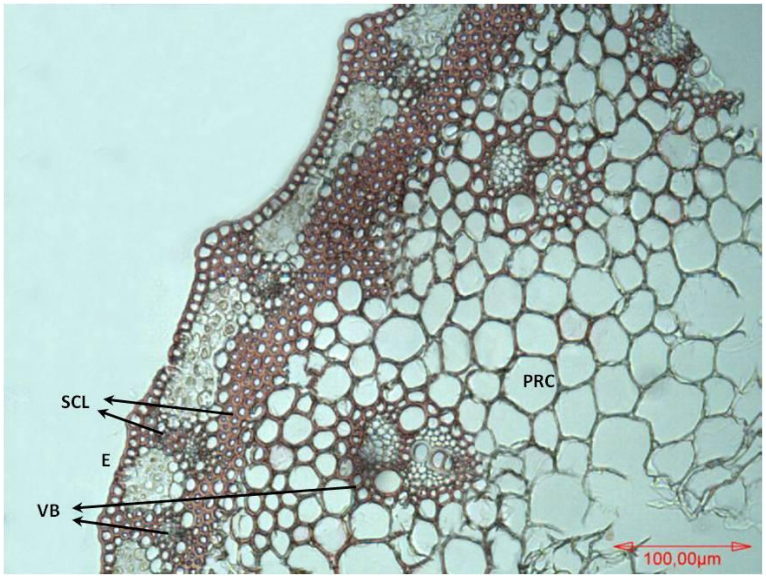

Figure 21. Transverse section of internode from L*eymus racemosus* subsp. *sabulosus* (Mavi *et al.* 2011c). **E**: Epidermis; **PRC**: Parenchyma; **SCL**: Sclerenchyma; **VB**: Vascular bundle.

Ae. speltoides var. *ligustica* and *Ae. comosa* (Figure 22) also have culms having waved outer frames. However, in these taxa, the epidermal cells are getting smaller in the connection points of the sclerenchyma cells. Moreover, only the large vascular bundles are connected to the epidermis (Figure 22).

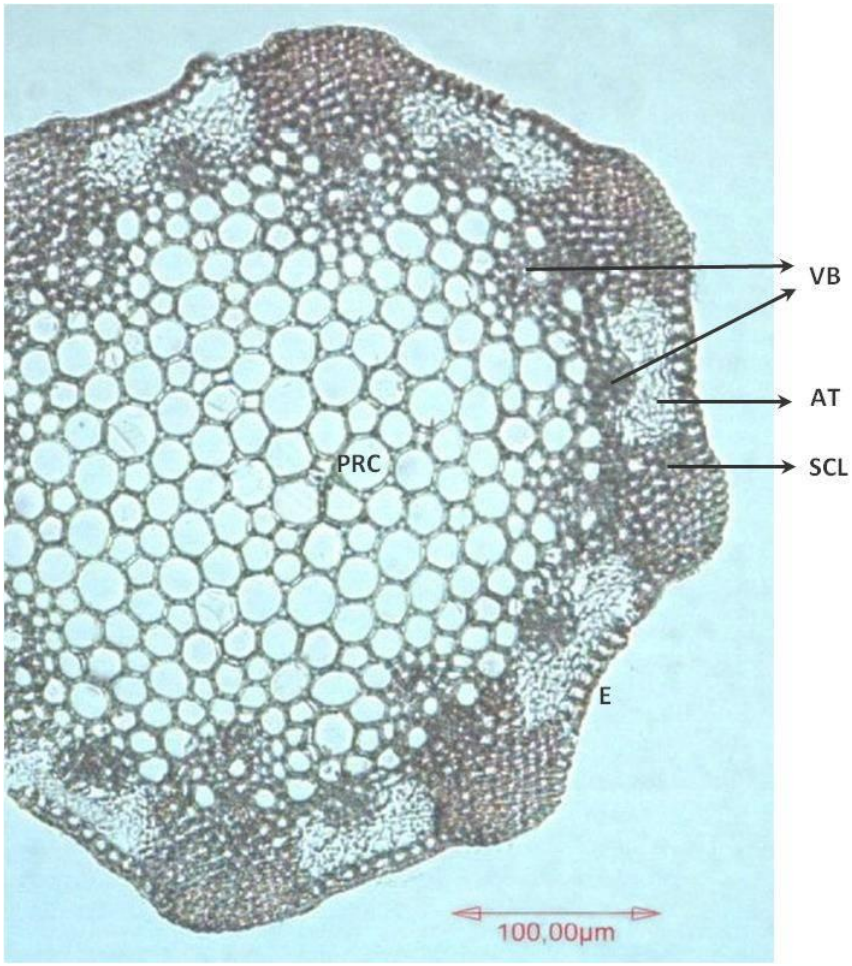

Figure 22. Transverse section of internode from *Aegilops comosa*. **AT**: Assimilatory tissue; **E**: Epidermis; **PRC:** Parenchyma; **SCL**: Sclerenchyma; **VB**: Vascular bundle.

The smooth outer frame within the culm sections is mostly seen in the taxa of the tribe *Triticeae*. Figure 23 shows the transverse section of the culm of *Hordelymus europeus*. In this structure, the same sized epidermal cells almost wholly cover 5-6 layers of sclerenchymatic cells which connect to the small vascular bundles. There are also two layers of vascular bundles, getting larger towards to the hollowed pith.

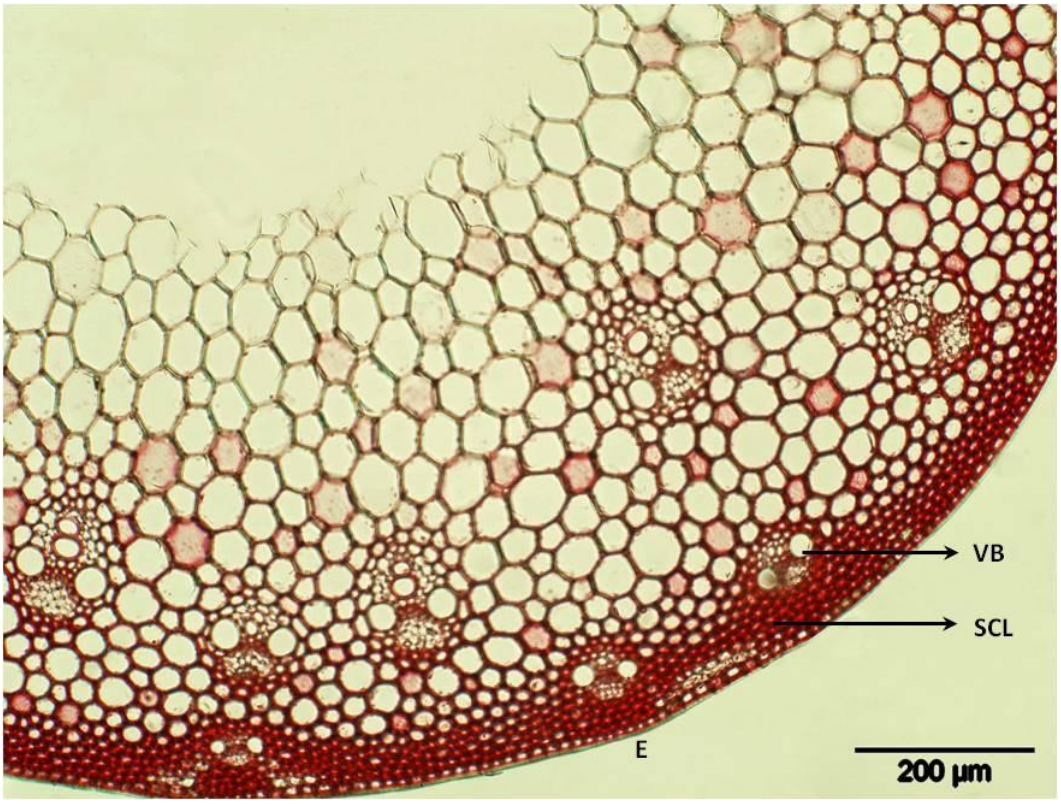

Figure 23. Transverse section of internode from *Hordelymus europeaus*. **E**: Epidermis; **SCL**: Sclerenchyma; **VB**: Vascular bundle.

Our research findings mostly support the previous studies concerning the culms of the family (Stover, 1934; Stover, 1951; Pohl and Lersten, 1975). However, the correlation with the hollowness of the culms and the arrangement of vascular bundles can not be generalized as given by Esau (1953)'s study. Esau (1953) mentioned that hollowed culms have only one layer of vascular bundles, one of which was closer to the epidermis. Moreover, in the solid culms, the vascular bundles were scattered (Esau, 1953). However, the hollowed culms of the tribe *Triticeae* have also three layers of vascular bundles, one or two of which are close to the epidermis and in the solid culms, there are both parenchymatic cells and scattered vascular bundles cover the piths. Furthermore, the outer frame of the culms show two types as waved and smooth. Lastly, because of the differences in the arrangement of sclerenchymatic cells under the epidermis, the assimilatory tissue may be wide or narrow. The general characters of the culms are summarized in Table 5.

Table 5. General anatomical characters of the culms

Taxa	hollowness	outer frame	*VB line number	connection of all *VB	diameter	connection of small *VB and epidermis	connection of large *VB and epidermis	*AT	Cellular length between epidermis and pith
Aegilops triuncialis subsp. *triuncialis*	hollowed	smooth	2	positive	630,25 ± 9,912	positive	positive	wide	232,56 ± 2,481
Aegilops kotschyi	hollowed	smooth	2	positive	851,74 ± 12,373	positive	positive	wide	296,21 ± 3,517
Aegilops markgrafii	hollowed	smooth	2	positive	661,71 ± 9,979	positive	positive	wide	202,90 ± 5,462
Ae. speltoides var *ligustica*	hollowed	waved	2	positive	966,69 ± 9,378	negative	positive	wide	222,41 ± 3,807
Ae. speltoides var *speltoides*	hollowed	smooth	2	positive	660,15 ± 5,871	positive	positive	wide	221,53 ± 6,435
Ae. tauchi	hollowed	smooth	2	positive	517,04 ± 7,747	positive	positive	wide	198,61 ± 4,594
Ae. umbellata	hollowed	smooth	2	positive	605,68 ± 6,770	positive	positive	narrow	136,84 ± 7,211
Ae. biuncialis	hollowed	smooth	2	positive	530,03 ± 9,890	positive	positive	wide	163,43 ± 7,441
Ae. geniculata	hollowed	smooth	2	positive	540,37 ± 7,461	positive	positive	wide	127,438 ± 2,731
Ae. crassa	hollowed	smooth	2	positive	984,07 ± 13,291	positive	positive	narrow	364,31 ± 7,987
Ae. peregrina	hollowed	smooth	2	positive	675,50 ± 13,516	positive	positive	wide	230,25 ± 8,926
Ae. juvenalis	hollowed	smooth	2	positive	677,52 ± 13,061	positive	positive	wide	204,85 ± 3,535
Ae. triuncialis subsp *bozdagensis*	hollowed	smooth	2	positive	749,12 ± 9,001	positive	positive	wide	266,05 ± 4,249
Ae. triuncialis subsp *persica*	hollowed	smooth	2	positive	607,56 ± 17,401	positive	positive	narrow	163,29 ± 2,903
Ae. vavilovi	hollowed	smooth	2	positive	968,76 ± 15,091	positive	positive	wide	302,85 ± 6,731

***AT**: Assimilatory tissue; **VB**: Vascular bundles

Table 5 Continue. General anatomical characters of the culms

Taxa	hollowness	outer frame	*VB line number	connection of all *VB	diameter	connection of small *VB and epidermis	connection of large *VB and epidermis	*AT	Cellular length between epidermis and pith
Ae. comosa	solid (parenchyma)	waved	2	positive	415,79 ± 10,471	negative	positive	wide	0
Agropyron incanum	hollowed	smooth	2	positive	626,25 ± 15, 277	positive	positive	wide	215,69 ± 8,304
Agr. cristatum subsp. *pectinatum* var. *pectinatum*	hollowed	smooth	2	positive	717,17 ± 14,851	negative	positive	wide	173,15 ± 7,481
Ambylopyrum muticum	hollowed	smooth	2	positive	798,24 ± 18,747	positive	positive	narrow	287,63 ± 2,274
Crithopsis delileana	solid (parenchyma)	smooth	2	positive	270,07 ± 15,582	negative	positive	wide	0
Dasypyrum villosum	hollowed	smooth	2	positive	1262,29 ± 13,870	positive	positive	wide	304,62 ± 2,632
Elymus pycnanthus	solid (vascular bundles)	smooth	2	positive	2131,37 ± 10,313	positive	positive	narrow	0
Thinopyrum erosiglumis	hollowed	smooth	2	positive	773,18 ± 3,721	positive	positive	wide	354,09 ± 6,985
Elytrigia sosnowskyi	hollowed	smooth	2	positive	852,88 ± 12,837	positive	positive	wide	350,56 ± 3,641
Elymus transhyrcanus	hollowed	smooth	2	negative	939,22 ± 1,829	positive	negative	narrow	505,97 ± 7,781
Elymus lazicus subsp *lazicus*	hollowed	smooth	2	positive	861,97 ± 9,299	positive	positive	wide	281,95 ± 9,356

***AT**: Assimilatory tissue; **VB**: Vascular bundles

Table 5 Continue. General anatomical characters of the culms

Taxa	hollowness	outer frame	*VB line number	connection of all *VB	diameter	connection of small *VB and epidermis	connection of large *VB and epidermis	*AT	Cellular length between epidermis and pith
Elymus longearistatus	hollowed	smooth	2	positive	683, 79 ± 10,785	positive	positive	wide	301,91 ± 7,853
Thinopyrum intermedium	solid (parenchyma)	smooth	2	positive	706, 23 ± 10,191	positive	positive	wide	0
Elytrigia canina	hollowed	smooth	2	positive	1382,87 ± 13,240	positive	positive	narrow	252,93 ± 6,425
Elymus farctus	solid (vascular bundles)	smooth	2	positive	1645,57 ± 14, 360	positive	positive	wide	0
Pseudoroegneria libanotica	solid (parenchyma)	smooth	3	negative	1075,49 ± 11,253	positive	negative	wide	0
Pseudoroegneria divaricata	hollowed	smooth	2	positive	564,03 ± 12,321	positive	positive	wide	182,29 ± 6,991
Eremophyrum confusum subsp. *sublanuginosum*	hollowed	smooth	2	positive	813,96 ± 12,277	positive	positive	narrow	159,76 ± 5,723
Eremophyrum distans	hollowed	smooth	2	positive	800,01 ± 11,724	positive	positive	wide	155,68 ± 6,094
Eremopyrum triticeum	hollowed	smooth	2	positive	791,57 ± 12,096	positive	positive	wide	149,96 ± 5,862
Eremopyrum orientale	hollowed	smooth	2	positive	800,58 ± 11,491	positive	positive	wide	158,94 ± 6,102
Eremopyrum boneopartis var *sinaicum*	hollowed	smooth	2	positive	797,56 ± 12,372	positive	positive	narrow	155,67 ± 5,911

***AT**: Assimilatory tissue; **VB**: Vascular bundles

Table 5 Continue. General anatomical characters of the culms

Taxa	hollowness	outer frame	*VB line number	connection of all *VB	diameter	connection of small *VB and epidermis	connection of large *VB and epidermis	*AT	Cellular length between epidermis and pith
Eremopyrum confusum var *confusum*	hollowed	smooth	2	positive	810,68 ± 10,582	positive	positive	narrow	159,96 ± 4,991
Eremopyrum confusum var *glabrum*	hollowed	smooth	2	positive	811,98 ± 11,475	positive	positive	narrow	159,67 ± 5,062
Henrardia persica var *persica*	hollowed	smooth	2	positive	360,45 ± 3,934	positive	positive	narrow	160,65 ± 1,601
Heteranthelium piliferum	hollowed	smooth	2	positive	435,55 ± 5,191	positive	positive	wide	106,33 ± 5,192
Hordelymus europeus	hollowed	smooth	3	negative	2062,85 ± 12,558	positive	negative	narrow	637,77 ± 6,779
H. brevisubulatum subsp *violaceum*	hollowed	smooth	2	positive	592,19 ± 10,157	positive	positive	narrow	242,50 ± 12,993
Hordeum geniculatum	hollowed	smooth	2	positive	1002,86 ± 10,826	positive	positive	narrow	254,65 ± 10,101
Hordeum bulbosum	hollowed	smooth	3	negative	1463,45 ± 15,385	positive	negative	wide	321,87 ± 4,333
Hordeum distichon	hollowed	smooth	2	positive	1459,25 ± 4,681	positive	positive	narrow	177,40 ± 6,571
H. spontaneum var anatolicum	hollowed	smooth	2	negative	2024,06 ± 14,823	positive	negative	wide	230,46 ± 4,255
Hordeum vulgare	hollowed	smooth	2	negative	1702,94 ± 15, 171	positive	negative	wide	306,66 ± 7,245
Hordeum murinum subsp *glaucum*	solid (parenchyma)	smooth	2	negative	684,41 ± 9,383	positive	negative	narrow	0
H. marinum var *pubescens*	hollowed	smooth	2	negative	797,19 ± 14,418	positive	negative	narrow	205,86 ± 0,696

***AT**: Assimilatory tissue; **VB**: Vascular bundles

Table 5 Continue. General anatomical characters of the culms

Taxa	hollowness	outer frame	*VB line number	connection of all *VB	diameter	connection of small *VB and epidermis	connection of large *VB and epidermis	*AT	Cellular length between epidermis and pith
Leymus racemosus subsp *sabulosus*	hollowed	waved	2	negative	1384,75 ± 15, 114	positive	negative	wide	376,33 ± 9,212
Leymus cappadocicus	hollowed	smooth	2	positive	715,73 ± 14, 471	positive	positive	wide	196, 64 ± 3,702
Psathyrostachys fragilis	hollowed	smooth	2	positive	881,26 ± 15,664	positive	positive	narrow	250,91 ± 1,373
Secale sylvestre	hollowed	smooth	2	positive	810,31 ± 14,211	positive	positive	wide	228,23 ± 2,125
Secale cereale	hollowed	smooth	3	negative	1769,14 ± 14,650	positive	negative	narrow	551,75 ± 9,941
Triticum monococcum	hollowed	smooth	2	negative	671,21 ± 14,561	positive	negative	narrow	211,05 ± 1,425
Triticum dicoccon	hollowed	smooth	2	positive	1504,42 ± 21,364	negative	positive	wide	363,67 ± 9,483
Triticum boeticum	hollowed	smooth	2	positive	1074,33 ± 7,290	positive	positive	wide	426,08 ± 4,493
Triticum arareticum	hollowed	smooth	2	positive	1216,36 ± 16,903	positive	positive	wide	245,09 ± 10,051
Taeniatherum caput medusa	hollowed	smooth	2	positive	793,48 ± 11,561	positive	positive	narrow	196,50 ± 4,663

***AT**: Assimilatory tissue; **VB**: Vascular bundles

3.3 Leaf anatomy and micromorphology

Leaves of the grasses are grown from an angle of every node and they are composed of two parts named as the leaf sheath, wrapping the culms and the leaf blade, the expanded part. The anatomical studies on the family Poaceae show that leaf anatomy includes more diagnostic characters than stem or root anatomy of the family (Vukolov, 1929; Gielwanowska *et al.*, 2005; Metcalfe,1960; Brown, 1958). In the light of these studies, it is valuable to say that the transverse sections of leaves of *Triticeae* show that, the leaf blades have important taxonomic characters. (Mavi *et al.*, 2011a,b,c).

The anatomical characters of the leaves in the tribe can be handled in two main groups as;

- The transverse sections of the leaves,
- The micromorphology of the leaf surfaces.

3.3.1 Transverse sections of leaves

The general structure of the transverse sections of the leaves is simply the same (Figure 24). In this structure, between the abaxial (lower) and the adaxial (upper) epidermises, there is mesophyll, which is not distinguished as palisade and spongy. The mesophyll of the leaves have two distinct structures, one of which is the vascular bundles surrounded by double type bundle sheaths. The inner sheaths of the bundles are formed by sclerenchymatic cells and this sheath wholly covers the bundles. The outer sheath is composed of large parenchymatic cells and halfly covers the bundles and the inner ones. The other structure in the mesophyll is the sclerenchymatic cells around the vascular bundles towards to the margins of the leaves.

Generally, the closed collateral vascular bundles of the leaves make a single layer through the sections. Mostly, there are two types of vascular bundles, small and large ones follow each other in one line. In a leaf including the midrib, the vascular bundle of the midrib is larger than the other vascular bundles of the leaf.

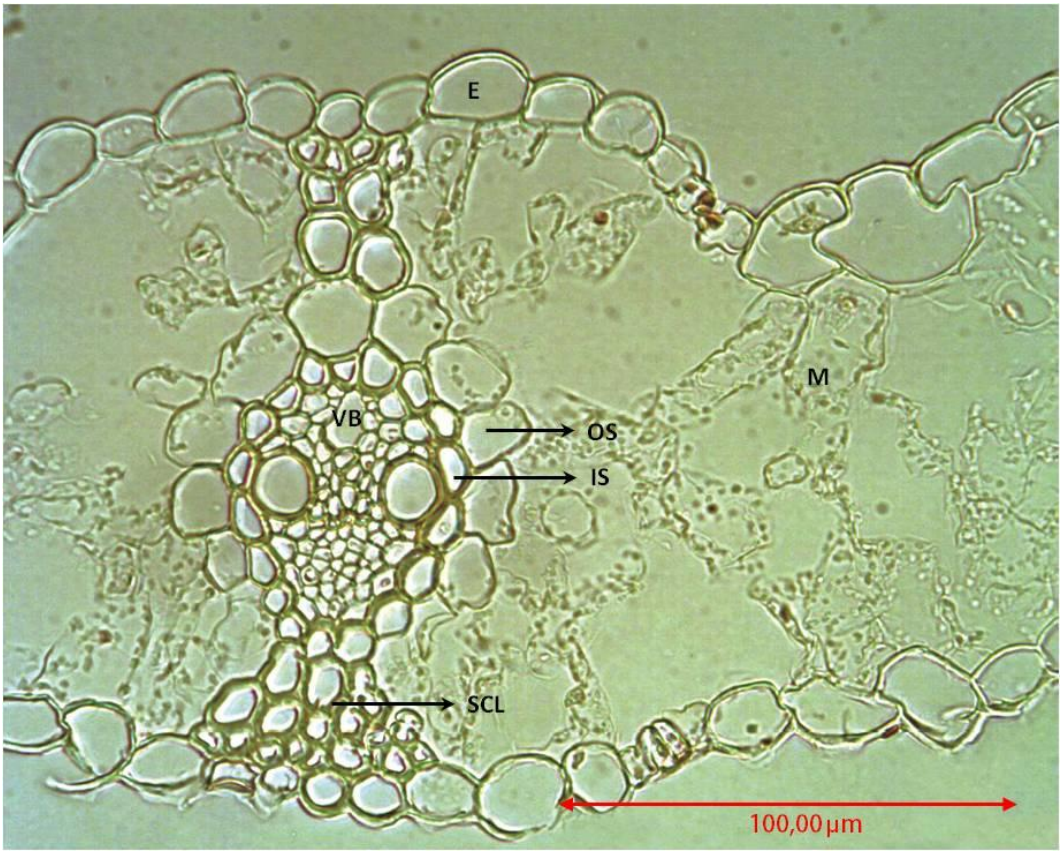

Figure 24. Transverse section of leaf from *Ae. triuncialis*. **E**: Epidermis; **IS**: Inner sheath; **M**: Mesophyll; **OS**: Outer sheath; **SCL**: Sclerenchyma; **VB**: Vascular bundle.

Despite the general appearance of the sections, the properties of all these anatomical characters can vary from one species to another. The general anatomical characters of leaf blades are summarized in Table 6.

3.3.1.1 Blade shapes in cross sections

Altough there are limited anatomical studies including the main types of the leaf blade shapes of the family (Metcalfe, 1960; Doğan, 1982; Doğan & Tosunoğlu, 1992), it is easly seen from the leaf transverse sections that, the shape of leaf blades in the tribe *Triticeae* can be grouped into 5 types as U-shaped, V-shaped, flat, convolute and rolled. These 5 types are exemplified in Figure 25.

Sometimes the margins of leaf blades are curved on both sides of the midvein. This type is called convolute and shown in Figure 25A. In convolute type of leaf blades,

the midvein may be certain or uncertain and do not make a rib on abaxial surface. Despite the convolute leaves, the V- shaped leaves have the mid-vein making a rib towards to the abaxial surfaces (Figure 25B). Whenever both margins of a leaf blade are very much curved and make a 'U' shape, this type of a blade is called U-shape (Figure 25C). Flat type leaf blades are completely flattened as shown in Figure 25D. Finally, in rolled type, the leaf margins are rolled inward from the edges as shown in Figure 25E. The types of blade shapes and the corresponding taxa can be summarized as below:

1- Flat type of blades are shown in the following genera: *Secale, Heteranthelium, Taeniatherum* and *Dasypyrum* with the taxa, *Ae. kotscyi, Ae. speltoides, Ae. umbellata, Triticum boeoticum, Triticum araraticum, Elytrigia repens, Elytrigia canina, Thinopyrum elongatum, Leymus racemosus subsp sabulosus, Thinopyrum intermedium, Hordeum bulbosum, H. spontaneum var anatolicum, H. vulgare, H. murinum* and *H. marinum* var *pubescens.*

2- V-shaped blades are observed in *Elytrigia sosnowskyi, Ae. geniculata, Agropyron cristatum* subsp *pectinatum* var. *pectinatum, Triricum monococcum, Triticum dicoccon, Eremopyrum boneopartis* var. *sinaicum* and the genera *Crithopsis, Hordelymus,* and *Pseudoroegneria.*

3- U-shaped blades are seen in the genus *Psathyrostachys* and *Ambylopyrum* with the taxa of *Elymus pycnanthus, Elymus farctus, Elymus transhyrcanus, Elymus longearistatus, Ae. crassa, Ae. cylindrica, Ae. tauschii, Ae. comosa, Agropyron incanum, Eremopyrum confusum* var *sublanuginosum, Eremopyrum distans, Eremopyrum orientale* and *Eremopyrum triticeum*.

4- The taxa having convolute leaves are as follows: *Ae. triuncialis, Ae. biuncialis, Ae. peregrina, Ae. columnaris, Ae. juvenalis, Agropyron cristatum subsp pectinatum var puberulum, H. brevisubulatum subsp violaceum, H. geniculatum* and *H. distichon.*

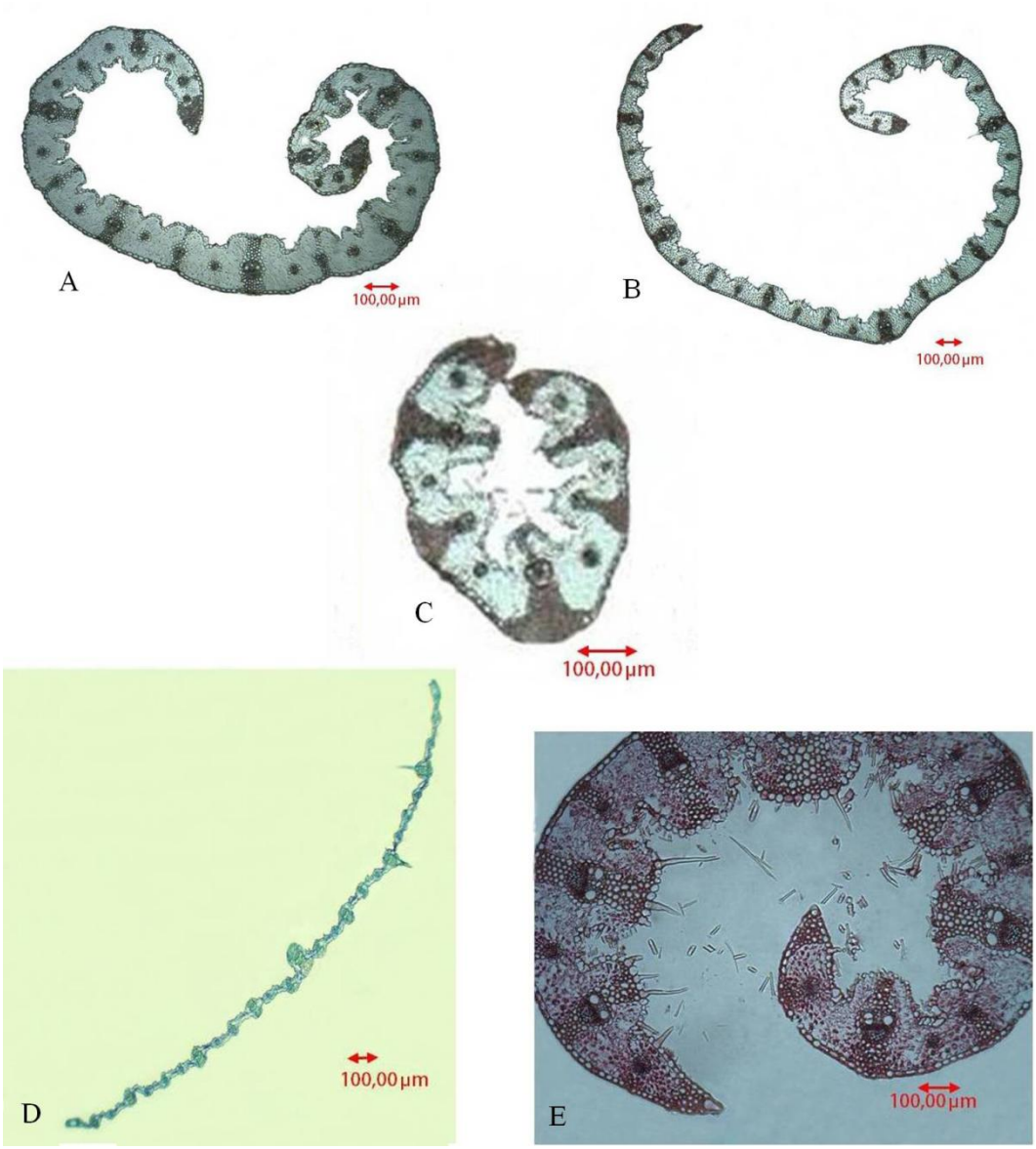

Figure 25. Transverse sections of leaves to exemplify the shapes of the blades. A) *Agropyron cristatum* subsp. *pectinatum* var. *puberulum* (Convolute); B) *A. cristatum* subsp. *pectinatum* var. *pectinatum* (V-shaped); C) *A. incanum* (U-shaped); D) *Dasypyrum villosum* (Straight); E) *Thinopyrum erosiglumis* (Rolled).

5- The rolled leaves are found in the taxa, *Thinopyrum erosiglumis, Elymus lazicus, Ae. markgrafii, Ae. vavilovii, Eremopyrum confusum* var *confusum* and var *glabrum, Leymus cappadocicus* and the genus *Henrardia.*

The anatomical properties of the leaves and the taxonomical relationships of the members in the genus *Agropyron*, depending on these anatomical characters, were

studied by Mavi *et al.* (2011b). It is easy to see from Figure 25 that, the genus *Agropyron* has three different taxa with three different blade shapes. Moreover, with the help of many other anatomical leaf properties, it was proved that each taxon has unique characteristics for each character (Mavi *et al.*, 2011b).

The furrows and ribs on the leaves in the tribe with the bulliform cells on their adaxial surfaces may give their shape. There is a high variation in length of the furrows on both adaxial and abaxial surfaces. For example, the minimum furrow length was measured as 15,42 µm on the adaxial surface of the leaf of *Ae. triuncialis.* However, the deepest furrows were measured as 126,54 µm and observed in *H. brevisubulatum* subsp *violaceum* on its adaxial surface. Because of the variation of the forrows on the leaf surfaces, to generalize this character, forrows which are more then 35 µm are referred as deep and the lower measurements are referred as shallow.

In some of the taxa such as the genera of *Secale, Leymus, Hordelymus, Taeniatherum*, most of the taxa of *Aegilops* and many of the members of *Elymus* sensu lato, except *Elymus transhyrcanus, Elymus lazicus* and *Elymus longearistatus* with the taxa A*gropyron cristatum* subsp *pectinatum* var *pectinatum*, *Eremopyrum orientale, Eremopyrum boneopartis, Eremopyrum confusum* var *confusum, H. bulbosum, H. geniculatum, H. brevisubulatum* subsp. *violaceum* don't have forrows on their abaxial surfaces. In these taxa, the adaxial furrows may be shallow (Figure 26) or deep (Figure 27) or seem not to be distinguishable as in Figure 28.

In the genera *Henrardia, Ambylopyrum, Psathyrostachys* and *Crithopsis* and the taxa *Elymus lazicus* subsp. *lazicus, Elymus longearistatus, Agropyron incanum, Hordeum distichon, H. murinum* subsp *glaucum, Eremopyrum distans, Eremopyrum confusum* var *sublanuginosum* and var *glabrum* the adaxial surface have deep furrows but the abaxial surface the furrows are shallow as shown in Figure 29.

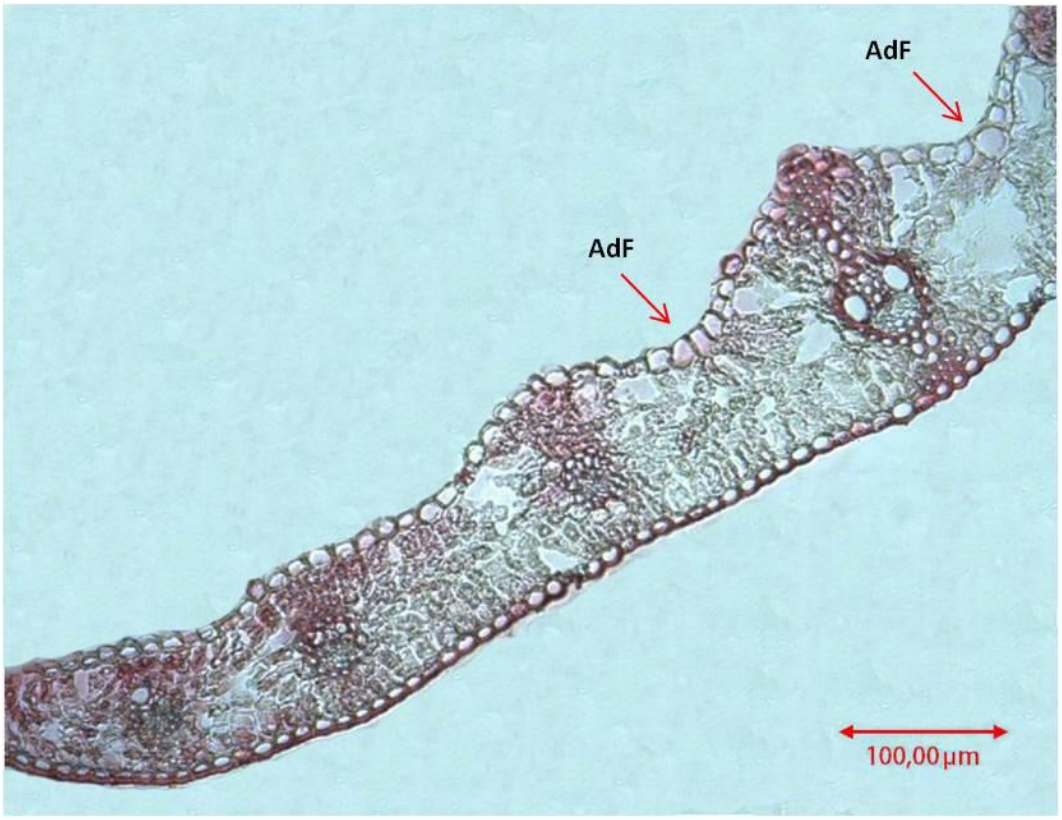

Figure 26. Transverse section of leaf from *Secale cereale*. **AdF:** Adaxial Furrow

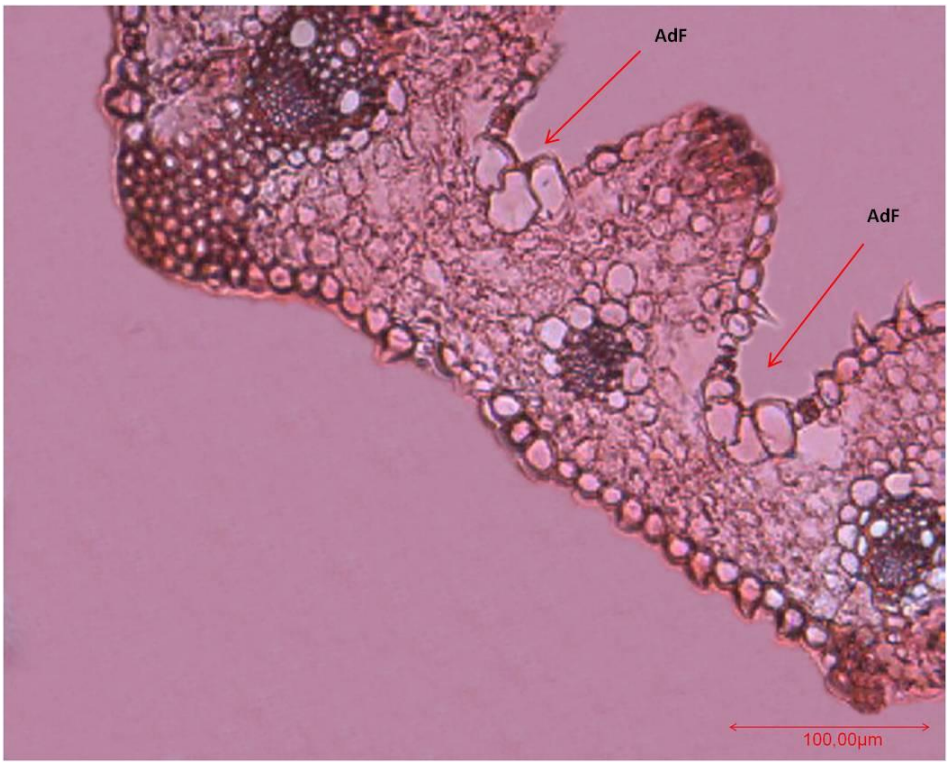

Figure 27. Transverse section of leaf from *Hordeum brevisubulatum* subsp *violaceum*. **AdF:** Adaxial Furrow

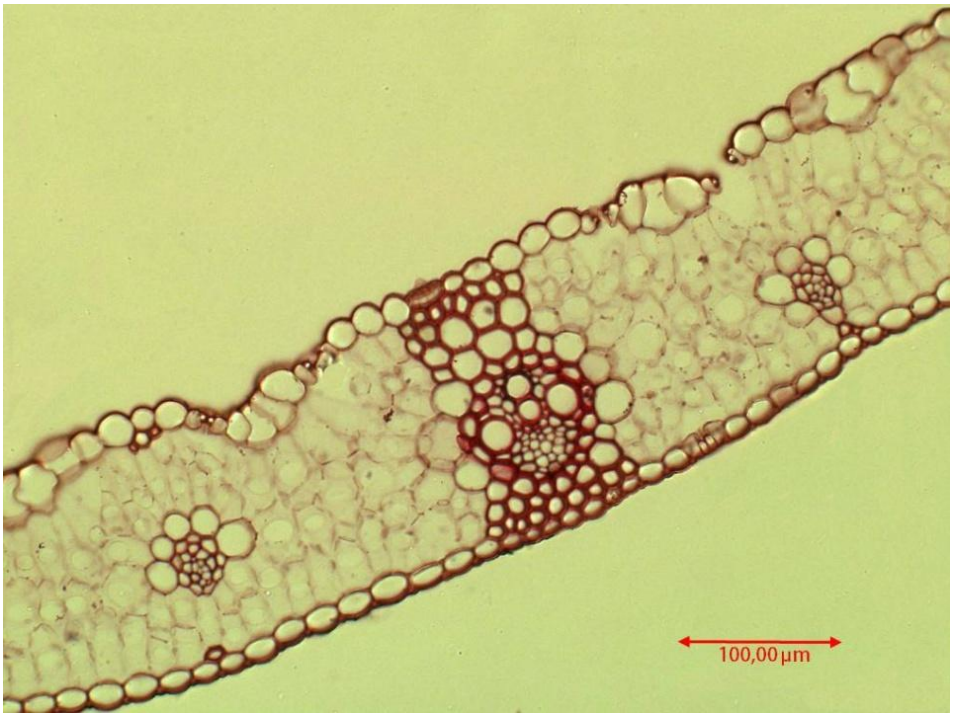

Figure 28. Transverse section of leaf from *Aegilops crassa.*

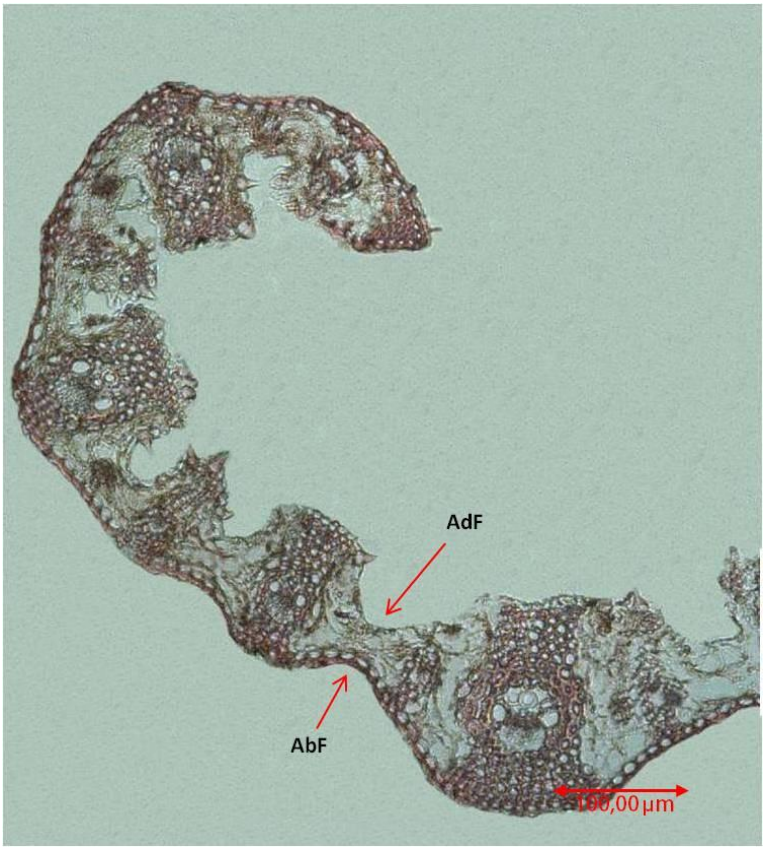

Figure 29. Transverse section of leaf from *Elymus longearistatus.* **AdF:** Adaxial Furrow, **AbF:** Abaxial Furrow.

In *Ae. umbellata, Agropyron cristatum* subsp *pectinatum* var *puberulum, H. vulgare, Triticum monococcum, Triticum araraticum, Eremopyrum triticeum,* and *Triticum boeoticum* forrows on both their adaxial and abaxial surfaces are not distinguishable. However, in *Triticum dicoccon, H. spontaneum* var *anatolicum, H. marinum* var *pubescens* (Figure 30), *Heteranthelium piliferum* and *Dasypyrum villosum* both adaxial and abaxial surfaces have deep furrows.

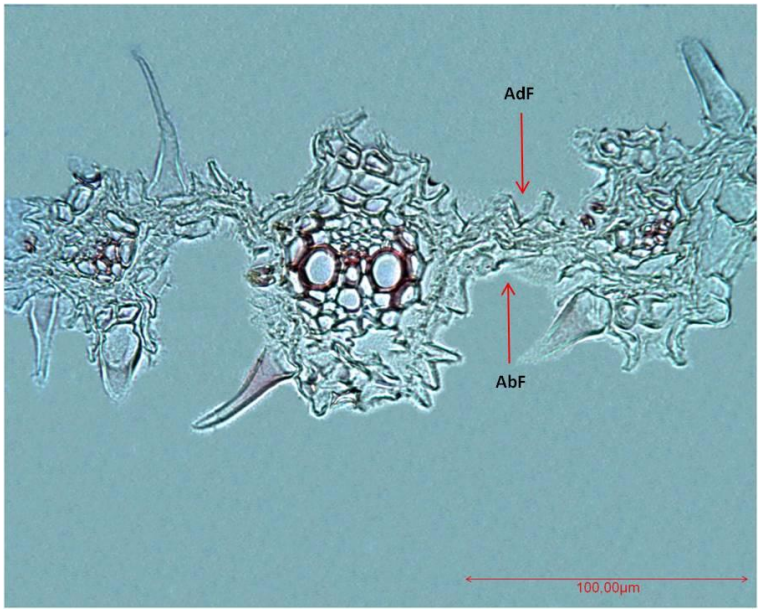

Figure 30. Transverse section of leaf from *H. marinum* var *pubescens.* **AdF:** Adaxial Furrow **AbF:** Abaxial Furrow.

3.3.1.2 Midvein

The appearance of midrib and the type of it may change according to the region of the leaf. In a mature leaf, the midrib gets larger through the lamina (Metcalfe, 1960). For this reason, the sections were obtained from the middle of the leaves. The midrib of the leaves have only one vascular bundle but, the size of it shows variation. There are three kinds of midrib properties of the tribe:

- In the first type, slightly appearing midvein is found. It is larger than the others and forms a rib through the abaxial side of the lamina (Figure 31A),

- In the second type, the midvein is larger than the other veins. It connects to the abaxial surface of the leaf with sclerenchyma. However, it does not form a rib through the abaxial side of the lamina (Figure 31B),

- In the third type, midvein is not the largest one. It is not certain. Moreover, it does not form a rib through the abaxial surface of the leaf (Figure 31C).

In the tribe Triticeae, the taxa having uncertain midvein in their leaves are as follows: *Thinopyrum elongatum, Elymus pycnanthus, Elymus farctus, Ae. biuncialis, Ae. crassa, H. spontaneum* var *anatolicum, A. muticum* and the genus *Leymus*. However, most of the taxa have slightly appeared midvein, that form a rib through the abaxial surface of their leaves. The taxa are *Elytrigia sosnowskyi, Elymus transhyrcanus, Elymus lazicus subsp lazicus, Elymus longearistatus, Elytrigia repens, Elytrigia canina, Pseudoroegneria libanotica, Pseudoroegneria divaricata, Ae. geniculata, Ae. cylindrica, Ae. tauschii, Ae. markgrafii, Ae. vavilovi, Agropyron incanum, Agropyron cristatum subsp pectinatum var pectinatum, Triticum monococcum, Triticum dicoccon, Eremopyrum confusum var sublanuginosum* and var *glabrum, Eremopyrum distans, Eremopyrum triticeum, Eremopyrum boneopartis var sinaicum,* and the genera *Secale, Heteranthelium, Henrardia, Crithopsis, Dasypyrum* and *Hordelymus.*

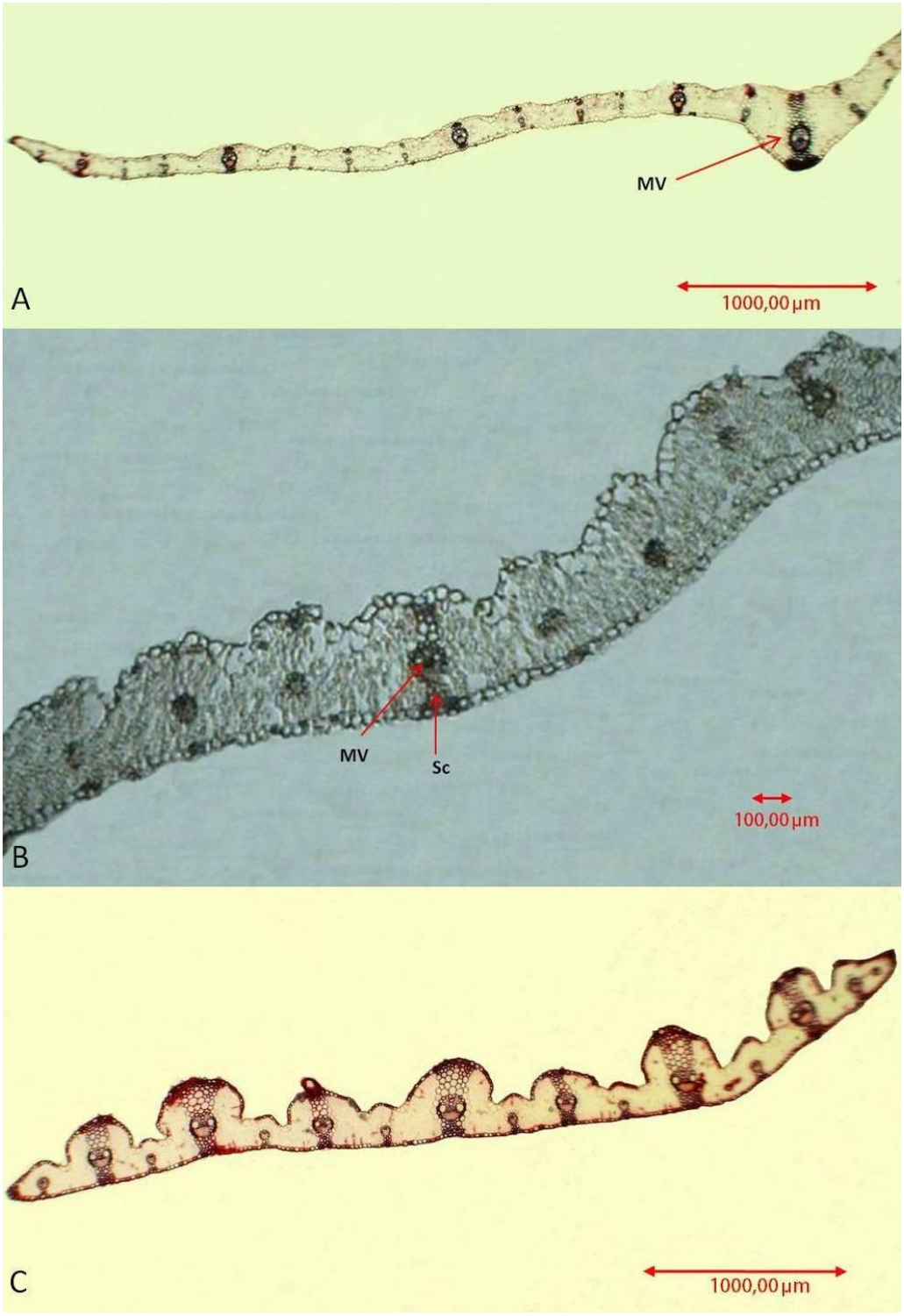

Figure 31. Midvein types **a)** *Hordelymus europeaus*; **b)** *Ae. kotschyi*; **c)** *Thinopyrum elongatum.* **MV:** Midvein, **Sc:** Sclerenchyma.

Taxa having large midvein, that connects to the abaxial surface of the leaves without forming a rib are *Thinopyrum erosiglumis, Thinopyrum intermedium, Ae. triuncialis, Ae. kotschyi, Ae. speltoides, Ae. umbellata, Ae. peregrina, Ae.*

columnaris, Ae. juvenalis, Ae. comosa, Agropyron cristatum subsp pectinatum var puberulum, Triticum boeoticum, Triticum araraticum, Eremopyrum orientale, Eremopyrum confusum var confusum, H. brevisubulatum subsp violaceum, H, geniculatum, H. bulbosum, H. distichon, H. vulgare, H. murinum subsp glaucum, H. marinum var pubescens, and the genera *Taeniatherum* and *Psathyrostachys.*

3.3.1.3 Epidermal cells in cross sections

Both the upper and the lower epidermal cells of the lamina can easily be seen from the transverse sections. The epidermal cells in cross sections are differentiated as hairs and bulliform cells. Mostly, the adaxial sides of the leaves have large bulliform cells. The arrangement of the epidermal cells on the abaxial sides of the leaves can be divided into two groups:

- Irregularly arranged epidermal cells are irregularly shaped (Figure 32)

- Regularly arranged epidermal cells have the same shape (Figure 33)

H. spontaneum, H. murinum, H. marinum, Heteranthelium piliferum, and *Crithopsis delileana* have irregularly arranged epidermal cells that are irregularly shaped. All the other taxa have regularly arranged epidermal cells.

Indumentum properties of leaf surfaces can also be clearly seen from transverse sections. Hairs of the surfaces are differentiated as prickles, hooks and macrohairs. The length of the hooks or prickles and the width of their bases are mostly the same in different specimens of the same genus. The differences between these 3 types and the variation of their height are discussed in the micromorphology of the leaf surfaces.

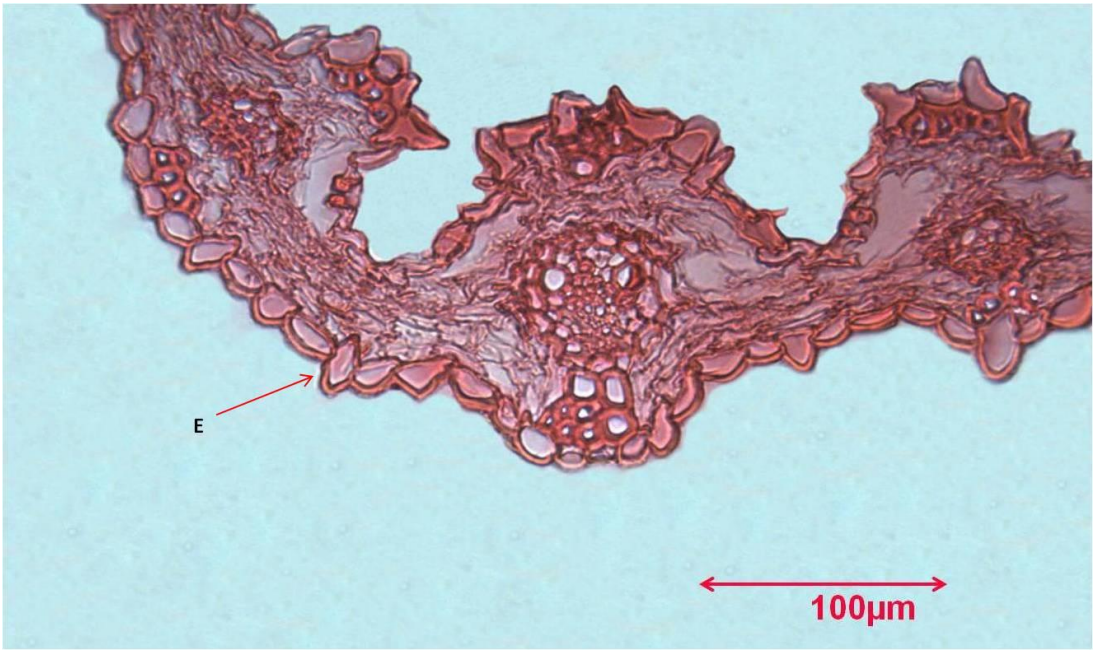

Figure 32. Transverse section of leaf from *Crithopsis delileana*. **E:** Epidermis

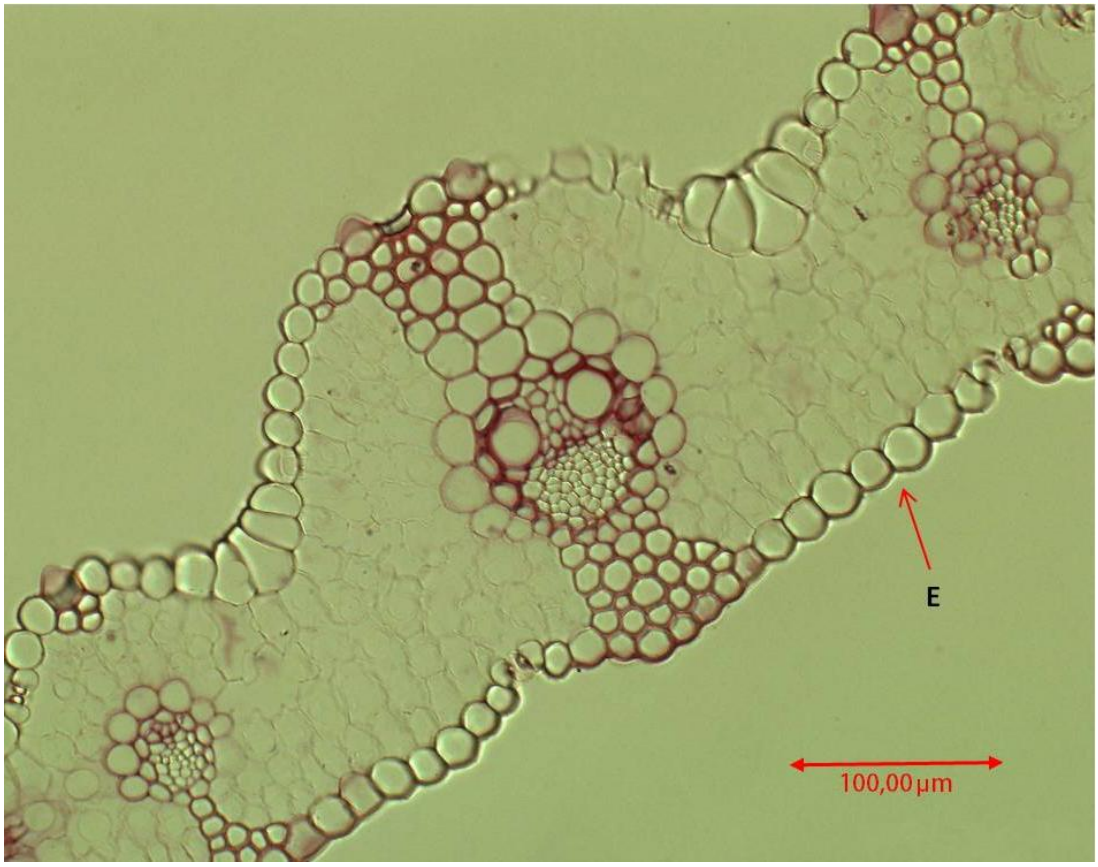

Figure 33. Transverse section of leaf from *Elytrigia repens*. **E:** Epidermis

3.3.1.4 Bulliform cells

Bulliform cells help folding of the leaves during water stress to reduce transpiration losses (Metcalfe, 1960). In arid regions, because of less moisture through vacuoles, bulliform cells help the leaves to close as the two edges of leaf blades. If the adequate water is available, these cells enlarge and the leaves open. Metcalfe (1960) grouped the bulliform cells and pointed out the taxonomical importance of them. According to Metcalfe (1960) the bulliform cells may be found in both adaxial and abaxial surfaces of leaves.

Transverse sections of the leaf blades show that, the bulliform cells are located on the adaxial surfaces of leaves in the tribe *Triticeae*. Moreover, most of the leaves have thin walls in the large bulliform cells, whereas in some of the leaves, they seem to be uncertain.

The shape, number and size differences of these bulliform cells create variation within the taxa of the tribe:

- Uncertain bulliform cells (Figure 34),

- Regularly shaped bulliform cells have nearly the same size of the adjacent cells (Figure 35),

- Regularly arranged bulliform cells have different sizes (Figure 36),

- Irregularly arranged bulliform cells have different sizes (Figure 37),

- Fan shaped bulliform cells (Figure 38).

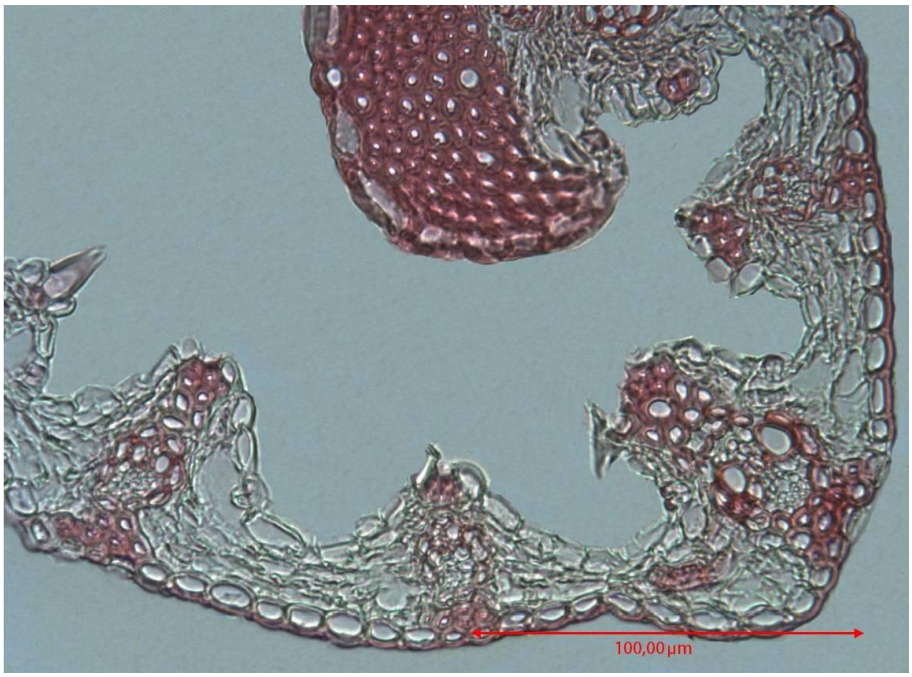

Figure 34. Transverse section of leaf from *Aegilops markgrafii*.

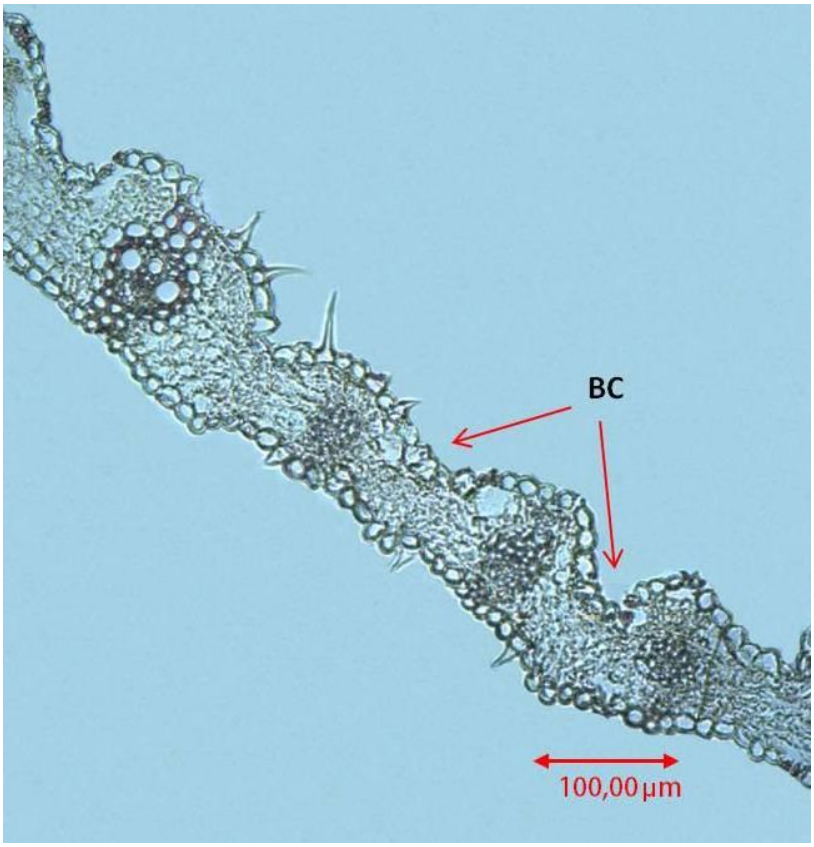

Figure 35. Transverse section of leaf from *Triticum monococcum*. **BC:** Bulliform cells

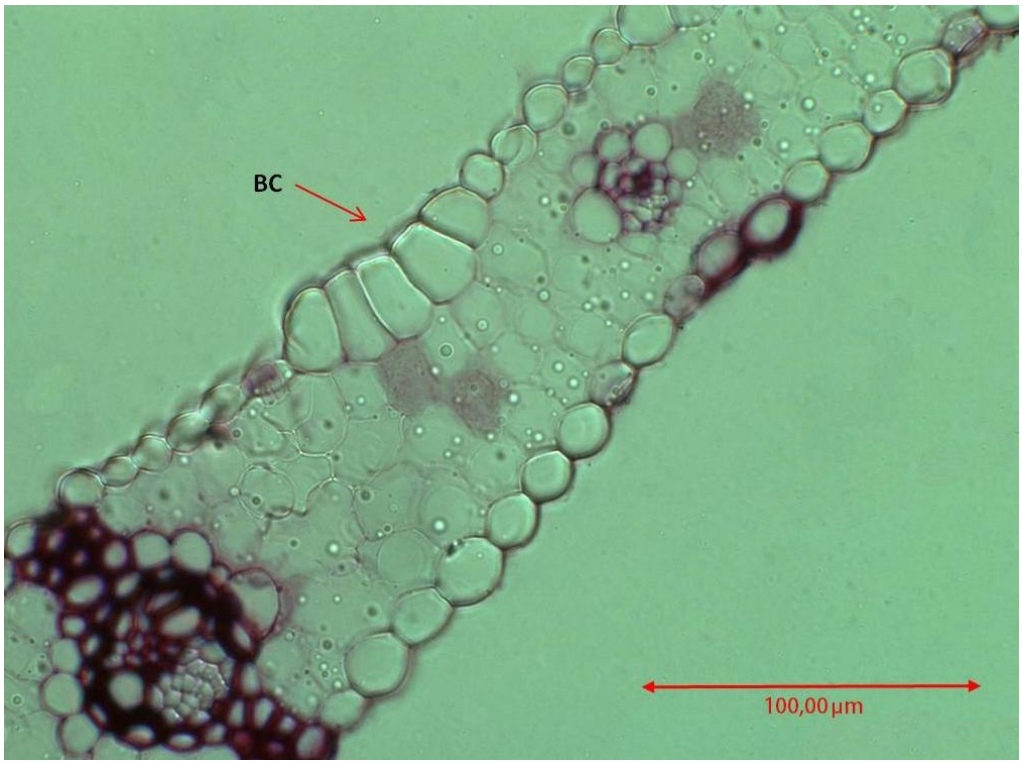

Figure 36. Transverse section of leaf from *Elyrigia canina* **BC:** Bulliform cells

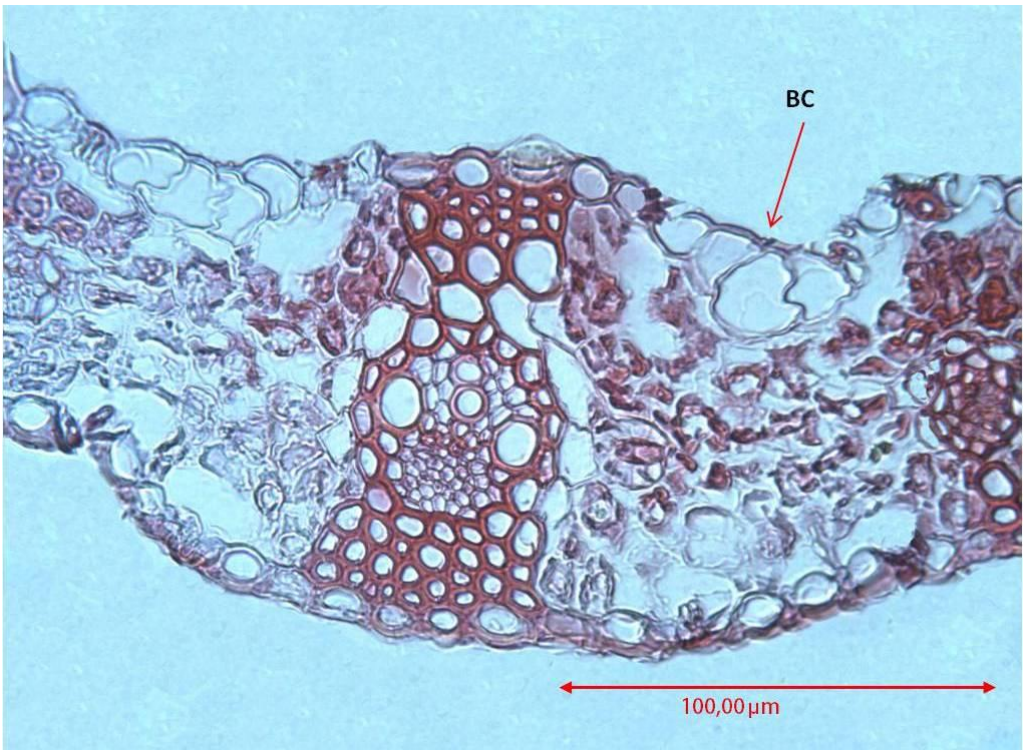

Figure 37. Transverse section of leaf from *Ae. triuncialis subsp persica.* **BC:** Bulliform cells.

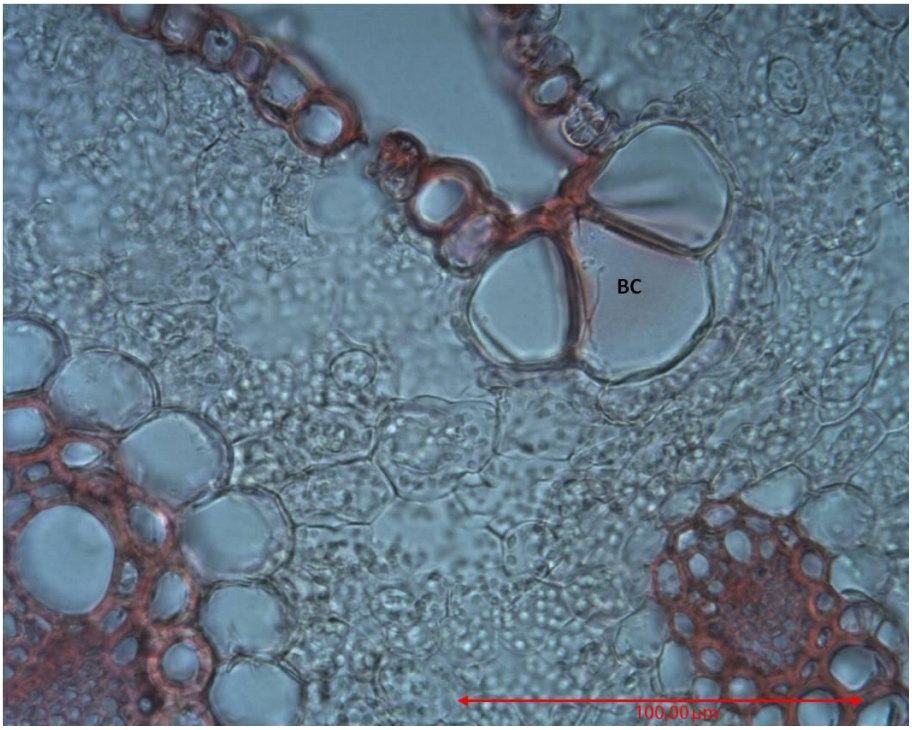

Figure 38. Transverse section of leaf from *Leymus racemosus* subsp *sabulosus* **BC:** Bulliform cells (Mavi *et al.*, 2011c).

Transverse sections of leaves show that, in *Elymus lazicus* subsp *lazicus, Elymus pycnanthus, Elymus farctus, Ae. cylindrica, Ae. markgrafii, Agropyron incanum, Leymus cappadocicus, H. marinum* var *pubescens* with the genera *Henrardia* and *Crithopsis* the bulliform cells seem not to be certain. However, *Elymus longearistatus, Ae. comosa, Eremopyrum confusum var confusum and var glabrum, Leymus racemosus* and *H. brevusubulatum* have fan shaped bulliform cells on their adaxial surfaces.

Moreover, regularly arranged bulliform cells may be in different shapes or nearly the same sizes with the adjacent cells on the adaxial surfaces of the leaves. *Elytrigia repens, Thinopyrum elongatum, Elytrigia canina, Ae. kotschyi, Ae. crassa, Ae. vavilovii, Agropyron cristatum subsp pectinatum var pectinatum,* and most of the genera such as *Heteranthelium, Taeniatherum, Ambylopyrum, Dasypyrum and Hordelymus* are included in the former group. The latter comprises the genera *Secale, Triticum* and *Psathyrostachys* with the taxa *Agropyron cristatum* subsp *pectinatum* var *puberulum* and *Thinopyrum intermedium.*

Most of the taxa of the Tribe have irregularly arranged bulliform cells in different sizes. The genus *Pseudoroegneria* and the taxa *Thinopyrum erosiglumis, Elytrigia sosnowskyi, Elymus transhyrcanus, Ae. triuncialis, Ae. speltoides, Ae. umbellata, Ae. biuncialis, Ae. geniculata, Ae. peregrina, Ae. columnaris, Ae. juvenalis, Ae. tauschii, Eremopyrum confusum var sublanuginosum, E. distans, E. orientale, E. triticeum, E boneopartis, H. geniculatum, H. bulbosum, H. distichon, H. spontaneum* var *anatolicum, H. vulgare and H. murinum* have irregularly shaped bulliform cells, which are also in different sizes.

3.3.1.5 Sclerenchymatic cells

According to Schwendener (1890), the sclerenchymatic zone around the vascular bundles of leaves is a characteristic structure for the family Poaceae. Moreover, Metcalfe (1960) noticed the importance of different sclerenchymatic arrangements in grass taxonomy and grouped these sclerenchymatic regions as I-shaped and T-shaped girders and strands. Furthermore, in some taxa of the family, there is a continuous sclerenchymatic layer through abaxial surface between two edges of leaf blades (Metcalfe, 1960; Doğan, 1982). According to Metcalfe (1960), there are also sclerenchymatic cells on the margins of the blades.

The transverse sections of leaf blades in the tribe *Triticeae* demonstrate that, all the taxa except *Elymus pycnanthus* (Figure 39) and *Elymus farctus* do not have continuous sclerenchyma through the abaxial side of their leaf blades. Moreover, all of the taxa except *Elymus pycnanthus* (Figure 39), *Elymus farctus, Elytrigia sosnowskyi, Thinopyrum elongatum, Thinopyrum erosiglumis, Leymus racemosus,* and the genera *Pseudoroegneria* and *Psathyrostachys* have I-shaped adaxial girders.

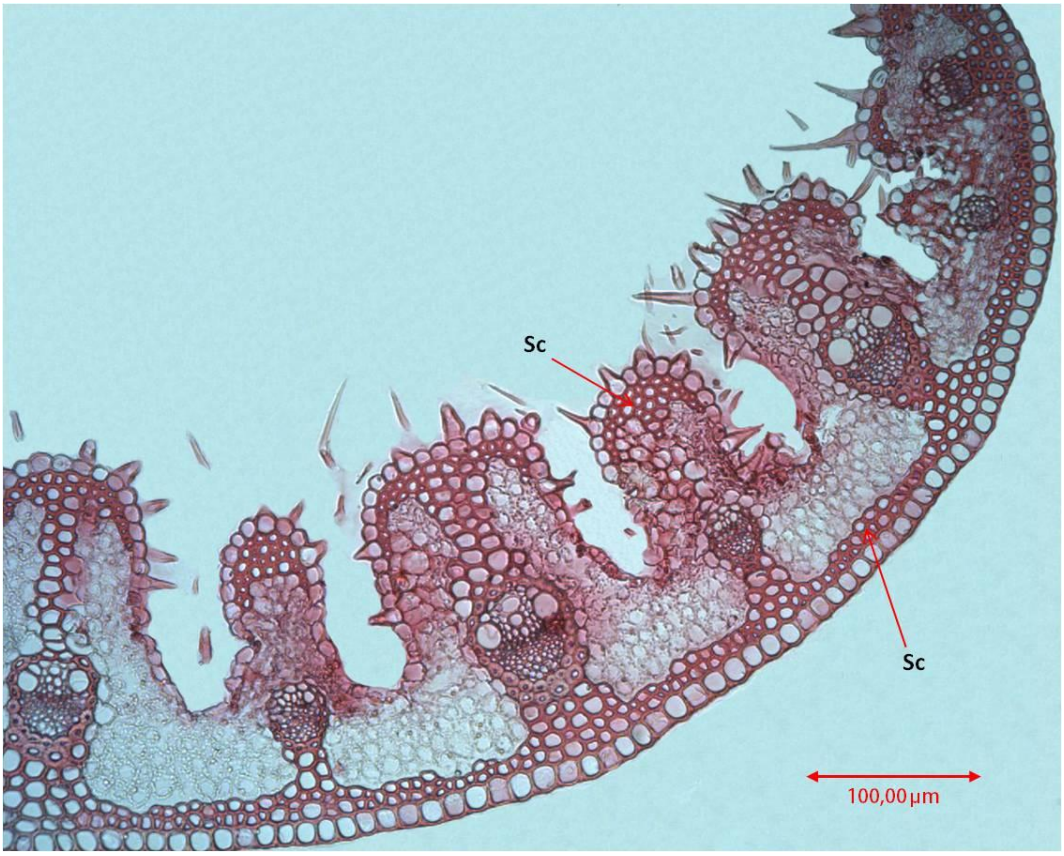

Figure 39. Transverse section of leaf from *Elymus pycnanthus*. **Sc:** Sclerenchyma

From the transverse sections of the leaf blades in *Triticeae*, it is clearly seen that, the sclerenchymatic strands through the adaxial and/or abaxial surfaces are very common. Moreover, the certain median vascular bundles of all the taxa have abaxial sclerenchymatic girders that enlarge through the epidermis. However, most of the taxa have vascular bundles, which are not connected to the epidermis by sclerenchyma. Generally, there are 4 different types of sclerenchymatic cell arrangements around the vascular bundles of leaves in the taxa of the tribe *Triticeae* and it is the most important point that, all these types can be observed together in a leaf of the taxa.

The arrangement types of sclerenchyma around the vascular bundles of the transverse sections of leaf blades can be listed as:

1. Sclerenchymatic cells are confined towards to the abaxial side of the leaf,

2. Sclerenchymatic cells are located towards the vascular bundles from adaxial or abaxial side of the leaf. In this type, these cells do not connect to the vascular bundles,

3. Sclerenchymatic cells are found both towards adaxial and abaxial sides of the leaf,

4. There is no sclerencyma around the vascular bundle.

With the help of these different types, the taxa of *Triticeae* can be classified in three major groups;

1. Leaf blades having vascular bundles, some of which do not have through adaxial or abaxial sclerenchyma (Figure 40),

2. Leaf blades having vascular bundles, some of which have abaxial girders (Figure 39),

3. Leaf blades having vascular bundles, which are all connected to the adaxial and abaxial epidermis with sclerenchyma (Figure 41).

Leaf blades of the genera *Pseudoroegneria, Leymus, Psathyrostachys, Crithopsis, Taeniatherum, Amblopyrum, Eremopyrum, Elytrigia,* and *Aegilops* except *Ae. markgrafii,* with the taxa *Elymus transhyrcanus, Thinopyrum intermedium, Hordeum geniculatum, Secale sylvestre, Triticum araraticum, T. boeticum, T. dicoccon, Agropyron cristatum subsp pectinatum var puberulum* and *Agropyron incanum* have vascular bundles, some of which are not connected to adaxial or abaxial surfaces with sclerenchymatic cells. However, in the genus *Hordelymus, Heteranthelium* and *Henrardia,* and the taxa *Secale cereale, Agropyron cristatum* subsp *pectinatum* var *pectinatum, Triticum monococcum, Ae. markgrafii, Elymus longearistatus* and *Elymus lazicus* subsp *lazicus* the vascular bundles of leaf blades have sclerenchymatic girders through both adaxial and abaxial surfaces.

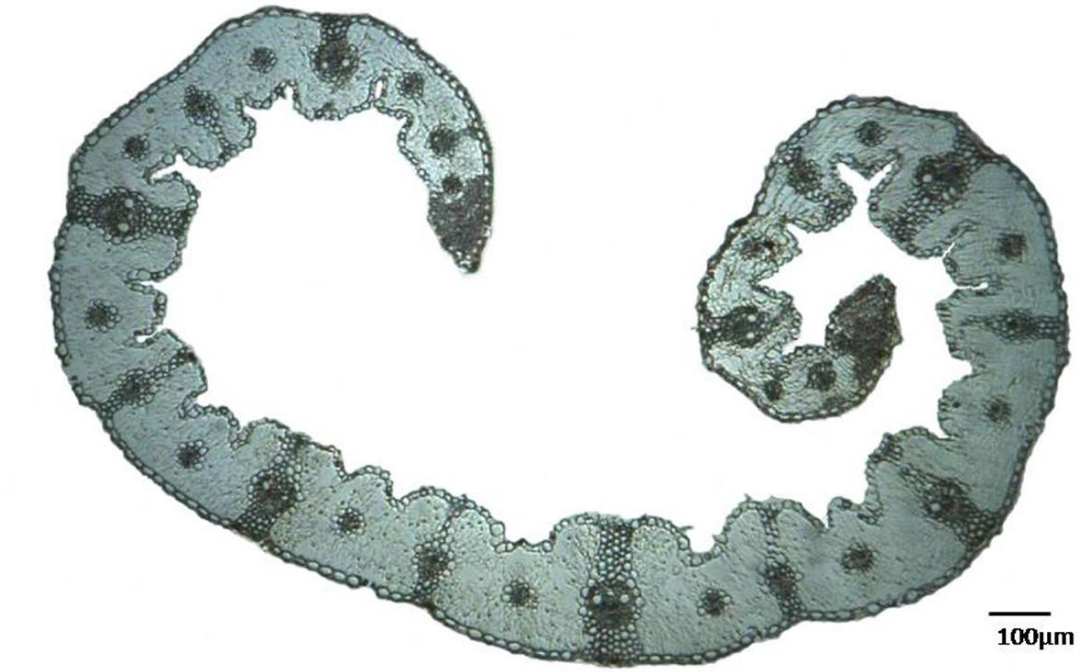

Figure 40. Transverse section of leaf from *Agropyron cristatum subsp pectinatum var puberulum* (Mavi *et al.*, 2011b).

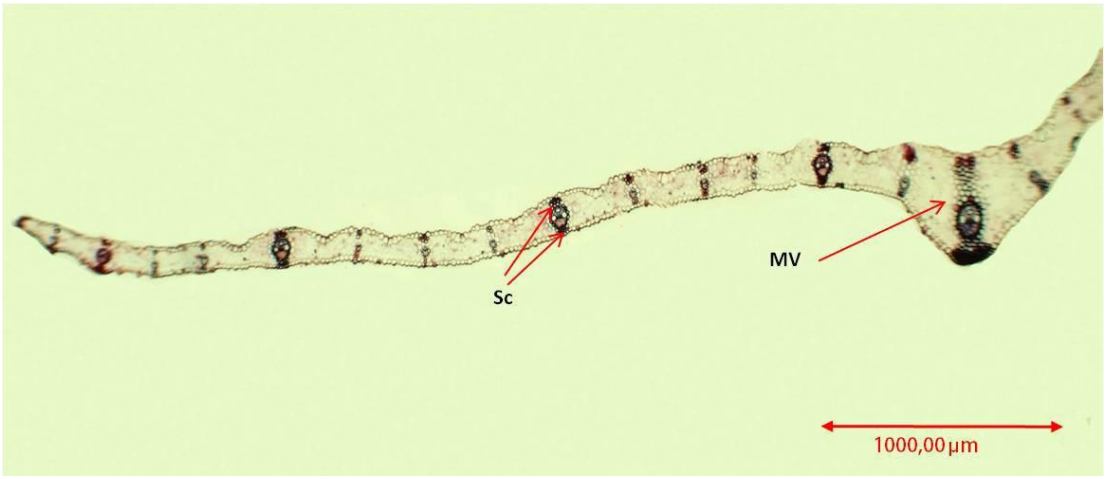

Figure 41. Transverse section of leaf from *Hordelymus europeaus* **MV:** Midvein, **Sc:** Sclerenchyma

The genera *Dasypyrum, Hordeum* (except *H. geniculatum*) and the taxa of *Elymus pycnanthus* and *Elymus farctus, Thinopyrum elongatum, Thinopyrum erosiglumis* have vascular bundles, in some of which have abaxial girders in their leaf blades.

The density of the marginal sclerenchyma varies with regard to row numbers of the cells. Some of the taxa, such as the genera *Triticum, Secale, Leymus, Crithopsis, Psathyrostachys, Dasypyrum,* and the taxa *Ae. speltoides* (Figure 42), *Ae geniculata, Ae. crassa, H. geniculatum, H. murinum* and *H. marinum* var *pubescens* have 2 or 3 layers of sclerenchymatic cells on their margins.

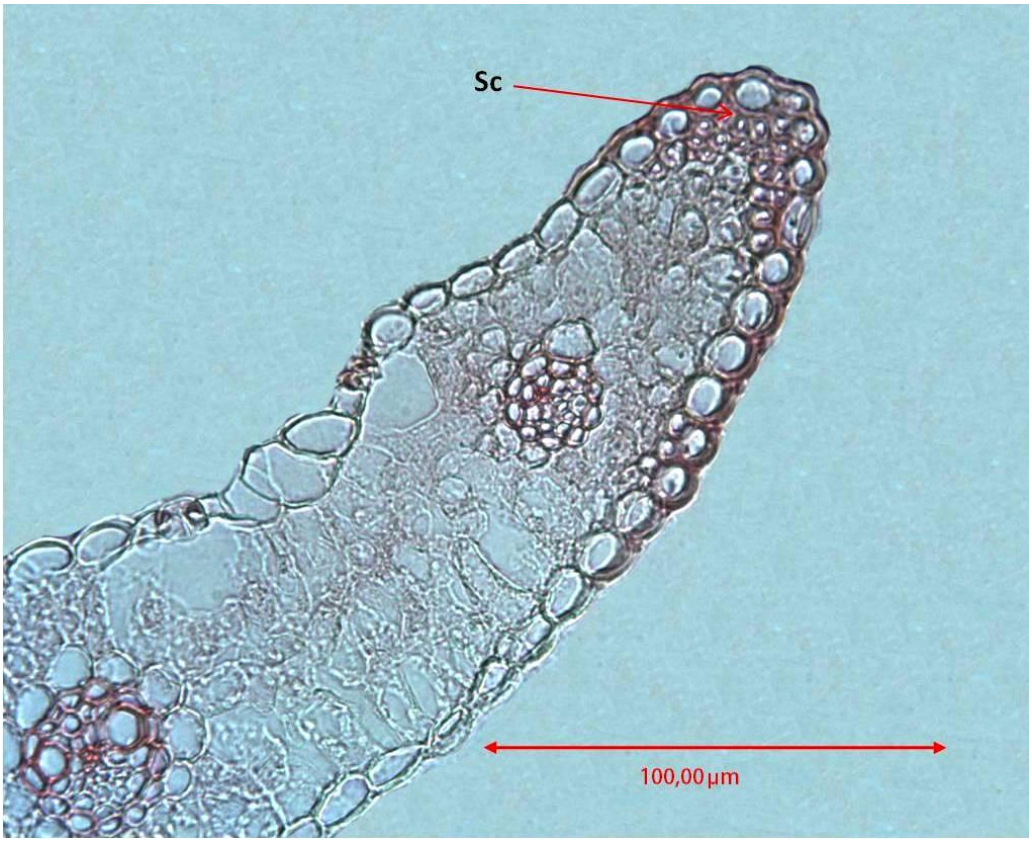

Figure 42. Transverse section of leaf from *Aegilops speltoides var ligustica* **Sc:** Sclerenchyma

Most of the taxa have 4 or 6 layers of sclerenchyma on their margins. These taxa can be listed as *Elymus transhyrcanus, Elymus lazicus, Elymus longearistatus, H. spontaneum* var *anatolicum, H. vulgare, H. distichon, H bubosum, H. brevisubulatum, Ae. vavilovii, Ae. tauschii, Ae. juvenalis, Ae. columnaris, Ae. cylindrica, Ae. biuncialis, Ae. kotschyii,* the genera *Thinopyrum, Elytrigia, Pseudoroegneria, Hordelymus, Taeniatherum, Henrardia, Heteranthelium and Eremopyrum* (Figure 43).

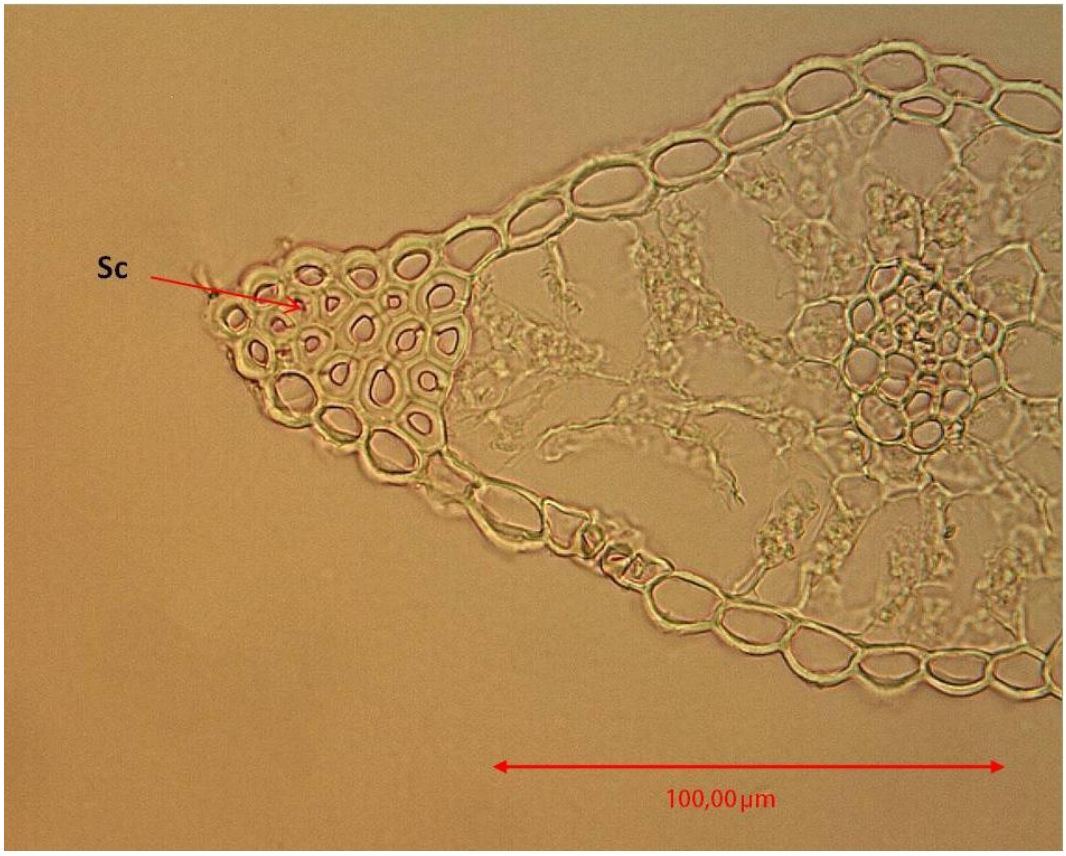

Figure 43. Transverse section of leaf from *Eremopyrum orientale* **Sc:** Sclerenchyma

There are also leaf blades having 7 or 9 layers of sclerenchymatic cells on their margins. These leaf blades are seen in the taxa *Agropyron cristatum* subsp *pectinatum* var *puberulum, Agropyron incanum, Ae. triuncialis, Ae. peregrina, Ae. umbellate* (Figure 44), *Ae. markgrafii, Elymus pycnanthus* and *Elymus farctus*. The margins of leaf blades in *Agropyron cristatum* subsp *pectinatum* var *pectinatum, Ae. comosa* and *Amblopyrum muticum* (Figure 45) have 10- 12 layers of sclerenchymatic cells.

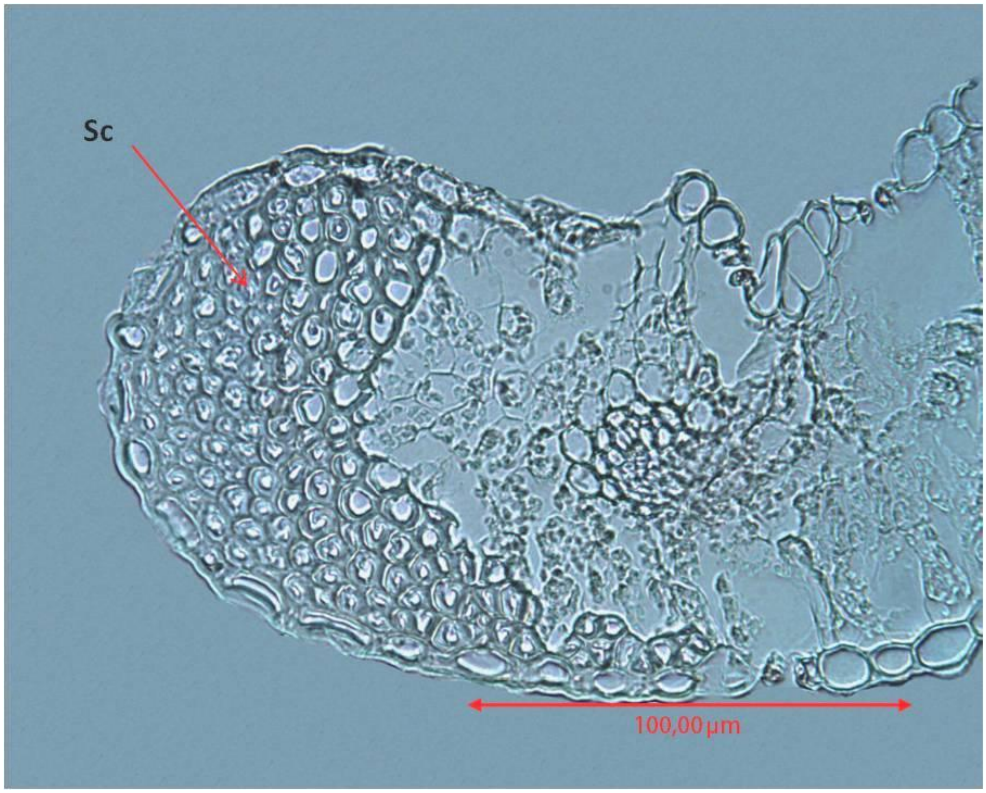

Figure 44. Transverse section of leaf from *Aegilops umbellata* **Sc:** Sclerenchyma

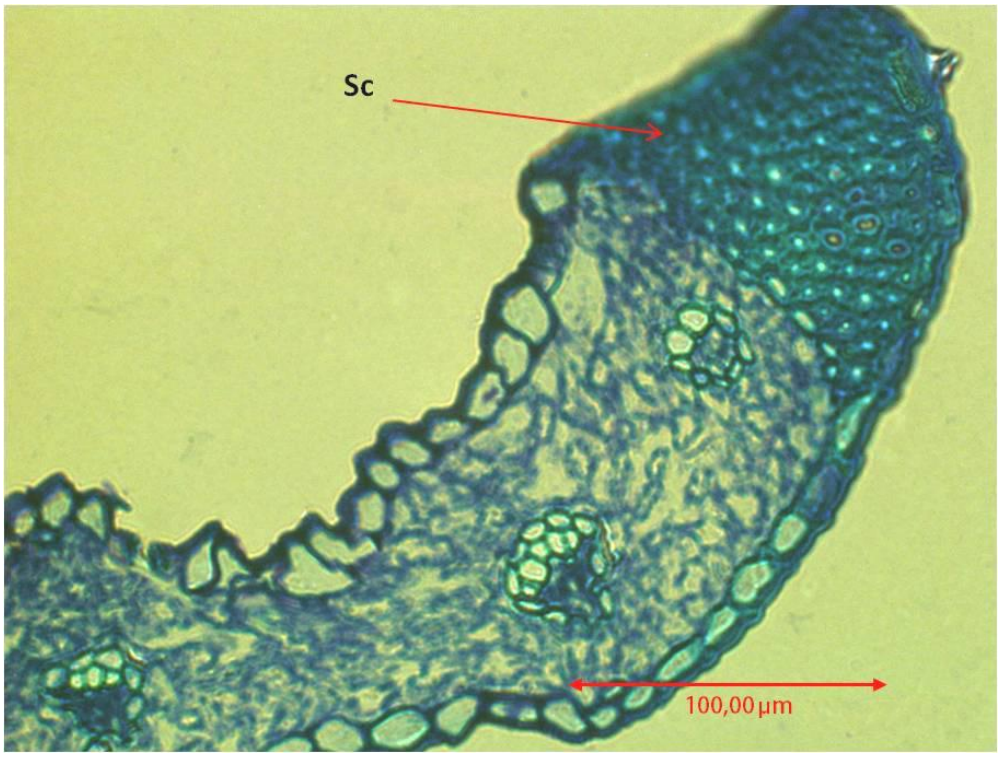

Figure 45. Transverse section of leaf from *Amblopyrum muticum* **Sc:** Sclerenchyma

Table 6. General characteristics of leaf transverse sections

Taxa	Shape of blade	Adaxial Furrows	Abaxial Furrows	Midvein	Outer bundle sheath	*Scl around the *VB	Continuous *Scl	*Scl line number on Margins	Epidermal cells in cross section	Types of bulliform cell	Adaxial *Scl type
Elymus pycnanthus	U-shaped	deep	absent	uncertain	without chl	with abaxial girder	present	9	regular	uncertain	T- shaped
Thinopyrum erosiglumis	rolled	shallow	absent	without rib	with chl	with abaxial girder	absent	4	regular	irregular	T- shaped
Elytrigia sosnowskyi	V-shaped	deep	absent	with rib	without chl	without scl	absent	5	regular	irregular	T- shaped
Elymus transhyrcanus	U-shaped	shallow	shallow	with rib	with chl	without scl	absent	4	regular	irregular	I- shaped
Elymus lazicus subsp lazicus	rolled	deep	shallow	with rib	without chl	adaxial and abaxial girder	absent	5	regular	uncertain	I- shaped
Elymus longearistatus	U-shaped	deep	shallow	with rib	with chl	adaxial and abaxial girder	absent	5	regular	fan shaped	I- shaped
Thinopyrum intermedium	flat	shallow	absent	without rib	with chl	without scl	absent	4	regular	regular + same	I- shaped
Elytrigia repens	flat	shallow	absent	with rib	without chl	without scl	absent	6	regular	regular + different	I- shaped
Thinopyrum elongatum	flat	shallow	absent	uncertain	with chl	with abaxial girder	absent	5	regular	regular + different	T- shaped
Elytrigia canina	flat	shallow	absent	with rib	without chl	without scl	absent	5	regular	regular + different	I- shaped
Elymus farctus	U-shaped	deep	absent	uncertain	without chl	with abaxial girder	present	9	regular	uncertain	T- shaped

* **Scl**: Sclerenchyma, **VB**: Vascular bundle

Table 6. Continue. General characteristics of leaf transverse sections

Taxa	Shape of blade	Adaxial Furrows	Abaxial Furrows	Midvein	Outer bundle sheath	*Scl around the *VB	Continuous *Scl	*Scl line number on Margins	Epidermal cells in cross section	Types of bulliform cell	Adaxial *Scl type
Pseudoroegneria libanotica	V-shaped	deep	absent	with rib	without chl	without scl	absent	4	regular	irregular	T- shaped
Pseudoroegneria divaricata	V-shaped	deep	absent	with rib	without chl	without scl	absent	6	regular	irregular	T- shaped
Ae. triuncialis subsp. triuncialis	convolute	shallow	absent	without rib	with chl	without scl	absent	7	regular	irregular	I- shaped
Ae. kotschyi	flat	shallow	absent	without rib	with chl	without scl	absent	4	regular	regular + different	I- shaped
Ae. markgrafii	rolled	deep	absent	with rib	with chl	adaxial and abaxial girder	absent	7	regular	uncertain	I- shaped
Ae. speltoides var ligustica	flat	shallow	absent	without rib	with chl	without scl	absent	3	regular	irregular	I- shaped
Ae. speltoides var speltoides	flat	shallow	shallow	without rib	with chl	without scl	absent	3	regular	irregular	I- shaped
Ae. umbellata	flat	shallow	shallow	without rib	with chl	without scl	absent	7	regular	irregular	I- shaped
Ae. biuncialis	convolute	shallow	absent	uncertain	without chl	without scl	absent	5	regular	irregular	I- shaped
Ae. geniculata	V-shaped	shallow	absent	with rib	with chl	without scl	absent	2	regular	irregular	I- shaped
Ae. crassa	U-shaped	absent	absent	uncertain	with chl	without scl	absent	3	regular	regular + different	I- shaped

* **Scl**: Sclerenchyma, **VB**: Vascular bundle

Table 6. Continue**.** General characteristics of leaf transverse sections

Taxa	Shape of blade	Adaxial Furrows	Abaxial Furrows	Midvein	outer bundle sheath	*Scl around the *VB	Continuous *Scl	*Scl line number on Margins	Epidermal cells in cross section	Types of bulliform cell	Adaxial *Scl type
Ae. peregrina	convolute	shallow	absent	without rib	without chl	without scl	absent	7	regular	irregular	I- shaped
Ae. cylindrica	U-shaped	shallow	absent	with rib	without chl	without scl	absent	5	regular	uncertain	I- shaped
Ae. columnaris	convolute	shallow	absent	without rib	without chl	without scl	absent	6	regular	irregular	I- shaped
Ae. juvenalis	convolute	shallow	absent	without rib	without chl	without scl	absent	5	irregular	irregular	I- shaped
Ae. tauschii	U-shaped	shallow	absent	with rib	without chl	without scl	absent	5	regular	irregular	I- shaped
Ae. triuncialis subsp. *bozdagensis*	convolute	shallow	absent	without rib	with chl	without scl	absent	7	regular	irregular	I- shaped
Ae. triuncialis subsp. *persica*	convolute	shallow	absent	without rib	with chl	without scl	absent	7	regular	irregular	I- shaped
Ae. comosa	U-shaped	shallow	absent	without rib	without chl	without scl	absent	11	regular	fan shaped	I- shaped
Ae. vavilovii	rolled	deep	absent	with rib	with chl	without scl	absent	6	regular	regular + different	I- shaped
Agr. incanum	U-shaped	deep	shallow	with rib	with chl	without scl	absent	9	regular	uncertain	I- shaped
Agr. cristatum subsp. *pectinatum* var. *puberulum*	convolute	shallow	shallow	without rib	with chl	without scl	absent	9	regular	regular + same	I- shaped

* **Scl**: Sclerenchyma, **VB**: Vascular bundle

Table 6. Continue**.** General characteristics of leaf transverse sections

Taxa	Shape of blade	Adaxial Furrows	Abaxial Furrows	Midvein	outer bundle sheath	*Scl around the *VB	Continuous *Scl	*Scl line number on Margins	Epidermal cells in cross section	Types of bulliform cell	Adaxial *Scl type
Agr. cristatum subsp. *pectinatum* var. *pectinatum*	V-shaped	shallow	absent	with rib	with chl	adaxial and abaxial girder	absent	10	regular	regular + different	I- shaped
Triticum monococcum	V-shaped	shallow	shallow	with rib	with chl	adaxial and abaxial girder	absent	2	regular	regular + same	I- shaped
Triticum dicoccon	V-shaped	deep	deep	with rib	with chl	without scl	absent	3	regular	regular + same	I- shaped
Triticum boeoticum	flat	shallow	shallow	without rib	without chl	without scl	absent	3	regular	regular + same	I- shaped
Triticum araraticum	flat	shallow	shallow	without rib	without chl	without scl	absent	3	regular	regular + same	I- shaped
Eremopyrum confusum var sublanuginosum	U-shaped	deep	shallow	with rib	without chl	without scl	absent	6	regular	irregular	I- shaped
Eremophyrum distans	U-shaped	deep	shallow	with rib	without chl	without scl	absent	5	regular	irregular	I- shaped
Eremopyrum triticeum	U-shaped	shallow	shallow	with rib	without chl	without scl	absent	5	regular	irregular	I- shaped
Eremopyrum orientale	U-shaped	deep	absent	without rib	without chl	without scl	absent	5	regular	irregular	I- shaped

* **Scl**: Sclerenchyma, **VB**: Vascular bundle

Table 6. Continue**.** General characteristics of leaf transverse sections

Taxa	Shape of blade	Adaxial Furrows	Abaxial Furrows	Midvein	outer bundle sheath	*Scl around the *VB	Continuous *Scl	*Scl line number on Margins	Epidermal cells in cross section	Types of bulliform cell	Adaxial *Scl type
Eremopyrum boneopartis var sinaicum	V-shaped	deep	absent	with rib	without chl	without scl	absent	5	regular	irregular	I- shaped
Eremopyrum confusum var confusum	rolled	deep	absent	without rib	without chl	without scl	absent	6	regular	fan shaped	I- shaped
Eremopyrum confusum var glabrum	rolled	deep	shallow	with rib	without chl	without scl	absent	6	regular	fan shaped	I- shaped
Secale sylvestre	flat	shallow	absent	with rib	with chl	without scl	absent	2	regular	regular + same	I- shaped
Secale cereale	flat	shallow	absent	with rib	with chl	adaxial and abaxial girder	absent	2	regular	regular + same	I- shaped
Leymus racemosus subsp sabulosus	flat	deep	absent	uncertain	without chl	without scl	absent	3	regular	fan shaped	T- shaped
Leymus cappadocicus	rolled	deep	absent	uncertain	without chl	without scl	absent	3	regular	uncertain	I- shaped
H. brevisubulatum subsp violaceum	convolute	deep	absent	without rib	without chl	with abaxial girder	absent	4	regular	fan shaped	I- shaped
H. geniculatum	convolute	deep	absent	without rib	without chl	without scl	absent	2	regular	irregular	I- shaped

* **Scl**: Sclerenchyma, **VB**: Vascular bundle

Table 6. Continue**.** General characteristics of leaf transverse sections

Taxa	Shape of blade	Adaxial Furrows	Abaxial Furrows	Midvein	outer bundle sheath	*Scl around the *VB	Continuous *Scl	*Scl line number on Margins	Epidermal cells in cross section	Types of bulliform cell	Adaxial *Scl type
H. bulbosum	flat	shallow	absent	without rib	with chl	with abaxial girder	absent	4	regular	irregular	I- shaped
H. distichon	convolute	deep	shallow	without rib	without chl	with abaxial girder	absent	4	regular	irregular	I- shaped
H, spontaneum var anatolicum	flat	deep	deep	uncertain	with chl	with abaxial girder	absent	4	irregular	irregular	I- shaped
H. vulgare	flat	shallow	shallow	without rib	with chl	with abaxial girder	absent	4	regular	irregular	I- shaped
H. murinum subsp glaucum	flat	deep	shallow	without rib	without chl	with abaxial girder	absent	2	irregular	irregular	I- shaped
H. marinum var pubescens	flat	deep	deep	without rib	without chl	with abaxial girder	absent	2	irregular	uncertain	I- shaped
Henrardia persica var persica	rolled	deep	shallow	with rib	without chl	adaxial and abaxial girder	absent	5	regular	uncertain	I- shaped
Heteranthelium piliferum	flat	deep	deep	with rib	with chl	adaxial and abaxial girder	absent	5	irregular	regular+different	I- shaped
Taeniatherum caput-medusae	flat	shallow	absent	without rib	without chl	without scl	absent	4	regular	regular + different	I- shaped

* **Scl**: Sclerenchyma, **VB**: Vascular bundle

Table 6. Continue**.** General characteristics of leaf transverse sections

Taxa	Shape of blade	Adaxial Furrows	Abaxial Furrows	Midvein	outer bundle sheath	*Scl around the *VB	Continuous *Scl	*Scl line number on Margins	Epidermal cells in cross section	Types of bulliform cell	Adaxial *Scl type
Ambylopyrum muticum	U-shaped	deep	shallow	uncertain	without chl	without scl	absent	12	regular	regular + different	I- shaped
Crithopsis delileana	V-shaped	deep	shallow	with rib	with chl	without scl	absent	2	irregular	uncertain	I- shaped
Psathyrostachys fragilis	U-shaped	deep	shallow	without rib	without chl	without scl	absent	2	regular	regular + same	T- shaped
Dasypyrum villosum	flat	deep	deep	with rib	with chl	with abaxial girder	absent	3	regular	regular + different	I- shaped
Hordelymus europaeus	V-shaped	absent	absent	with rib	without chl	adaxial and abaxial girder	absent	4	regular	regular + different	I- shaped

* **Scl**: Sclerenchyma, **VB**: Vascular bundle

3.3.2 Leaf Micromorphology

The researches about the anatomy of the family has shown that the surface properties of the leaves are significant for the proximity of relationships of the taxa (Sylvester *et al.*, 2001; Mejia-Saulés and Bisby, 2003; Motomura, 2004; Keshavarzi *et al.*, 2007; Vieira *et al.*, 2003; Iio *et al.*, 2005).

In *Triticeae*, it is clearly observed both from the adaxial and abaxial surfaces of the leaves that, the epidermal cells are differentiated as long cells, hooks, prickles, macrohairs and short cells as stomata and silica bodies. Moreover, there are 2 different zones namely, the costal zone and the intercostals zone. The intercostal zones comprise stomata (Figure 46). To clarify all these characters, the leaf surfaces are examined both with the help of Light Microscope (LM) and Scanning Electron Microscope (SEM).

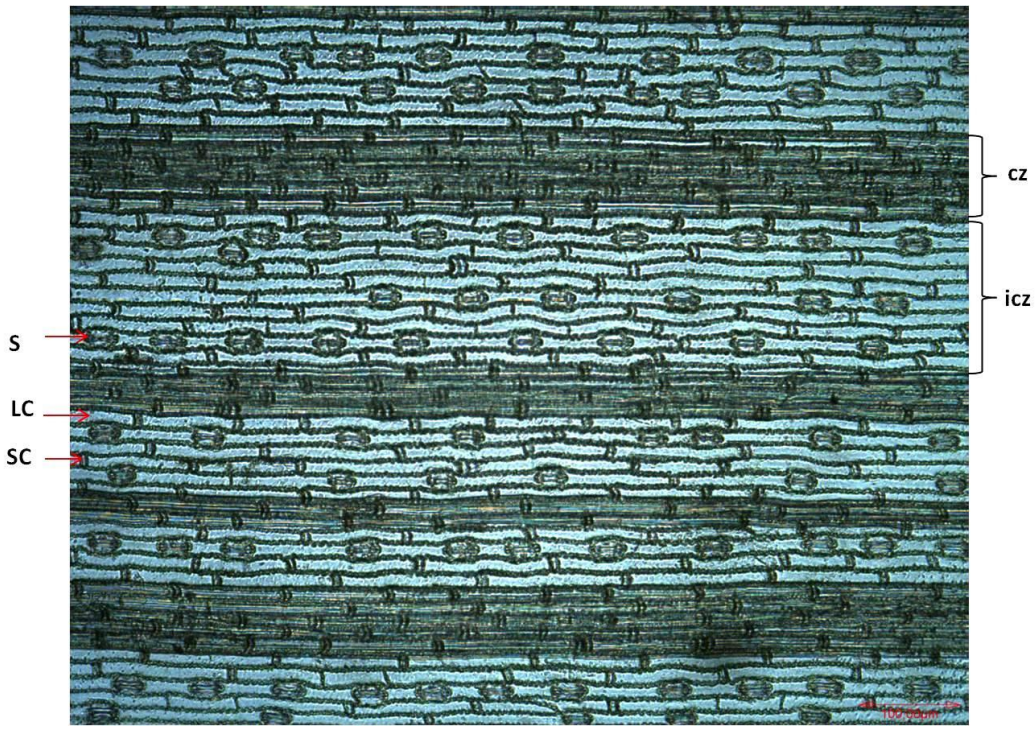

Figure 46. Abaxial leaf surface of *Secale sylvestre*. **cz:** costal zone, **icz:** intercostals zone, **LC:** Long cell, **SC:** Short cell, **S:** Stoma

Figure 46 is a LM observation from the abaxial leaf surface of *Secale sylvestre.* In this structure, it is clear that, the intercostal zones and the costal zones follow eachother in the leaf surface. Both zones comprise long cells, short cells and silica cells. However, stomata are parallelly arranged in the intercostals zones.

3.3.2.1 Long cells and Short cells

In the studies about the leaf surfaces of grasses, the shape of the long epidermal cells was recognized as a diagnostic character (Metcalfe, 1960; Keshavarzi *et al.*, 2007).

The long cell properties are most clearly seen in observations with Light Microscope. According to the surface views, there are 2 types of long cells on the abaxial sides of leaves in the tribe as smooth (Figure 47) and sinuous walled (Figure 48) long epidermal cells. In most of the taxa, the long epidermal cells are smooth walled. For example, *Elytrigia sosnowskyi, Thinopyrum elongatum, Elytrigia canina, Elymus farctus, Ae. triuncialis* subsp *triuncialis* and subsp *bozdagensis, Ae. markgrafii, Ae. juvenalis, Ae. columnaris, Ae. cylindrica, Ae. umbellata, Ae. peregrine, Ae. geniculata, Ae. speltoides, Ae. kotschyii, Ae. comosa, Ae. vavilovii, Triticum* except *T. araraticum, Eremopyrum confusum*, *E. boneopartis, E. triticeum* and the genera *Heteranthelium, Dasypyrum* and *Hordelymus*.

Moreover, in the genus *Hordeum*, except *H. brevisubulatum,* all the other taxa have abaxial epidermal long cells with straight walls. However, in the genera *Elymus* (except *E. farctus*), *Agropyron, Secale, Pseudoroegeria, Leymus, Henrardia, Taeniatherum, Ambylopyrum, Crithopsis, Psathyrostachys* and the taxa *Triticum araraticum, Thinopyrum erosiglumis, Thinopyrum intermedium, Elytrigia repens, Ae. biuncialis, Ae. crassa, Ae. tauschii, Ae. triuncialis* subsp *persica, Eremopyrum distans, Eremopyrum orientale, H. brevisubulatum* the epidermal cell walls of abaxial leaf surfaces are sinuous.

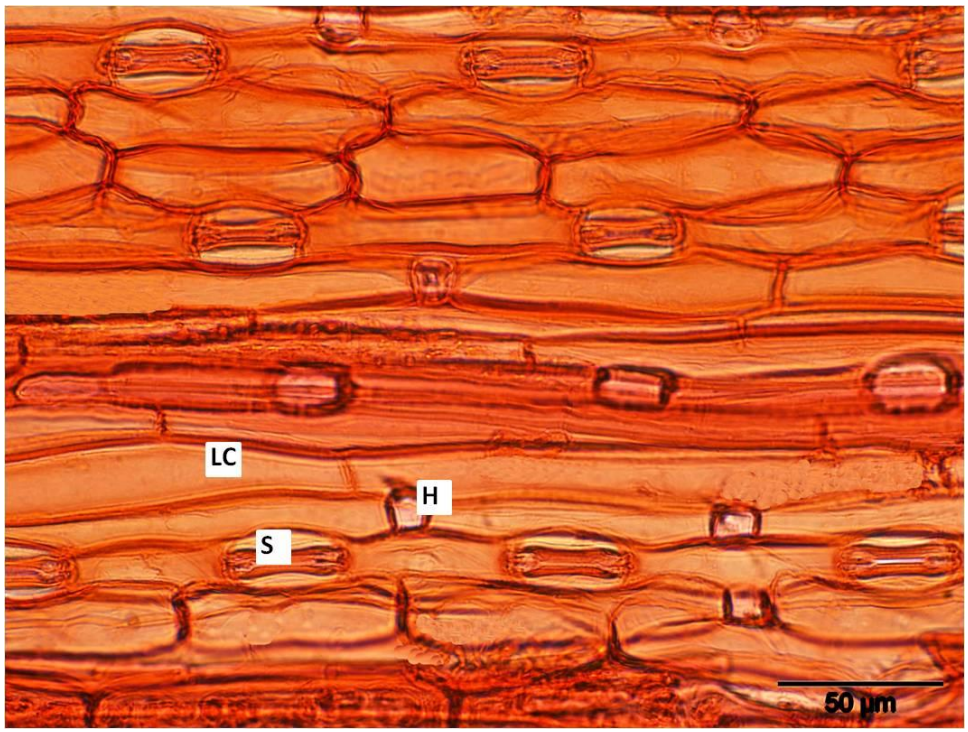

Figure 47. Abaxial leaf surface of *Dasypyrum villosum* **H:** hook, **LC:** Long cell, **S:** Stoma

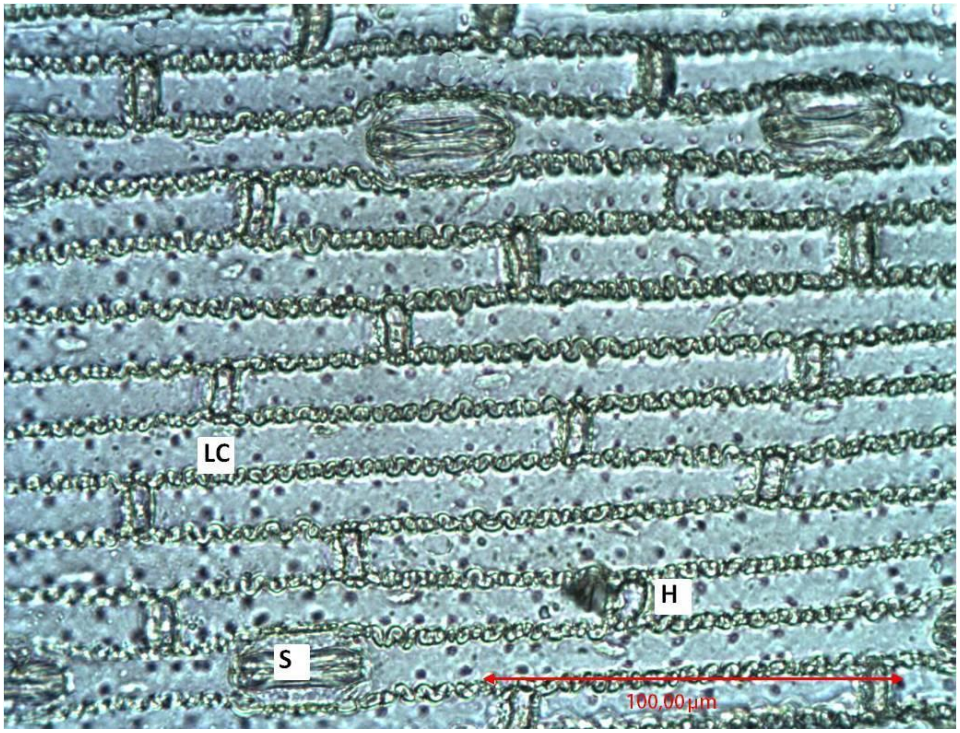

Figure 48. Abaxial leaf surface of *Elymus transhyrcanus* **H:** hook, **LC:** Long cell, **S:** Stoma

There are also short epidermal cells between the long epidermal cells on the leaf surfaces. Generally on the abaxial surfaces, there are 1 or 2 short cells, one of which is silica cell, between two long cells (Figure 49). However, in adaxial surfaces, 3 short cells between two long cells have also been observed (Figure 50). Moreover, in intercostal zones of leaf surfaces, there are parallelly arranged stomata.

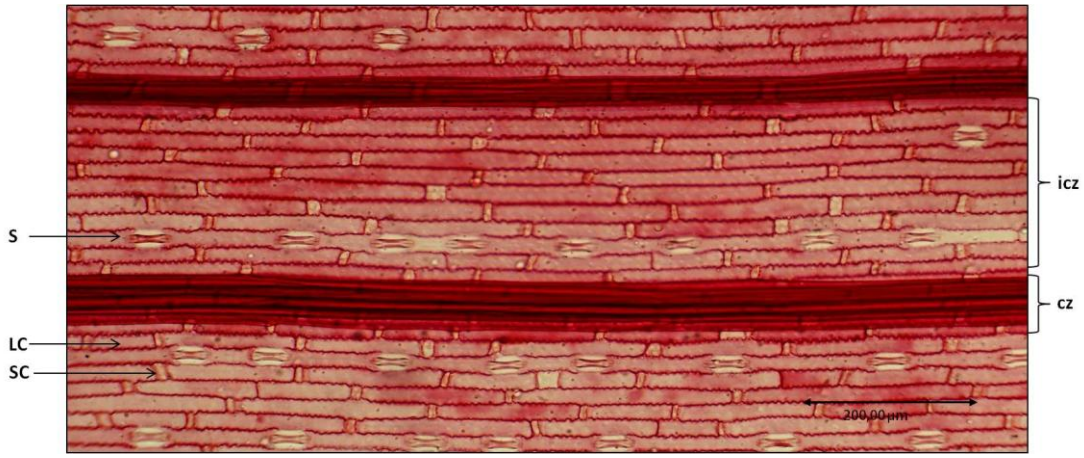

Figure 49. Abaxial leaf surface of *Elytrigia repens*. **cz:** costal zone, **icz:** intercostals zone, **LC:** Long cell, **SC:** Short cell, **S:** Stoma

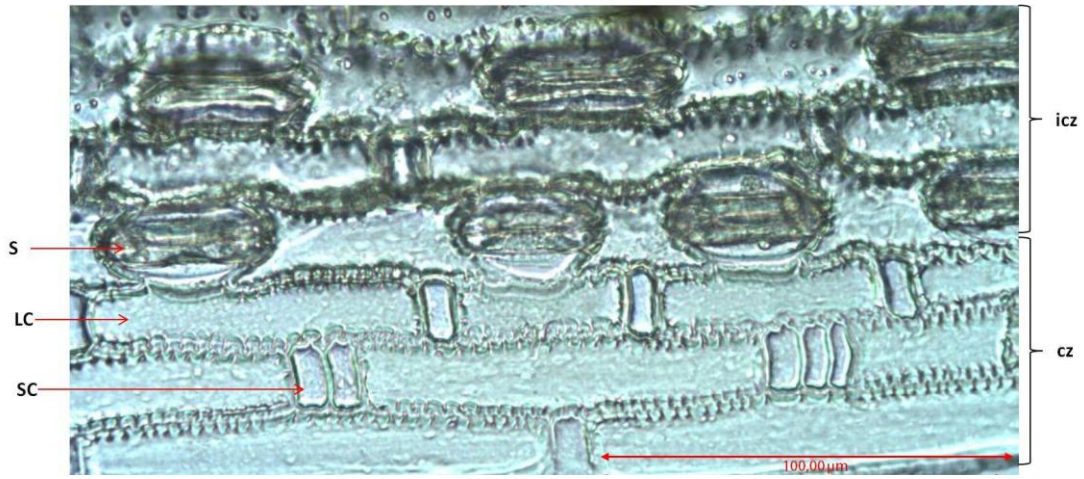

Figure 50. Abaxial leaf surface of *Leymus racemosus* subsp *sabulosus* **cz:** costal zone, **icz:** intercostals zone, **LC:** Long cell, **SC:** Short cell, **S:** Stoma

3.3.2.2 Stoma Architecture

Metcalfe (1960) classified stomata of the leaves of the family according to the adjacent subsidiary cells. With reference to this study, it can be observed that the stomata of the studied taxa of the tribe have variance according to their adjacent subsidiary cells. Most of the stomata have long and paralel shaped adjacent cells. Also there are stomata in different shapes, such as dome shape and low dome shape.

Acording to Metcalfe (1960), it is important to measure the length and the width of stomata. These measurements help to decide the type of the stomata. For example, in dome shaped stomata, the width of them are more close to the measurements of the length.

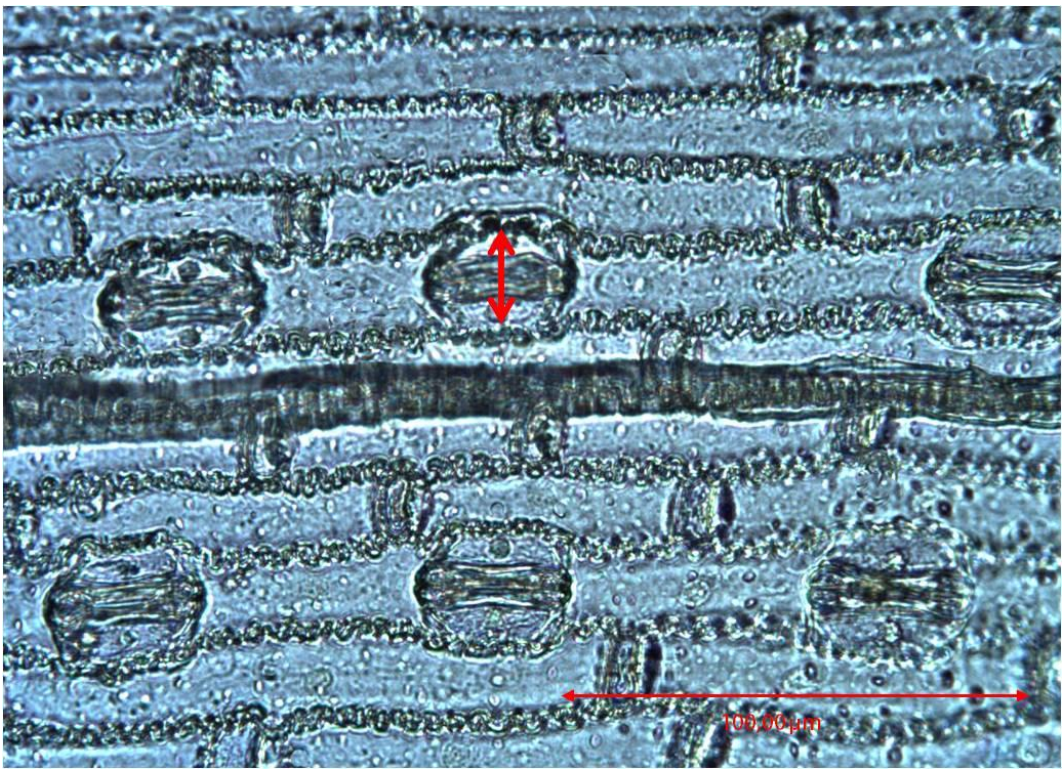

Figure 51. Abaxial leaf surface of *Thinopyrum intermedium*. The arrow indicates the witdh of stomata.

The longest width was measured in *Ae. kotschyi* as 23,65 ± 1,644 µm and the shortest length was measured in *Ae. geniculata* as 23,20 ± 0,991 µm. However, to decide the stomata shapes, it is important to calculate the length/width ratio of them.

The smallest ratio, 1,50 ,was obtained from *Thinopyrum intermedium* (Figure 51). The measurements from stomata show that *Elytrigia sosnowskyi* has the shortest width as 10,50 ± 0,456 µm, within the leaf abaxial surfaces of the tribe. Moreover, the longest length was measured on abaxial surfaces of *A. cristatum* subsp *pectinatum* var *puberulum* as 50,20 ± 2,312 µm. However, the highest ratio was measured as 3,29 from the stomata of the leaf abaxial surfaces of *Eremopyrum confusum* var *sublanuginosum* (Figure 52).

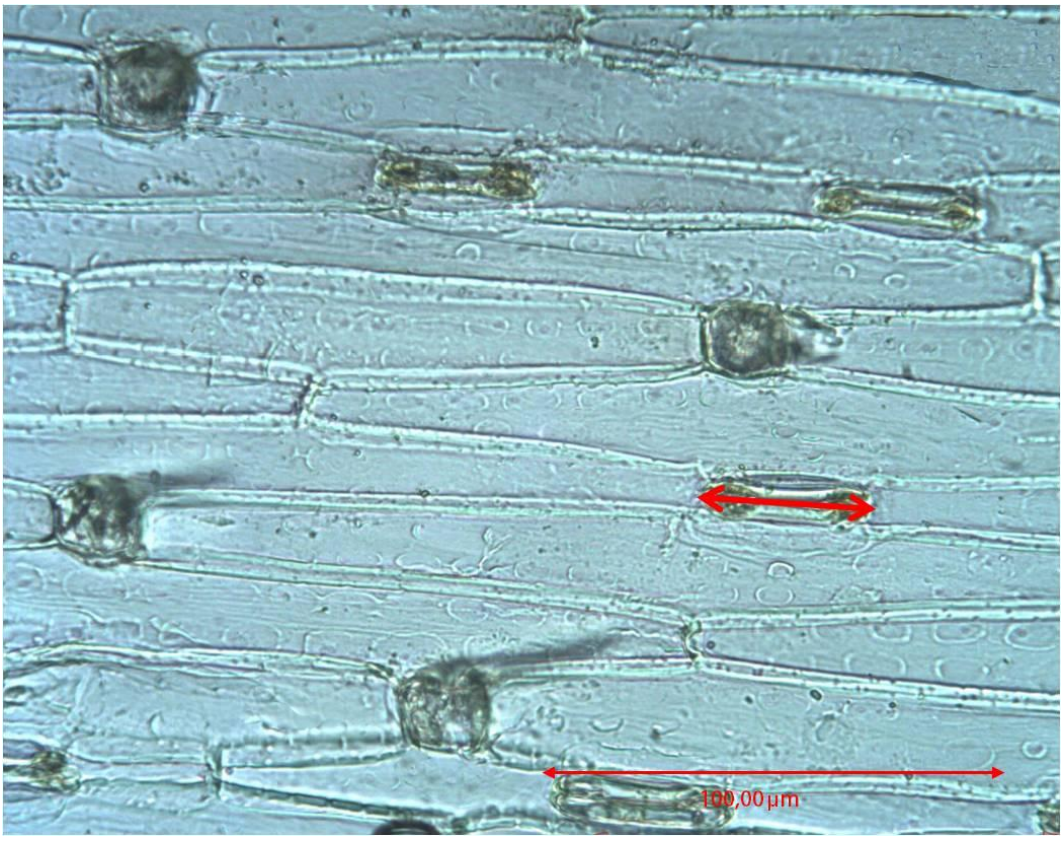

Figure 52. Abaxial leaf surface of *Eremopyrum confusum* var *sublanuginosum*. The arrow indicates the length of stomata.

When the observations from the abaxial surfaces of leaves are examined, it is seen that, *Elymus pycnanthus* (Figure 53) and *Elymus farctus* do not have any stomata on their abaxial sides of leaves. This is because of the abaxially continuous sclerenchyma in their leaves. From this point of view, it can be said that, the abaxial sides of the leaves of *Elymus pycnantus* and *Elymus farctus* have only costal zones, including long cells and short cells.

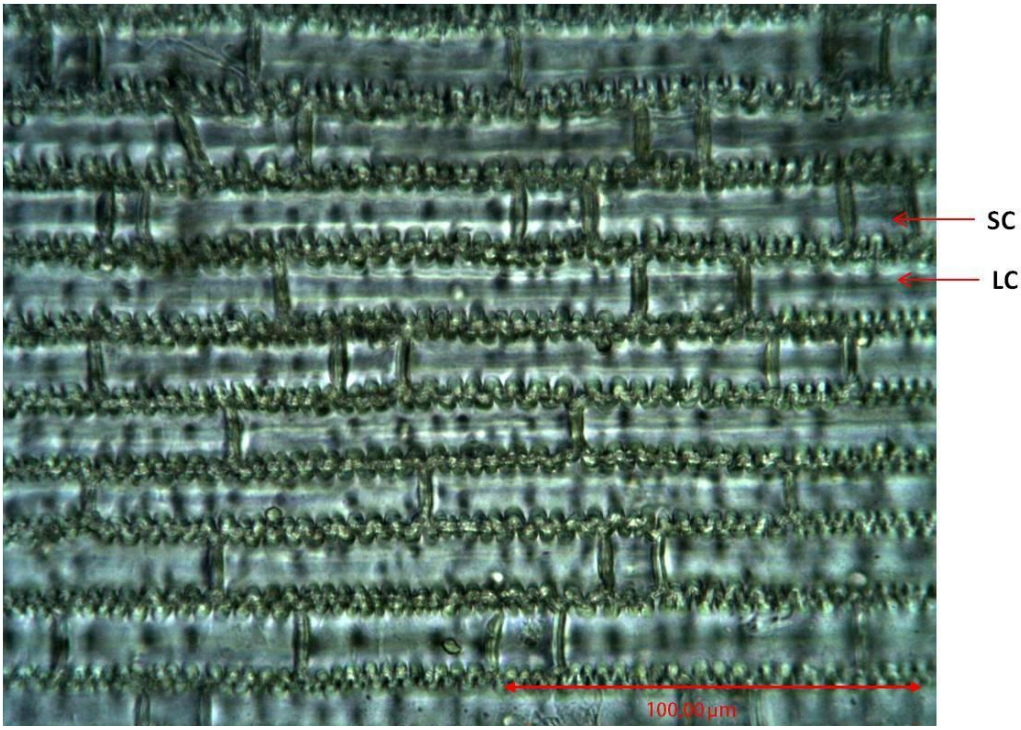

Figure 53. Abaxial leaf surface of *Elymus pycnanthus* **LC:** Long cell, **SC:** Short cell.

Observations from the leaf surfaces of the tribe show that, there are maximum 4 layers, at least 1 layer of stomata on the intercostal zones of leaves. Moreover, there are maximum 9 layers and minimum 1 layer of long cells were measured. The number of stomata layers or the numbers of long cells between these layers have not seemed to be a diagnostic character since the beginning of the anatomical studies including the leaf surfaces. However, it is such an important character to distinguish the taxa (Mavi *et al.*, 2011a). In their abaxial leaf surfaces, most of the taxa have 2 layers of stomata and 4 layers of long cells between them (Figure 54).

The density of stomata on leaf surfaces seems to be another important diagnostic character (Metcalfe, 1960). Within the tribe, the average stomata number of leaf surfaces varies from 3,18 ± 0,536 µm in *E. transhyrcanus* to 12,13 ± 0,498 µm measured in *H. geniculatum*. Generally, in large genera such as *Aegilops*, the average stomatal density is about 6,04, whereas in *Elymus* the density is about 3,81. Moreover, *Secale* and *Eremopyrum* have nearly the same stoma numbers as 5,72 and 5,39 respectively.

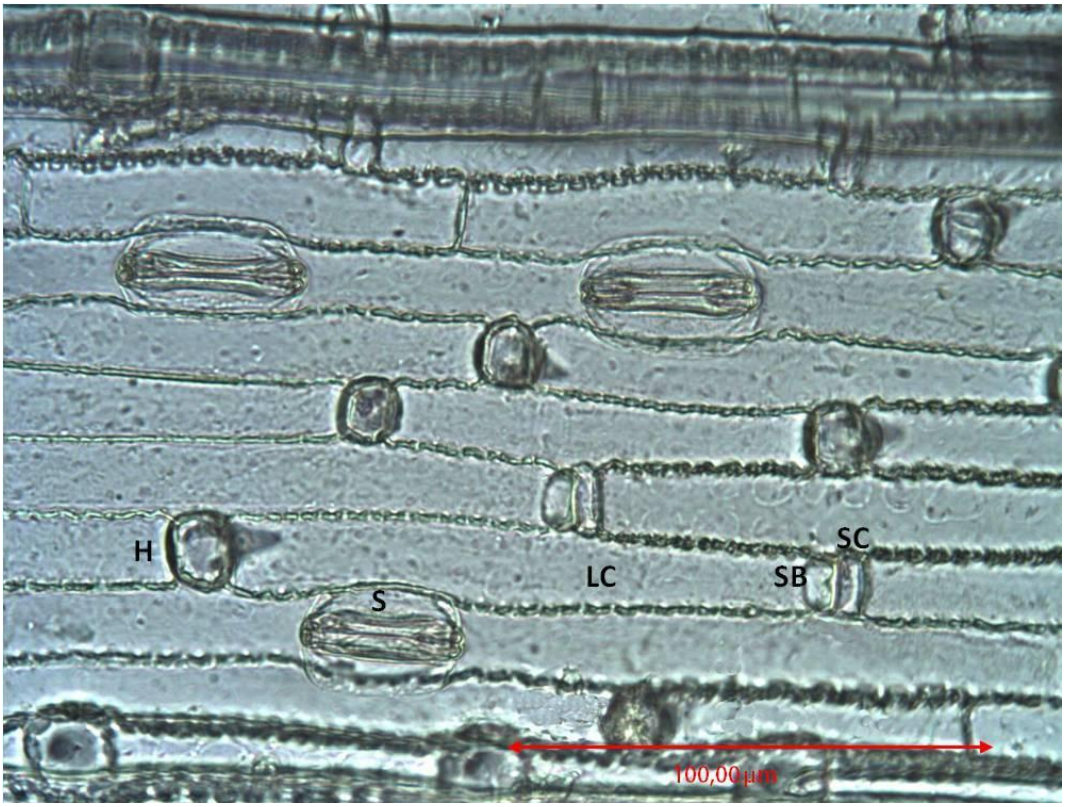

Figure 54. Abaxial leaf surface of *Aegilops juvenalis*. **H:** Hook, **LC:** Long cell, **SB:** Silica body, **SC:** Short cell, **S:** Stoma

Hordeum has the most stomata on the leaf abaxial surfaces as 8,58. On the leaf surfaces of *Agropyron* (Figure 55a) and Leymus stomatal density was measured as 4,47 and 4,10 respectively. In the tribe, there are also genera represented by only one species. In *Crithopsis*, the number of stomata was measured as 9,75 ± 0,197 µm. *Heteranthelium* and *Hordelymus* (Figure 55b) follow it with the average densities as 8,09 ± 0,728 µm and 7,01 ± 0,256 µm.

The general leaf surface characteristics of the tribe are given in Table 7.

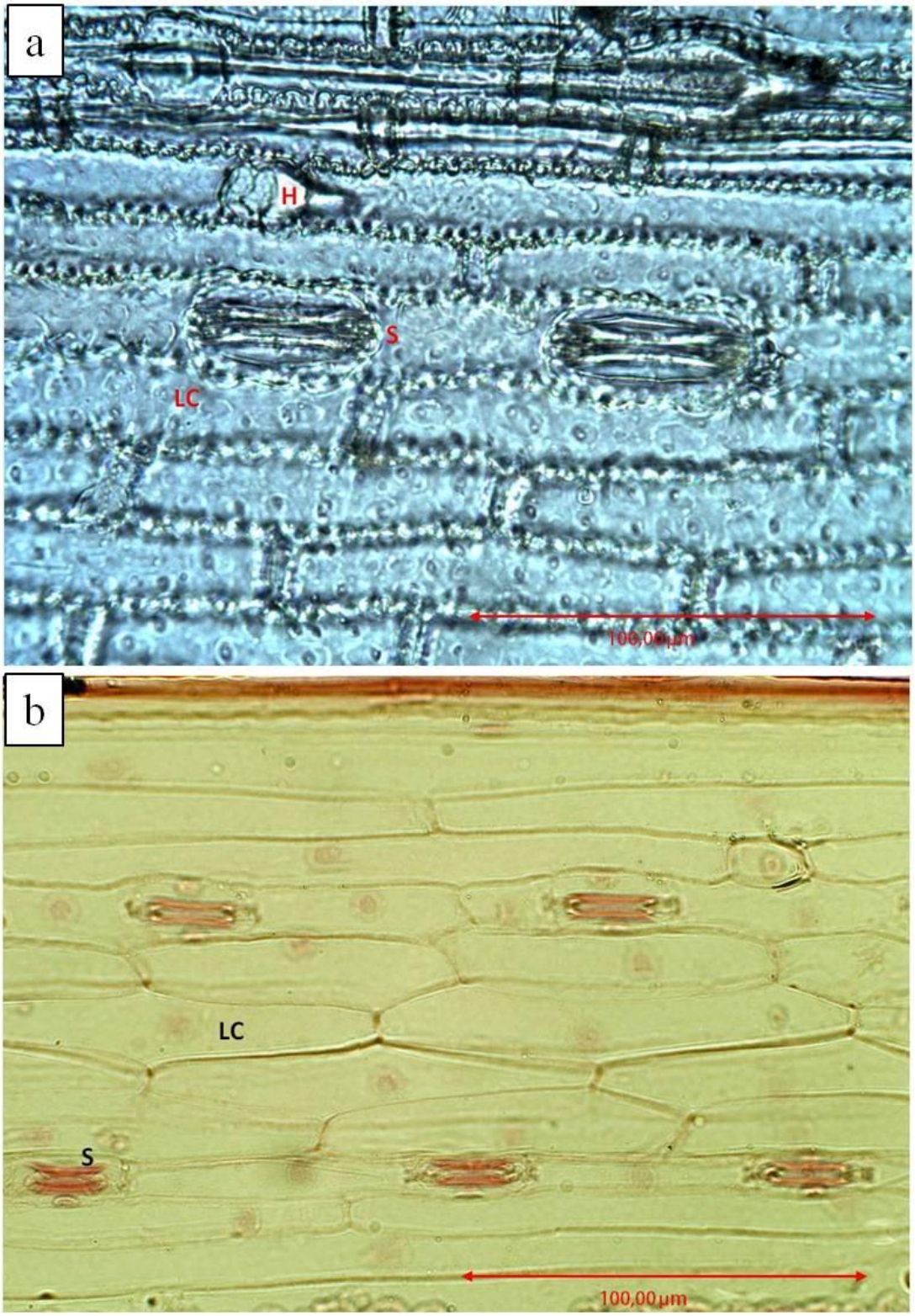

Figure 55. Abaxial leaf surfaces **a)** *Agropyron cristatum* subsp *pectinatum* var *puberulum*. **b)** *Hordelymus europeous*. **H:** Hook, **LC:** Long cell, **S:** Stoma.

3.3.2.3 Silica cells

According to Prychid *et al.* (2004), many plants take up soluble monosilicic acid from the soil and some of these plants subsequently deposit it as cell inclusions of characteristic structures. Moreover, epidermal silica cells mostly located over sclerenchyma that is called costal zones of leaf surfaces, but such as in Poaceae, silica also occurs in intercostal cells. Silica cells occur both adaxially and abaxially in the leaf epidermis, but the frequency varies according to the number of subepidermal sclerenchyma strands or girders, which in some genera are nearly equally numerous on both surfaces, while in others they are more numerous abaxially (Prychid *et al.*, 2004). Prychid *et al.* (2004) also pointed out the possibility of the number of bodies and rows as diagnostic characters. However, it was also added that, the age of leaf and the environmental conditions must be taken into account (Ollendorf, 1992).

In the tribe *Triticeae*, silica cells on the leaf surfaces are costally or intercostally located. Costally located silica bodies may be equidimentional, conical and mostly horizontally elongated, but they are equidimentional or conical in intercostal zones (Mavi *et al.*, 2011a).

Figure 56a shows the abaxial leaf surface of *Taeniatherum caput-medusa*. In this taxon, the conical silica cells are both costally and intercostally located on the abaxial surface. However, silica cells was observed only in costally located on the adaxial surface of its leaves. From the observations of SEM studies, the adaxial surfaces of leaves were examined. The costally located silica bodies and intercostal stomata of the adaxial leaf surface of *Taeniatherum caput-medusa* can be seen in Figure 56b. There are also taxa having silica cells only their costal zones of both adaxial and abaxial surfaces. These taxa can be listed as *Ae. kotschyi, Ae. umbellata, Ae. columnaris, Ae. markgrafii, H. distichon, H. spontaneum* var *anatolicum* (Figure 57) and *H. vulgare*.

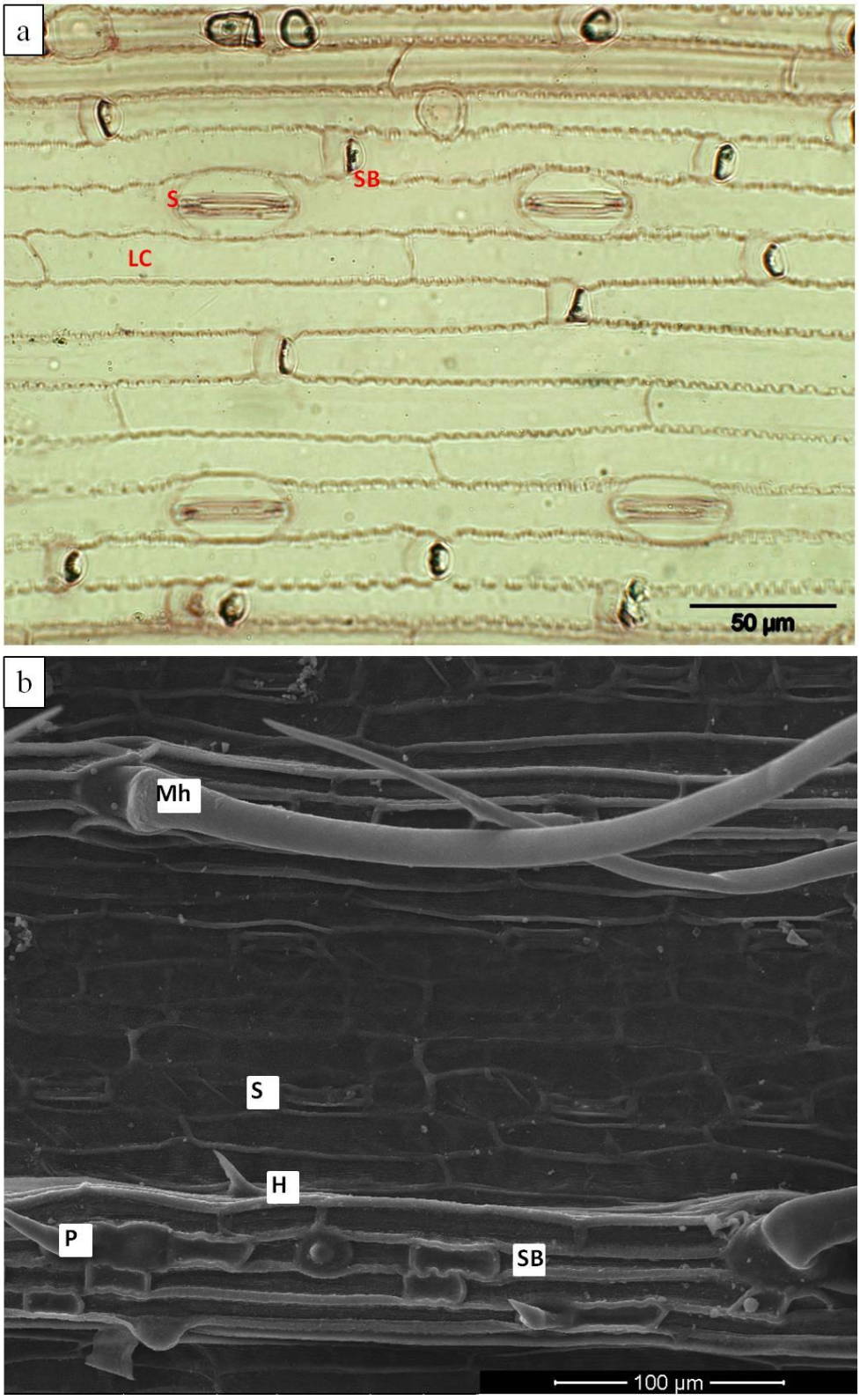

Figure 56. Abaxial leaf surface of *Taeniatherum caput-medusa*. **a)** abaxial surface, **b)** adaxial surface. **H:** Hook, **LC:** Long cell, **Mh:** Macrohairs, **P:** Prickle, **SB:** Silica body, **S:** Stoma.

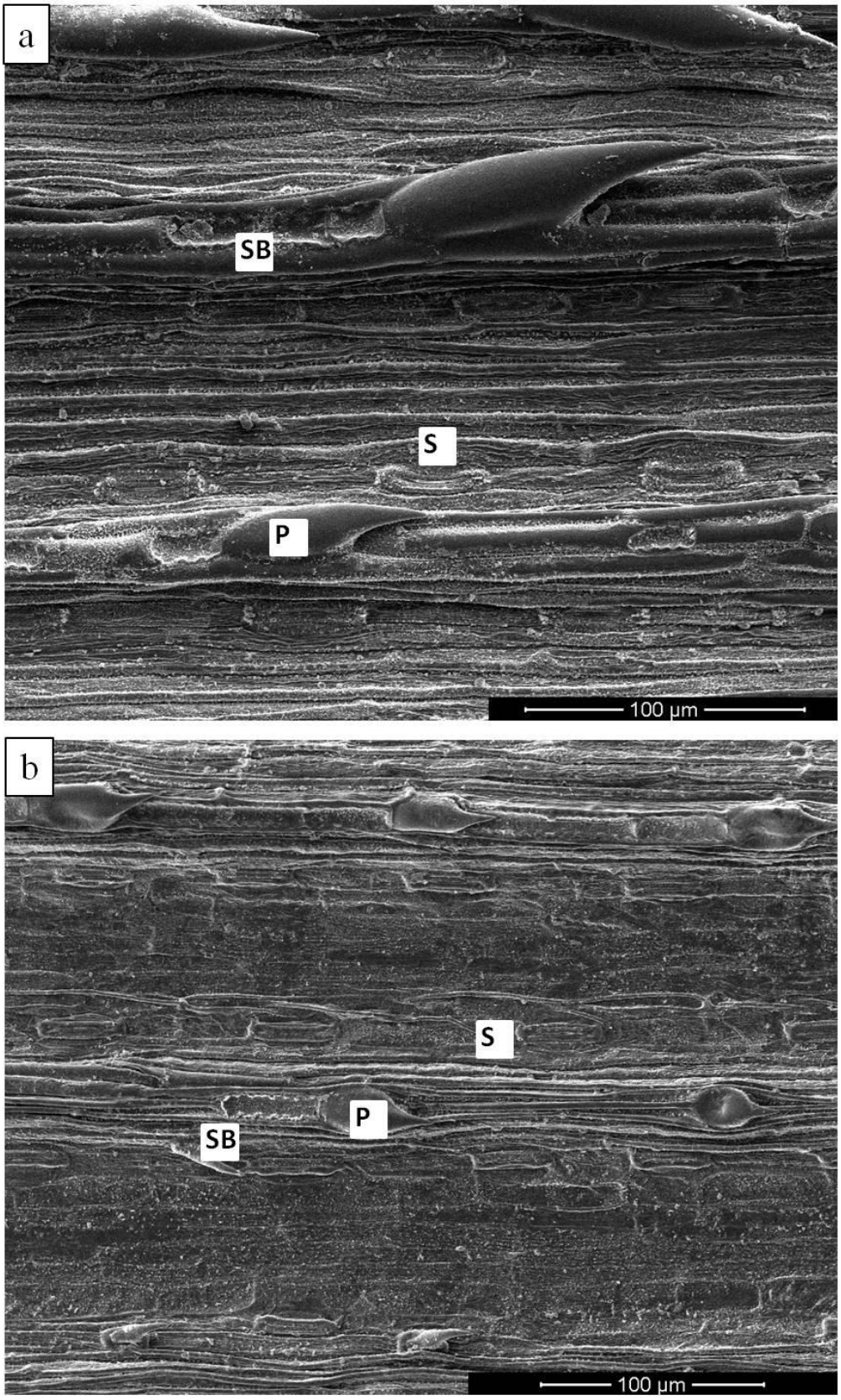

Figure 57. Leaf surfaces of *H. spontaneum* var *anatolicum* **a)** abaxial surface, **b)** adaxial surface. **P:** Prickle, **SB:** Silica body, **S:** Stoma.

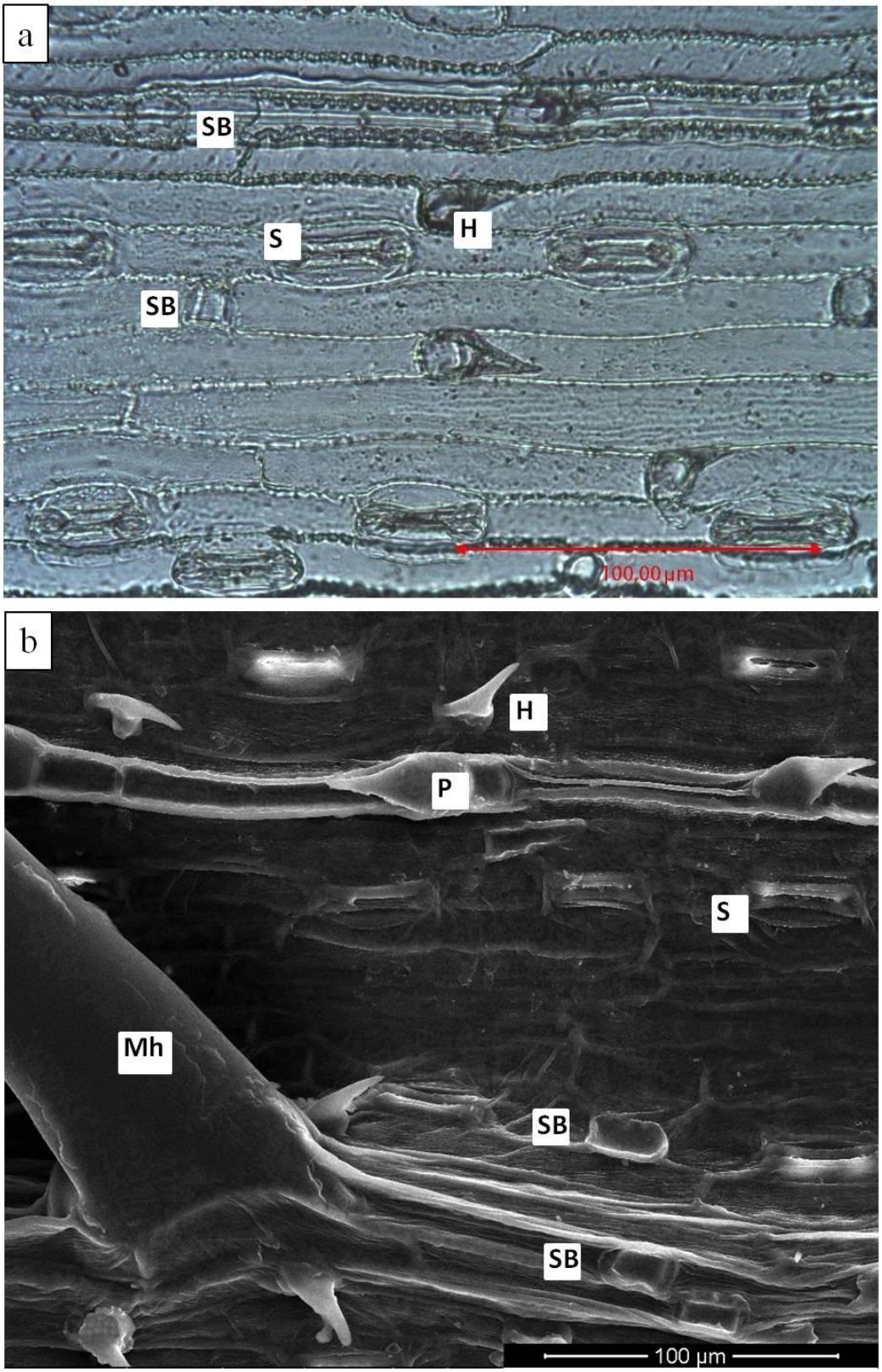

Figure 58. Leaf surfaces of *Ae. triuncialis* subsp *bozdagensis* **a)** abaxial surface, **b)** adaxial surface. **H:** Hook, **Mh:** Macrohair, **P:** Prickle, **SB:** Silica body, **S:** Stoma.

In most of the taxa of the tribe, silica cells located both in the costal and the intercostal zones of both their abaxial and adaxial surfaces of leaves. Examples for this structure are the genera *Elytrigia, Thinopyrum, Agropyron, Leymus, Crithopsis, Psathyrostachys, Triticum* (except *T. araraticum*) and the taxa of *Pseudoroegneria divaricata, Ae. triuncialis* (Figure 58), *Elymus pycnanthus, Elymus farctus, Ae. markgrafii, Ae. biuncialis, Ae. tauschii, Ae. vavilovi, Hordeum brevisubulatum, H. geniculatum, H. bulbosum, H. marinum* var *pubescens* and *H. murinum*.

3.3.2.4 Leaf Hairs

The indumentum property of the leaves in the tribe includes hooks, prickles and unicellular macrohairs. Hooks are the shortest type of hairs. The longest hooks are seen on the leaves of *Psathyrostachys fragilis* as 29,53 ± 1,827 µm and the shortest hooks are measured as 6, 37 ± 1,972 µm in *Taeniatherum caput-medusa*. Generally, in each leaf of any of the taxa, prickles are longer than their hooks. The longest hooks are also longer than the shortest prickles, measured as 18,36 ± 1,654 µm in *Ambylopyrum muticum*. However, hooks on the leaf surfaces of *Ambylopyrum* are measured as 8,14 ± 2,388 µm. Moreover the longest prickle are seen on the leaf surfaces of *Elymus pycnanthus* as 60,57 ± 2,049 µm. All the measurements are given in Table 8.

The macrohair type of all the leaves in the tribe *Triticeae* is single-celled simple hairs. Length of the macrohairs on the leaf surfaces varies between 75,23 ± 10,744 µm and 1124,00 ± 15,660 µm in *Triticum araraticum* and *Dasypyrum villosum* respectively. There are 2 different subcategories of the indumentum properties of the leaf surfaces of the tribe:

- The appearance of the hooks, the prickles and/or the macrohairs are located costally and/or intercostally on the abaxially and/or adaxially surfaces of leaves.

- Macrohair and the prickle line numbers are in the costal zones on the abaxial and/or the adaxial surfaces of the leaves.

The appearance of the hooks, the prickles and the macrohairs on the adaxial or abaxial surfaces of the leaves and the corresponding taxa can be listed as;

1- The leaves have all the types of hairs on their abaxial and adaxial surfaces: *Ae. triuncialis, Ae. speltoides* (Figure 59), *Ae. umbellata, Ae. biuncialis, Ae. crassa, cylindrica, Ae. juvenalis, Ae. peregrina, Ae. tauschii, Ae. markgrafii*, *Ae. vavilovi, Pseudoroegneria divaricata*, *H. marinum* var *pubescens, H. geniculatum,* the genera *Crithopsis, Henrardia* and *Dasypyrum*.

2- The leaves have hooks and prickles on their adaxial and abaxial surfaces, but don't have macrohairs neither on their abaxial nor adaxial surfaces: *Ambylopyrum* (Figure 60), *Psathyrostachys,* and *Triticum monococcum, T. dicoccon, T. boeoticum, Elymus transhyrcanus, Elymus lazicus, Ae. columnaris, Eremopyrum boneopartis* var *sinaicum, Leymus racemosus, Hordeum bulbosum, H. distichon, H. spontaneum* var *anatolicum,* and *H. vulgare*.

3- The leaves have hooks and prickles on their adaxial and abaxial surfaces and macrohairs appear only on their adaxial surfaces: *Ae. comosa, Elytrigia sosnowskyi, Elytrigia canina, Agropyron cristatum subsp pectinatum*, *Triticum araraticum, Eremopyrum triticeum, Eremopyrum orientale, Leymus cappadocicus, H. brevisubulatum, H. murinum, Hordelymus europeaus* (Figure 61).

4- The leaves do not contain any of the hair types on their abaxial surfaces: T. *erosiglumis, E. pycnanthus, E. longearistatus, Thinopyrum elongatum* (Figure 62), *E. farctus, E. repens, Eremopyrum distans*, *Pseudoroegneria libanotica, E. confusum* var *glabrum* (Figure 63), *Secale sylvestre* and *Taeniatherum.*

5- The leaves have macrohairs on their adaxial and abaxial surfaces and have prickle and/or hooks on their adaxial or abaxial sides: *Henrardia persica, Agropyron incanum, Ae. peregrina, Ae. geniculata* (Figure 64), *Ae. kotchyi*.

6- The leaves have adaxially located macrohairs and have prickles and/or hooks on their adaxial and/or abaxial surfaces: *Thinopyrum intermedium, Eremopyrum*

confusum var *sublanuginosum* (Figure 65).

7- The leaves don't have any prickles or hooks, but have macrohairs on their adaxial and abaxial surfaces: *Heteranthelium piliferum* (Figure 66).

Figure 59 shows the abaxial and adaxial surfaces of *Ae. speltoides* var *ligustica*. On both of these surfaces, the leaves have all the hair types, hooks, prickles and macrohairs. Moreover, it is clearly seen in Figure 60 that, *Ambylopyrum muticum* has prickles and hooks on both adaxial and abaxial surfaces of their leaves, but macrohairs are not seen. However, the leaves of *Hordelymus europeaus* (Figure 61) have adaxial macrohairs which can be used for differentiating it from *Ambylopyrum muticum*.

Figure 62 shows the abaxial leaf surface of *Thinopyrum elongatum*. This species doesn't have any macrohairs, hooks or prickles on its leaf surfaces. However, the adaxial leaf surface of the species has only one layer of macrohairs, whose length was measured as 287,64 ± 11,237 μm. Moreover, *Thinopyrum erosiglumis* also has the same property. However, the costally located adaxial macrohair layer number is 7. On the other hand, *Eremopyrum confusum* var. *glabrum* (Figure 63) has only 1 layer of prickle on its costal zone of the adaxial leaf surface. Moreover, the number of macrohair lines on the costal zones of leaves varies between 1 and 7 on the adaxial surfaces. However, on abaxial sides the number seems to vary from 1 to 3, but extremely the biggest macrohair line number was measured in *Agropyron incanum* as 8 on its abaxial leaf surfaces.

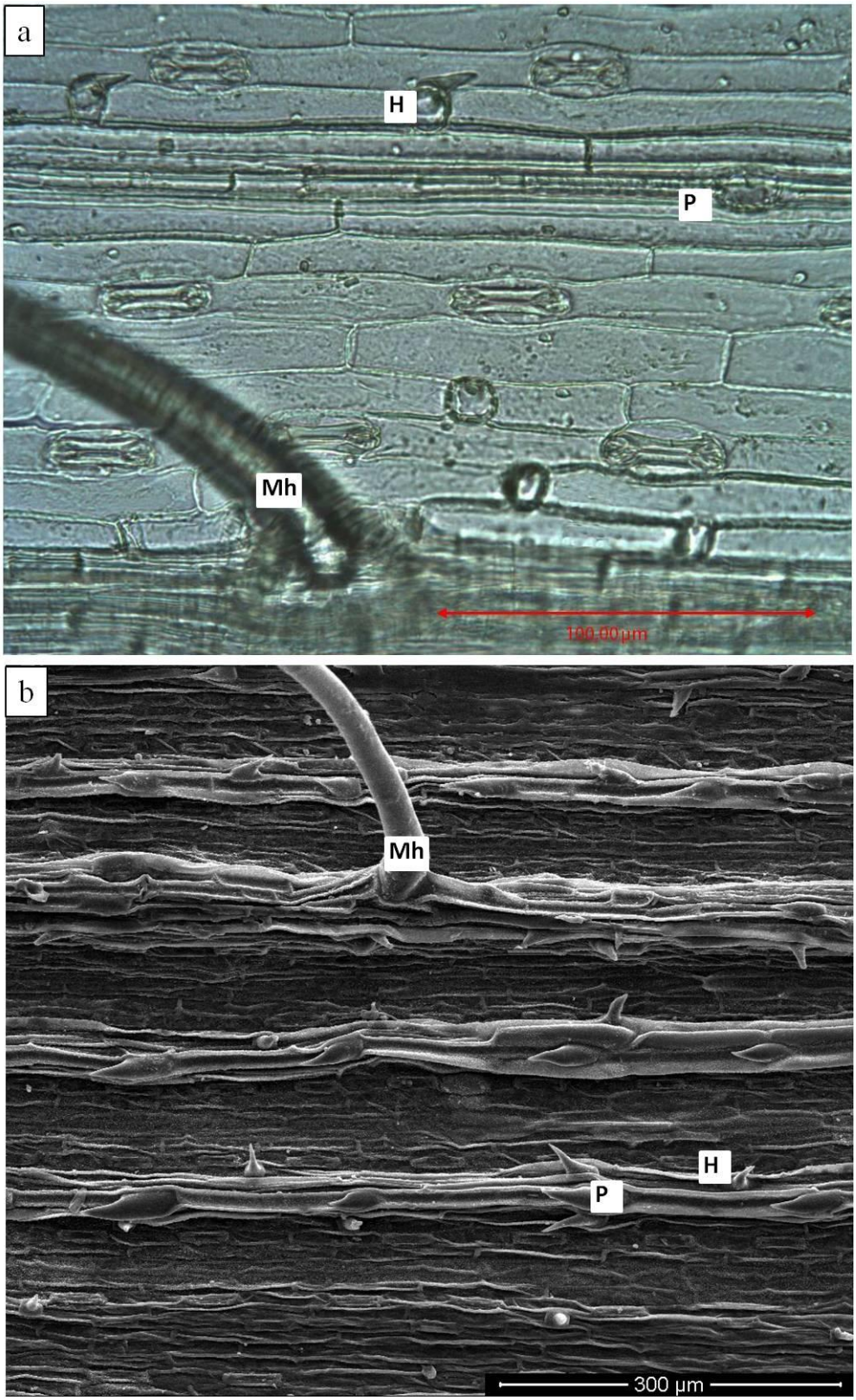

Figure 59. Leaf surfaces of *Aegilops speltoides* var *ligustica* **a)** abaxial surface, **b)** adaxial surface. **H:** Hook, **Mh:** Macrohair, **P:** Prickle.

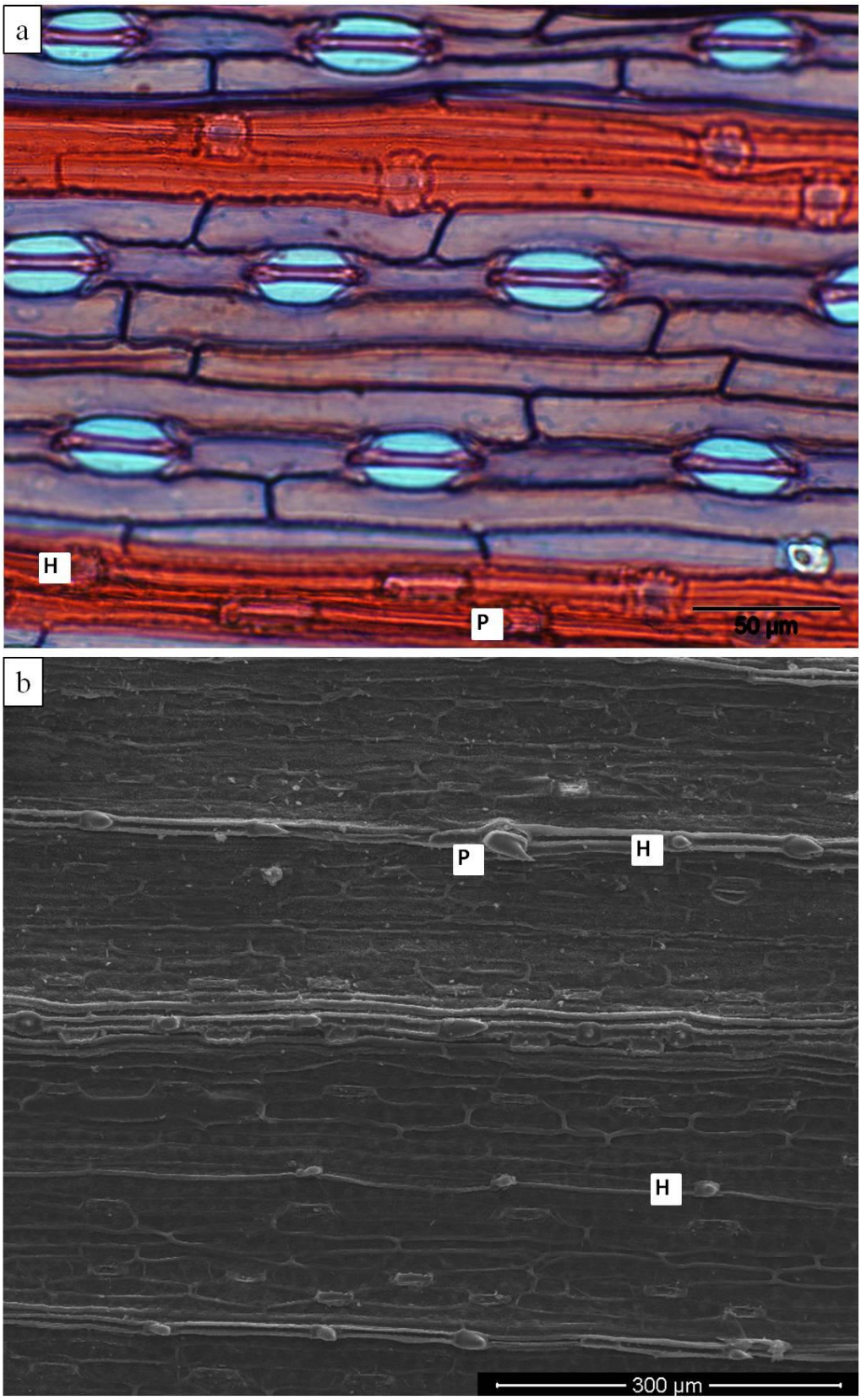

Figure 60. Leaf surfaces of *Ambylopyrum muticum* **a)** abaxial surface, **b)** adaxial surface. **H:** Hook, **P:** Prickle.

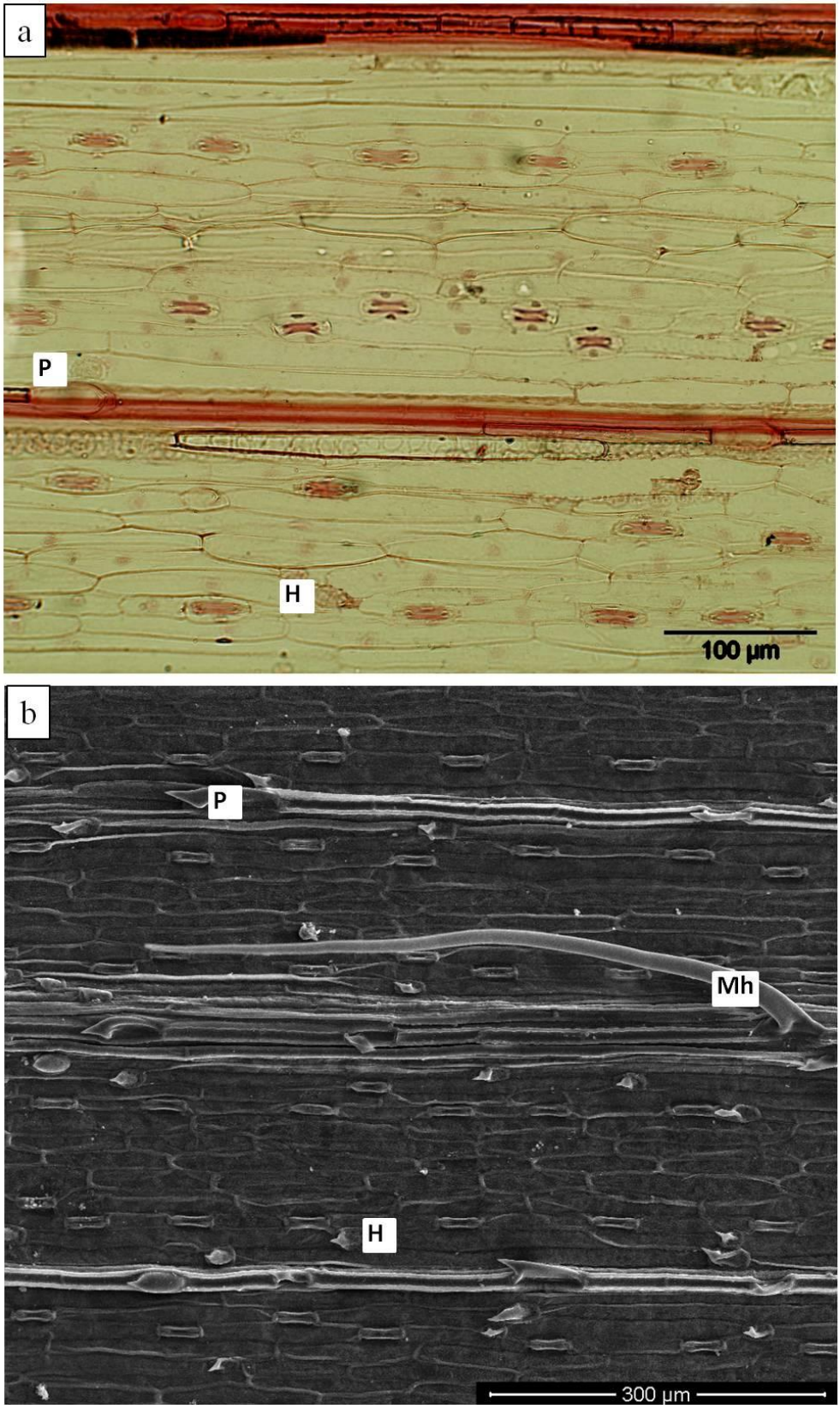

Figure 61. Leaf surfaces of *Hordelymus europeaus* **a)** abaxial surface, **b)** adaxial surface. **H:** Hook, **Mh:** Macrohair, **P:** Prickle.

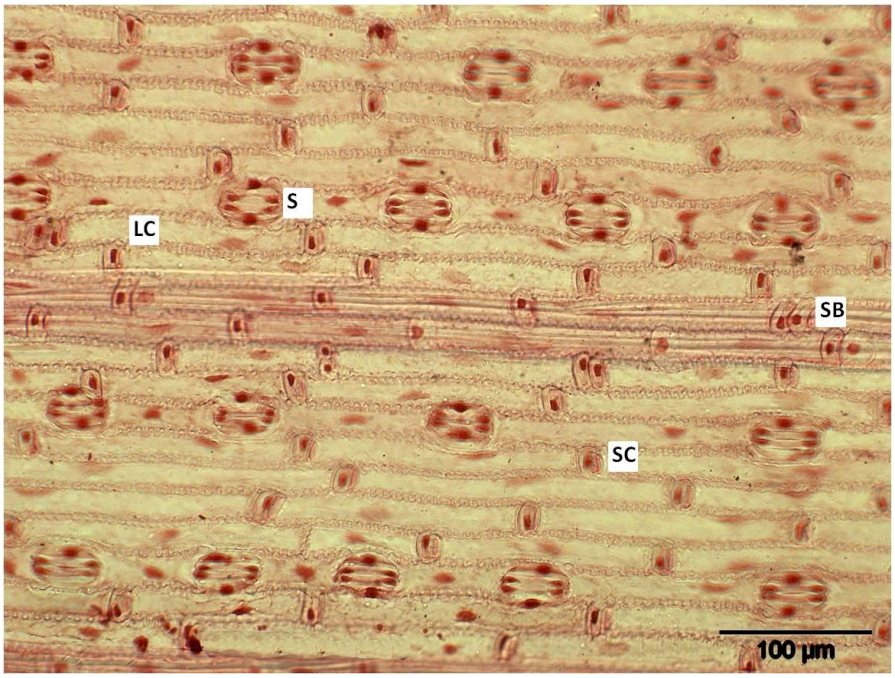

Figure 62. Abaxial leaf surface of *Thinopyrum elongatum* **LC:** Long cell, **S:** Stoma, **SB:** Silica body, **SC:** Short cell.

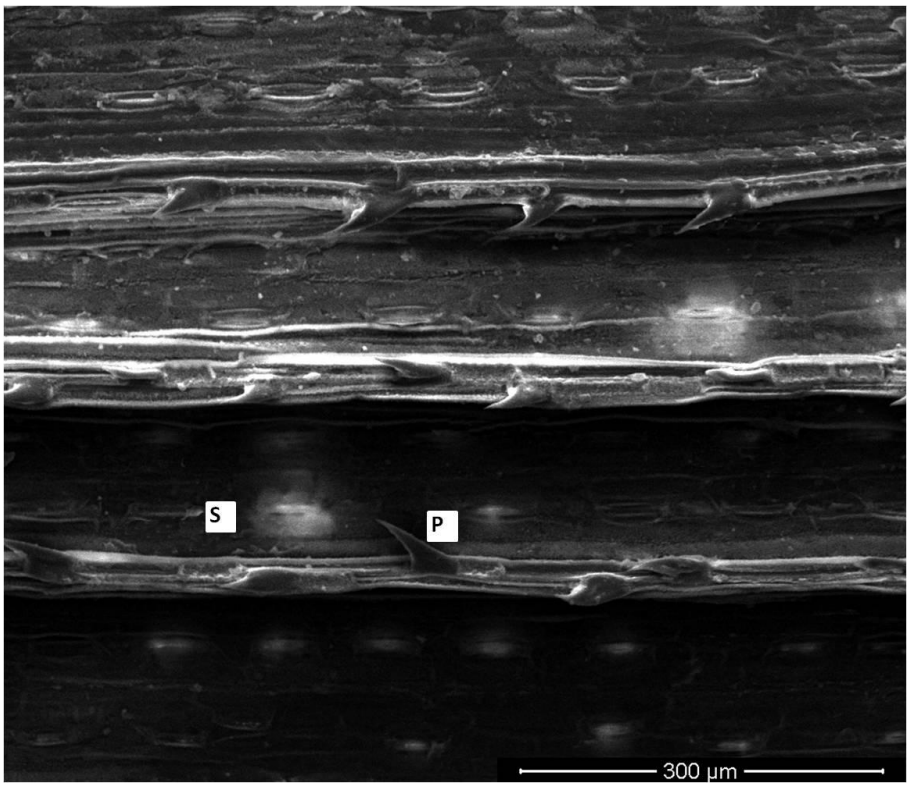

Figure 63. Adaxial leaf surface of *Eremopyrum confusum var glabrum.* **P:** Prickle, **S:** Stoma.

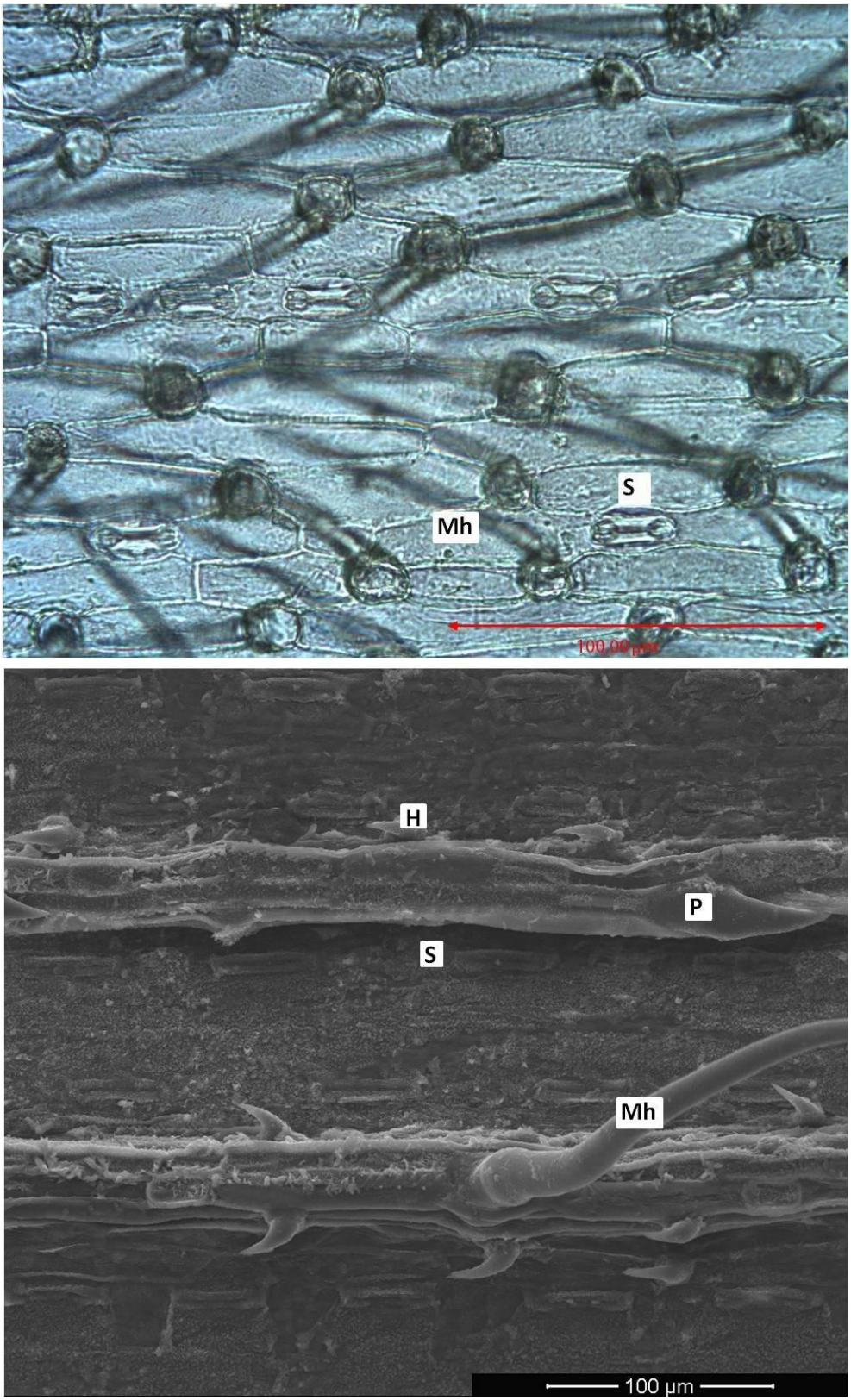

Figure 64. Leaf surfaces of *Aegilops geniculata* **a)** abaxial surface, **b)** adaxial surface. **H:** Hook, **Mh:** Macrohair, **P:** Prickle, **S:** Stoma.

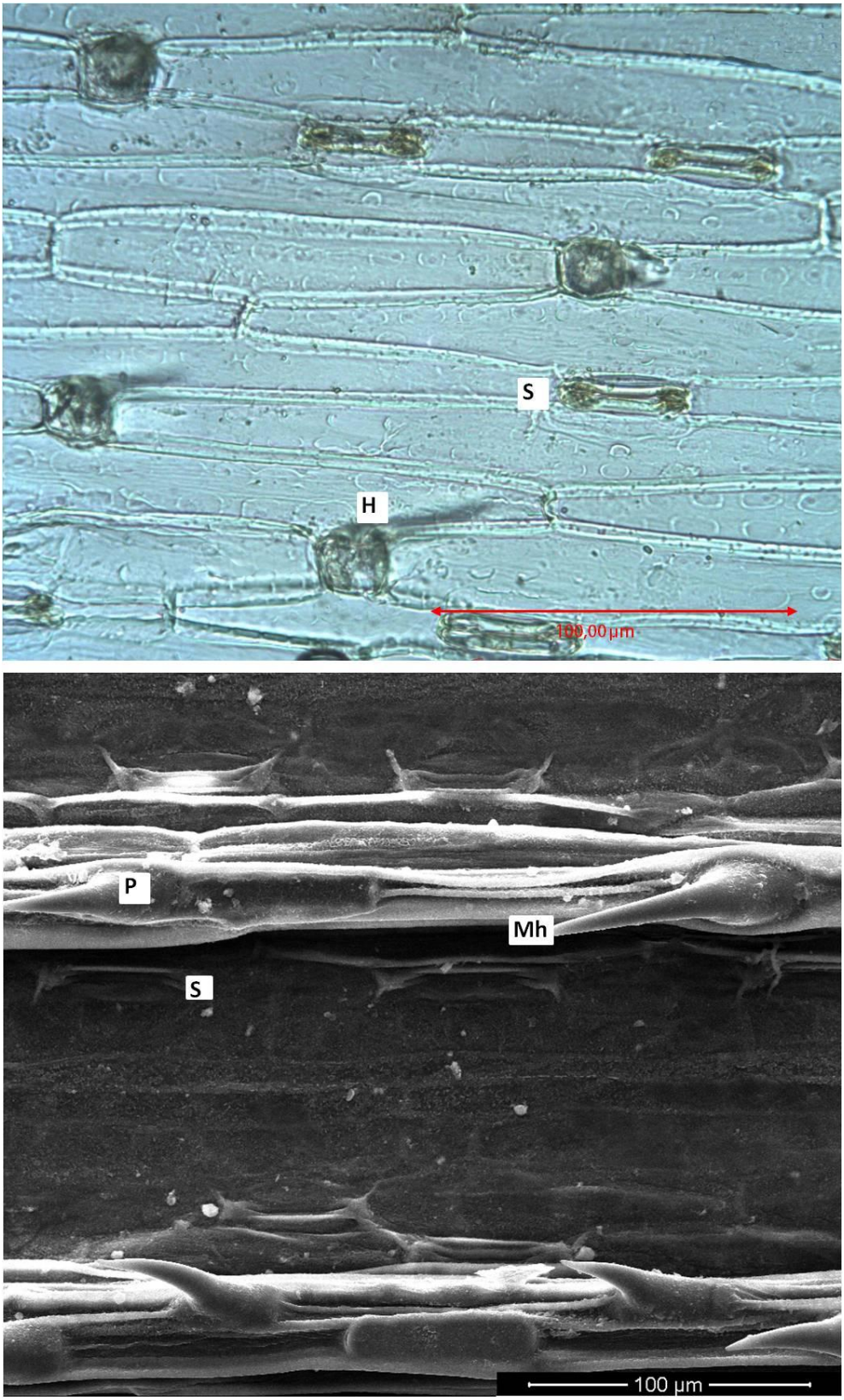

Figure 65. Leaf surfaces of *Eremopyrum confusum* subsp *sublanuginosum* **a)** abaxial surface, **b)** adaxial surface. **H:** Hook, **Mh:** Macrohair, **P:** Prickle, **S:** Stoma.

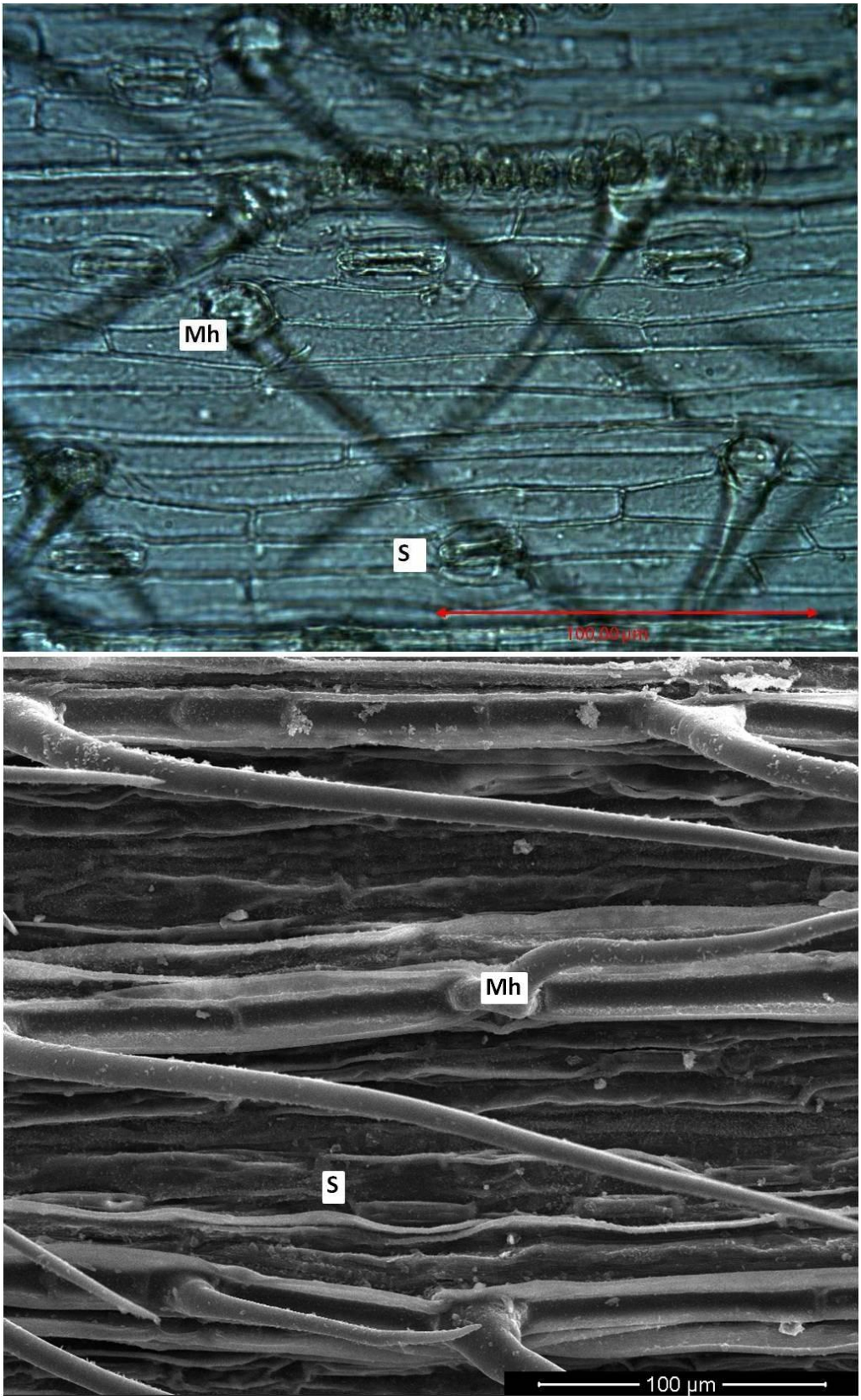

Figure 66. Leaf surfaces of *Heteranthelium piliferum* **a)** abaxial surface, **b)** adaxial surface. **Mh:** Macrohair, **S:** Stoma.

Table 7. General characteristics of leaf surfaces

Taxa	Cell wall undulation	hooks on adaxial surface	hooks on abaxial surface	*Mh on adaxial surface	*Mh on abaxial surface	Adaxial silica bodies	Abaxial silica bodies	Costal Mh line number on adaxial	Costal Mh line number on abaxial	Costal prickle line number on adaxial	Costal prickle line number on abaxial	Stomata line number on abaxial	Max *IS cell line
Elymus pycnanthus	present	absent	absent	*C	absent	both *C and *IC	both *C and *IC	7	0	3	0	0	0
Thinopyrum erosiglumis	present	absent	absent	*C	absent	both *C and *IC	both *C and *IC	7	0	0	0	2	3
Elytrigia sosnowskyi	absent	both *C and *IC	both *C and *IC	*C	absent	both *C and *IC	both *C and *IC	1	0	5	1	2	4
Elymus transhyrcanus	present	*C	both *C and *IC	absent	absent	*C	both *C and *IC	0	0	2	1	2	8
Elymus lazicus subsp *lazicus*	present	*C	*C	absent	absent	*C	both *C and *IC	0	0	4	1	1	0
Elymus longearistatus	present	*C	absent	absent	absent	*C	both *C and *IC	0	0	4	0	1	0
Thinopyrum intermedium	present	absent	*C	*C	absent	both *C and *IC	both *C and *IC	1	0	3	1	2	4
Elytrigia repens	present	both *C and *IC	absent	absent	absent	both *C and *IC	both *C and *IC	0	0	5	0	2	4

*C: Costal, *IC: Intercostal, *IS: Interstomatal, *Mh: Macrohair

Table 7 Continue. General characteristics of leaf surfaces

Taxa	Cell wall undulation	hooks on adaxial surface	hooks on abaxial surface	*Mh on adaxial surface	*Mh on abaxial surface	Adaxial silica bodies	Abaxial silica bodies	Costal Mh line number on adaxial	Costal Mh line number on abaxial	Costal prickle line number on adaxial	Costal prickle line number on abaxial	Stomata line number on abaxial	Max *IS cell line
Thinopyrum elongatum	absent	absent	absent	*C	absent	both *C and *IC	both *C and *IC	1	0	0	0	2	3
Elytrigia canina	absent	both *C and *IC	both *C and *IC	*C	absent	both *C and *IC	both *C and *IC	1	0	5	1	2	1
Elymus farctus	absent	both *C and *IC	absent	*C	absent	both *C and *IC	both *C and *IC	7	0	3	0	0	0
Pseudoroegneria libanotica	present	*C	absent	*C	absent	*C	both *C and *IC	5	0	4	0	2	4
Pseudoroegneria divaricata	present	*C	both *C and *IC	*C	both *C and *IC	both *C and *IC	both *C and *IC	5	1	4	2	2	3
Ae. triuncialis subsp. *triuncialis*	absent	*C	both *C and *IC	*C	*C	both *C and *IC	both *C and *IC	1	1	3	1	2	5
Ae. kotschyi	absent	*C	*C	*C	*C	*C	*C	1	1	1	0	2	5
Ae. markgrafii	absent	both *C and *IC	both *C and *IC	*C	*C	both *C and *IC	both *C and *IC	1	1	1	1	4	3

*C: Costal, *IC: Intercostal, *IS: Interstomatal, *Mh: Macrohair

Table 7 Continue. General characteristics of leaf surfaces

Taxa	Cell wall undulation	hooks on adaxial surface	hooks on abaxial surface	*Mh on adaxial surface	*Mh on abaxial surface	Adaxial silica bodies	Abaxial silica bodies	Costal Mh line number on adaxial	Costal Mh line number on abaxial	Costal prickle line number on adaxial	Costal prickle line number on abaxial	Stomata line number on abaxial	Max *IS cell line
Ae. speltoides var. *ligustica*	absent	both *C and *IC	both *C and *IC	*C	*C	both *C and *IC	*C	1	1	2	2	2	4
Ae. speltoides var. *speltoides*	absent	both *C and *IC	both *C and *IC	*C	*C	both *C and *IC	*C	1	1	1	1	2	5
Ae. umbellata	absent	*C	*C	*C	*C	*C	*C	1	1	3	1	2	4
Ae. biuncialis	present	*C	both *C and *IC	*C	*C	both *C and *IC	both *C and *IC	1	1	1	1	2	3
Ae. geniculata	absent	both *C and *IC	absent	*C	both *C and *IC	both *C and *IC	*C	1	1	1	0	3	7
Ae. crassa	present	both *C and *IC	both *C and *IC	*C	*C	*C	both *C and *IC	1	1	2	1	2	3
Ae. peregrina	absent	*C	*C	*C	*C	*C	both *C and *IC	1	1	1	0	2	3
Ae. cylindrica	absent	*C	*C	*C	*C	*C	both *C and *IC	1	1	1	1	2	4

*C: Costal, *IC: Intercostal, *IS: Interstomatal, *Mh: Macrohair

Table 7 Continue. General characteristics of leaf surfaces

Taxa	Cell wall undulation	hooks on adaxial surface	hooks on abaxial surface	*Mh on adaxial surface	*Mh on abaxial surface	Adaxial silica bodies	Abaxial silica bodies	Costal Mh line number on adaxial	Costal Mh line number on abaxial	Costal prickle line number on adaxial	Costal prickle line number on abaxial	Stomata line number on abaxial	Max *IS cell line
Ae. columnaris	absent	both *C and *IC	both *C and *IC	absent	absent	*C	*C	0	0	1	1	2	3
Ae. juvenalis	absent	both *C and *IC	both *C and *IC	*C	*C	*C	both *C and *IC	1	1	1	1	2	4
Ae. tauschii	present	*C	*C	*C	*C	both *C and *IC	both *C and *IC	1	1	1	1	2	3
Ae. triuncialis subsp. *bozdagensis*	absent	*C	both *C and *IC	*C	*C	both *C and *IC	both *C and *IC	1	1	1	1	2	4
Ae. triuncialis subsp. *persica*	present	*C	both *C and *IC	*C	*C	both *C and *IC	both *C and *IC	1	1	1	1	2	3
Ae. comosa	absent	both *C and *IC	both *C and *IC	*C	absent	*C	both *C and *IC	1	0	1	1	2	3
T. caput-medusae	present	*C	absent	*C	absent	*C	both *C and *IC	1	0	3	0	2	7

*C: Costal, *IC: Intercostal, *IS: Interstomatal, *Mh: Macrohair

Table 7 Continue. General characteristics of leaf surfaces

Taxa	Cell wall undulation	hooks on adaxial surface	hooks on abaxial surface	*Mh on adaxial surface	*Mh on abaxial surface	Adaxial silica bodies	Abaxial silica bodies	Costal Mh line number on adaxial	Costal Mh line number on abaxial	Costal prickle line number on adaxial	Costal prickle line number on abaxial	Stomata line number on abaxial	Max *IS cell line
Ae. vavilovii	absent	both *C and *IC	both *C and *IC	*C	*C	both *C and *IC	both *C and *IC	1	1	1	1	3	5
Agr. incanum	present	absent	both *C and *IC	both *C and *IC	*C	both *C and *IC	both *C and *IC	3	8	0	6	2	4
Agr. cristatum subsp *pectinatum var. puberulum*	present	both *C and *IC	both *C and *IC	*C	absent	both *C and *IC	both *C and *IC	1	0	2	2	2	4
Agr. cristatum subsp *pectinatum* var. *pectinatum*	present	both *C and *IC	both *C and *IC	*C	absent	both *C and *IC	both *C and *IC	1	0	2	4	3	9
T. monococcum	absent	both *C and *IC	both *C and *IC	absent	absent	both *C and *IC	both *C and *IC	0	0	3	3	2	4
T. dicoccon	absent	both *C and *IC	both *C and *IC	absent	absent	both *C and *IC	both *C and *IC	0	0	3	3	2	4
T. boeoticum	absent	both *C and *IC	both *C and *IC	absent	absent	both *C and *IC	both *C and *IC	0	0	3	1	2	4

*C: Costal, *IC: Intercostal, *IS: Interstomatal, *Mh: Macrohair

Table 7 Continue. General characteristics of leaf surfaces

Taxa	Cell wall undulation	hooks on adaxial surface	hooks on abaxial surface	*Mh on adaxial surface	*Mh on abaxial surface	Adaxial silica bodies	Abaxial silica bodies	Costal Mh line number on adaxial	Costal Mh line number on abaxial	Costal prickle line number on adaxial	Costal prickle line number on abaxial	Stomata line number on abaxial	Max *IS cell line
T. araraticum	present	both *C and *IC	both *C and *IC	*C	absent	*C	both *C and *IC	1	0	3	2	2	4
E. confusum var *sublanuginosum*	absent	*C	both *C and *IC	*C	absent	*C	both *C and *IC	1	0	2	0	4	3
E. distans	present	*C	absent	*C	absent	*C	both *C and *IC	1	0	1	0	3	7
E. triticeum	absent	both *C and *IC	both *C and *IC	*C	absent	*C	both *C and *IC	1	0	2	2	2	6
E.orientale	present	*C	both *C and *IC	*C	absent	*C	both *C and *IC	1	0	2	2	2	5
E.boneopartis var *sinaicum*	absent	*C	both *C and *IC	absent	absent	*C	both *C and *IC	0	0	2	2	2	7
E. confusum var *confusum*	absent	absent	absent	*C	*C	*C	both *C and *IC	1	1	1	0	4	3
E. confusum var *glabrum*	absent	absent	absent	absent	absent	*C	both *C and *IC	0	0	1	0	4	3

*C: Costal, *IC: Intercostal, *IS: Interstomatal, *Mh: Macrohair

Table 7 Continue. General characteristics of leaf surfaces

Taxa	Cell wall undulation	hooks on adaxial surface	hooks on abaxial surface	*Mh on adaxial surface	*Mh on abaxial surface	Adaxial silica bodies	Abaxial silica bodies	Costal Mh line number on adaxial	Costal Mh line number on abaxial	Costal prickle line number on adaxial	Costal prickle line number on abaxial	Stomata line number on abaxial	Max *IS cell line
S. sylvestre	present	absent	absent	both *C and *IC	absent	*C	both *C and *IC	1	0	0	0	3	3
S. cereale	present	both *C and *IC	absent	absent	absent	0	both *C and *IC	0	0	0	1	3	3
L. racemosus subsp *sabulosus*	present	*C	*C	absent	absent	both *C and *IC	both *C and *IC	0	0	1	1	3	1
L. cappadocicus	present	*C	*C	*C	absent	both *C and *IC	both *C and *IC	1	0	1	1	3	1
H. brevisubulatum subsp *violaceum*	present	both *C and *IC	both *C and *IC	*C	absent	both *C and *IC	both *C and *IC	1	0	2	2	2	3
H. geniculatum	absent	both *C and *IC	both *C and *IC	both *C and *IC	both *C and *IC	both *C and *IC	both *C and *IC	3	3	4	4	2	3
H. bulbosum	absent	both *C and *IC	both *C and *IC	absent	absent	both *C and *IC	both *C and *IC	0	0	1	3	2	9

*C: Costal, *IC: Intercostal, *IS: Interstomatal, *Mh: Macrohair

Table 7 Continue. General characteristics of leaf surfaces

Taxa	Cell wall undulation	hooks on adaxial surface	hooks on abaxial surface	*Mh on adaxial surface	*Mh on abaxial surface	Adaxial silica bodies	Abaxial silica bodies	Costal Mh line number on adaxial	Costal Mh line number on abaxial	Costal prickle line number on adaxial	Costal prickle line number on abaxial	Stomata line number on abaxial	Max *IS cell line
H. distichon	absent	both *C and *IC	both *C and *IC	absent	absent	*C	*C	0	0	1	1	4	9
H, spontaneum var *anatolicum*	absent	*C	*C	absent	absent	*C	*C	0	0	1	1	4	9
H. vulgare	absent	*C	*C	absent	absent	*C	*C	0	0	1	1	4	9
H. murinum subsp *glaucum*	absent	both *C and *IC	both *C and *IC	*C	absent	both *C and *IC	both *C and *IC	1	0	1	1	2	6
H. marinum var *pubescens*	absent	both *C and *IC	both *C and *IC	*C	both *C and *IC	both *C and *IC	both *C and *IC	3	3	4	4	2	6
H. persica var *persica*	present	*C	both *C and *IC	*C	both *C and *IC	*C	both *C and *IC	4	3	0	1	2	5
H. piliferum	absent	absent	absent	*C	both *C and *IC	both *C and *IC	*C	2	2	0	0	2	7
H. europaeus	absent	both *C and *IC	both *C and *IC	*C	absent	*C	both *C and *IC	1	0	2	1	2	5

*C: Costal, *IC: Intercostal, *IS: Interstomatal, *Mh: Macrohair

Table 7 Continue. General characteristics of leaf surfaces

Taxa	Cell wall undulation	hooks on adaxial surface	hooks on abaxial surface	*Mh on adaxial surface	*Mh on abaxial surface	Adaxial silica bodies	Abaxial silica bodies	Costal Mh line number on adaxial	Costal Mh line number on abaxial	Costal prickle line number on adaxial	Costal prickle line number on abaxial	Stomata line number on abaxial	Max *IS cell line
A. muticum	present	both *C and *IC	*C	absent	absent	*C	both *C and *IC	0	0	1	1	2	3
C. delileana	present	*C	*C	*C	*C	both *C and *IC	both *C and *IC	1	1	1	1	2	9
P.fragilis	present	both *C and *IC	*C	absent	absent	both *C and *IC	both *C and *IC	0	0	7	3	2	2
D. villosum	absent	both *C and *IC	both *C and *IC	*C	*C	*C	both *C and *IC	1	1	1	1	2	2

*C: Costal, *IC: Intercostal, *IS: Interstomatal, *Mh: Macrohair

Table 8. Measurements from the leaf surfaces (µm).

Taxa	average density of stomata	average length of stomata	average breadth of stomata	Length/ Breath ratio of stomata	Prickle length	hook length	macrohair length
Elymus pycnanthus	0,00	0,00	0,00	0,00	60,57 ± 2,049	_	145,92 ± 12,981
Elymus farctus	0,00	0,00	0,00	0,00	59,64 ± 1,177	_	147,71 ± 12,745
Thinopyrum erosiglumis	4,88 ± 0,175	30,79 ± 0,384	18,77 ± 0,331	1,64	_	_	287,64 ± 10,830
Thinopyrum intermedium	4,87 ± 0,243	32,05 ± 0,271	21,39 ± 0,839	1,50	30,22 ± 1,029	10,51 ± 1,603	286,98 ± 10,492
Thinopyrum elongatum	4,89 ± 0,145	31,81 ± 1,283	19,12 ± 1,293	1,66	_	_	281,49 ± 11,237
Elymus transhyrcanus	3,18 ± 0,536	28,95 ± 1,923	16,25 ± 1,630	1,78	29,69 ± 1,714	9,87 ± 1,320	_
Elymus lazicus subsp *lazicus*	3,66 ± 0,356	33,26 ± 1,374	12,41 ± 0,930	2,68	29,68 ± 0,934	9,86 ± 2,307	_
Elymus longearistatus	4,60 ± 0,234	37,74 ± 2,193	14,07 ± 1,293	2,68	27,95 ± 1,171	11,33 ± 1,470	_
Elytrigia canina	4,66 ± 0,738	36,08 ± 0,236	17,78 ± 1,890	2,02	27,94 ± 1,294	11,36 ± 1,529	251,59 ± 10,834
Elytrigia repens	4,26 ± 0,483	35,94 ± 0,943	17,59 ± 1,377	2,04	29,70 ± 1,029	9,86 ± 1,739	_
Elytrigia sosnowskyi	3,93 ± 0,324	31,50 ± 2,384	10,5 ± 0,456	3,00	28,75 ± 1,332	10,65 ± 1,748	117,46 ± 11,741
Pseudoroegneria libanotica	3,74 ± 0,857	46,32 ± 1,283	18,03 ± 0,882	2,56	28,77 ± 0,923	10,62 ± 1,379	116,69 ± 12,667
Pseudoroegneria divaricata	3,72 ± 0,284	46,43 ± 1,578	18,06 ± 0,762	2,62	28,76 ± 1,439	10,66 ± 2,077	115,92 ± 12,510
Ae. kotschyi	5,89 ± 0,043	38,86 ± 2,073	23,65 ± 1,644	1,64	23,66 ± 1,483	15,05 ± 1,964	314,38 ± 10,448
Ae. markgrafii	8,10 ± 0,384	28,22 ± 2,182	17,71 ± 0,982	1,59	23,53 ± 0,637	15,51 ± 2,097	313,37 ± 12,313
Ae. speltoides var. *ligustica*	7,13 ± 0,203	30,76 ± 1,927	13,22 ± 1,660	2,33	24,87 ± 2,419	15,55 ± 2,934	311,26 ± 11,532
Ae. speltoides var. *speltoides*	7,63 ± 0,254	31,44 ± 1,894	15,72 ± 0,912	2,00	24,65 ± 1,029	15,65 ± 2,477	311,39 ± 10,743
Ae. umbellata	5,07 ± 0,594	31,53 ± 1,960	14,27 ± 1,196	2,21	23,61 ± 2,109	15,16 ± 2,098	315,36 ± 11,518
Ae. biuncialis	5,35 ± 0,738	34,73 ± 1,278	18,77 ± 0,903	1,85	23,60 ± 1,897	15,29 ± 2,471	312,79 ± 10,426
Ae. geniculata	6,13 ± 0,289	23,20 ± 0,991	12,19 ± 1,106	1,90	24,51 ± 2,275	15,14 ± 2,033	315,24 ± 11,386

Table 8 Continue. Measurements from the leaf surfaces (µm).

Taxa	average density of stomata	average length of stomata	average breadth of stomata	Length/ Breath ratio of stomata	Prickle length	hook length	macrohair length
Ae. crassa	6,59 ± 0,582	28,18 ± 2,093	18,29 ± 0,685	1,54	24, 58 ± 1,756	15,56 ± 2,756	317,35 ± 10,315
Ae. peregrina	6,67 ± 0,187	35,63 ± 2,453	23,34 ± 1,557	1,53	23, 50 ± 0,593	15,44 ± 2,081	313,73 ± 12,657
Ae. cylindrica	3,75 ± 0,159	36,70 ± 1,920	16,39 ± 0,738	2,24	23,55 ± 0,839	15,32 ± 1,962	316,36 ± 11,876
Ae. columnaris	7,27 ± 0,359	30,05 ± 0,928	18,89 ± 2,018	1,59	23,69 ± 0,478	15,71 ± 2,094	_
Ae. juvenalis	3,80 ± 0,839	35,67 ± 1,907	21,75 ± 0,966	1,64	23,52 ± 0,391	15,34 ± 1,877	313,98 ± 12,911
Ae. tauschii	7,88 ± 0,491	25,86 ± 1,293	13,68 ± 0,724	1,89	23,59 ± 0,693	15,17 ± 2,703	314,65 ± 10,682
Ae. triuncialis subsp. *triuncialis*	4,35 ± 0,921	39,10 ± 2,473	18,78 ± 1,283	2,08	23,88 ± 0,485	14,96 ± 1,664	310,58 ± 12,463
Ae. triuncialis subsp. *bozdagensis*	5,18 ± 0,741	36,91 ± 2,093	17,39 ± 1,737	2,12	23,89 ± 0,396	14,98 ± 1,907	310,96 ± 11,194
Ae. triuncialis subsp. *persica*	4,64 ± 0,698	29,60 ± 1,839	14,09 ± 0,920	2,10	23,86 ± 0,371	14,98 ± 2,883	310,04 ± 10,662
Ae. comosa	6,51 ± 0,327	34,52 ± 1,389	20,45 ± 1,094	1,68	23, 66 ± 0,749	15,51 ± 2,392	314,74 ± 10,369
Ae. vavilovii	3,75 ± 0,392	36,18 ± 2,075	18,03 ± 0,837	2,01	24,46 ± 0,823	15,77 ± 1,922	315,73 ± 11,258
Agr. incanum	5,75 ± 0,912	31,60 ± 2,098	13,15 ± 1,198	2,40	30,24 ± 0,536	17,24 ± 2,096	141,37 ± 12,714
Agr. cristatum subsp. *pectinatum* var. *puberulum*	3,50 ± 0,510	50,20 ± 2,312	19,60 ± 1,699	2,60	32,21 ± 0,328	17,45 ± 1,807	151,36 ± 10,535
Agr. cristatum subsp. *pectinatum* var. *pectinatum*	4,16 ± 0,905	26,30 ± 2,102	12,30 ± 1,599	2,10	31,67 ± 0,593	17,36 ± 1,962	139,66 ± 12,384
Triticum monococcum	7,00 ± 0,027	29,00 ± 1,839	15,42 ± 1,564	1,88	20,62 ± 0,497	16,06 ± 2,077	_
Triticum dicoccon	4,86 ± 0,293	33,80 ± 2,192	19,67 ± 0,494	1,72	20,59 ± 1,937	16,09 ± 1,908	_
Triticum boeoticum	8,00 ± 0,203	31,17 ± 1,483	17,39 ± 1,293	1,79	19,97 ± 1,899	16,04 ± 2,039	_

Table 8 Continue. Measurements from the leaf surfaces (μm).

Taxa	average density of stomata	average length of stomata	average breadth of stomata	Length/ Breath ratio of stomata	Prickle length	hook length	macrohair length
Triticum araraticum	6,06 ± 0,091	34,99 ± 0,946	18,37 ± 1,578	1,90	20,66 ± 0,933	16,06 ± 2,104	75,23 ± 10,744
Eremopyrum confusum var *sublanuginosum*	5,52 ± 0,128	41,45 ± 2,102	12,58 ± 0,859	3,29	36,74 ± 2,091	27,00 ± 1,293	129,36 ± 11,954
Eremophyrum distans	5,13 ± 0,239	29,63 ± 2,493	13,53 ± 1,283	2,19	37,69 ± 1,584	28,53 ± 2,102	126,45 ± 13,097
Eremopyrum triticeum	5,50 ± 0,983	24,32 ± 2,763	12,27 ± 0,957	1,98	37,74 ± 1,736	28,62 ± 2,081	125,95 ± 12,325
Eremopyrum orientale	5,57 ± 0,738	27,97 ± 2,182	13,93 ± 1,283	2,01	37,54 ± 1,688	27,76 ± 1,094	124,63 ± 12,282
Eremopyrum boneopartis var *sinaicum*	5,00 ± 0,028	27,18 ± 2,849	13,67 ± 0,382	1,99	37,71 ± 2,467	28,54 ± 2,662	_
Eremopyrum confusum var *confusum*	5,50 ± 0,930	42,19 ± 1,778	13,00 ± 1,891	3,25	36,73 ± 1,854	_	129,52 ± 11,660
Eremopyrum confusum var *glabrum*	5,51 ± 0,748	42,16 ± 1,920	13,00 ± 2,017	3,24	36,75 ± 0,907	_	_
Secale sylvestre	5,73 ± 0,827	29,71 ± 2,338	15,35 ± 0,677	1,94	_	_	378,45 ± 10,087
Secale cereale	5,71 ± 0,261	29,49 ± 2,839	15,89 ± 1,739	1,86	51,66 ± 1,737	12,34 ± 1,293	_
Leymus racemosus subsp *sabulosus*	4,01 ± 0,791	39,01 ± 0,599	15,00 ± 0,911	2,60	38,83 ± 1,150	18,36 ± 2,101	_
Leymus cappadocicus	4,19 ± 0,801	29,79 ± 0,201	18,49 ± 1,029	1,61	39,75 ± 1,207	18,35 ± 2,234	92,97 ± 10,353
H. brevisubulatum subsp *violaceum*	10,09 ± 0,528	31,51 ± 1,003	13,32 ± 0,714	2,31	35,15 ± 2,738	24,32 ± 2,102	351,48 ± 13,293

Table 8 Continue. Measurements from the leaf surfaces (µm).

	average density of stomata	**average length of stomata**	**average breadth of stomata**	**Length/ Breath ratio of stomata**	**Prickle length**	**hook length**	**macrohair length**
H. geniculatum	12,13 ± 0,498	38,10 ± 1,512	13,01 ± 0,791	2,90	38,66 ± 2,364	24,37 ± 2,584	341,47 ± 12,373
H. bulbosum	7,61 ± 0,093	29,61 ± 1,299	17,74 ± 0,892	1,70	40,24 ± 2,134	26,27 ± 2,736	195,62 ± 14,287
H. distichon	7,62 ± 0,089	31,90 ± 1,900	15,21 ± 0,696	2,09	39,98 ± 2,529	25,26 ± 2,528	194,78 ± 14,382
H. spontaneum var *anatolicum*	7,15 ± 0,584	33,70 ± 1,712	13,80 ± 1,202	2,40	39,31 ± 2,198	26,35 ± 2,019	193,58 ± 13,928
H. vulgare	6,89 ± 0,602	41,70 ± 2,091	16,40 ± 1,097	2,50	39,31 ± 2,637	26,32 ± 2,839	193,67 ± 14,394
H. murinum subsp *glaucum*	6,10 ± 0,523	35,00 ± 1,702	15,90 ± 1,203	2,20	40,51 ± 2,091	26,22 ± 2,182	195,74 ± 12,993
H. marinum var *pubescens*	11,04 ± 0,543	40,01 ± 2,012	13,30 ± 1,012	3,00	38,66 ± 2,593	25,03 ± 2,278	343,78 ± 12,394
Henrardia persica var *persica*	6,99 ± 0,207	_	_	_	33,87 ± 2,074	16,96 ± 1,733	110,26 ± 12,886
Heteranthelium piliferum	8,09 ± 0,728	26,82 ± 1,903	12,85 ± 1,034	2,09	_	_	154,54 ± 11,902
Taeniatherum caput-medusae	5,60 ± 0,192	_	_	_	18,98 ± 1,340	6,37 ± 1,972	453,24 ± 10,734
Ambylopyrum muticum	6,04 ± 0,918	31,53 ± 1,374	17,86 ± 0,907	1,77	18,36 ± 1,654	8,14 ± 2,388	_
Crithopsis delileana	9,75 ± 0,197	23,24 ± 0,723	11,98 ± 1,389	1,94	41,38 ± 1,713	13,77 ± 3,425	586,54 ± 12,392
Psathyrostachys fragilis	4,20 ± 0,793	39,00 ± 2,449	18,49 ± 1,268	2,1	55,22 ± 0,935	29,53 ± 1,827	_
Dasypyrum villosum	6,10 ± 0,539	_	_	_	35,53 ± 2,690	14,28 ± 1,982	1124,00 ± 15,660
Hordelymus europaeus	7,01 ± 0,256	41,14 ± 2,384	17,5 ± 1,388	2,3	33,39 ± 1,727	15,06 ± 2,718	666,69 ± 13,293

3.4 Numerical Taxonomic Analysis

Numerical taxonomy has been applied earlier in the classification of plant taxa in Turkey (Togan *et al.*, 1983; Dogan *et al.*, 1992). Numerical taxonomic methods such as Cluster Analysis (CA) and Principal Coordinate (PCO) analysis have been carried out for determination of the anatomical similarity among the taxa of the tribe *Triticeae*. The characters used for this purpose and the character states are given in Table 3. For numerical taxonomical methods, all the taxa (65 cases) and totally 44 variables were used. Most of the genera are represented by one species and as seen in Figure 67, the results of the cluster analysis help to decide the overall similarity of the genera within the tribe. According to the results of the cluster analysis, the most closely related taxa are *Ae. triuncialis* subsp *triuncialis* and subsp *bozdagensis* with the similarity of 0,956. The similarity of the other subspecies, subsp *persica,* closes to this node with the similarity of 0,921. The most closely related species to *Ae. triuncialis* are *Ae. biuncialis* and *Ae. tauschii* with the similarity of 0,879.

Another outcome of the analysis includes the varieties of *Eremopyrum confusum*. The similarity of *E. confusum* var *confusum* and var *glabrum* is 0,946. The third var of the species, var *sublanuginosum*, resembles these two with the similarity of 0,874. Especially the leaf blade shapes, the indumentum properties of the leaf surfaces and the hair density of roots cause the first two var to become close rather than the third one. In the genus *Eremopyrum*, *E. boneopartis* and *E. orientale* are related with the similarity of 0,913. This node is close to *E. triticeum*. The most recent anatomical study of *Eremopyrum* established the differences between the taxa of the genus and added that, *Eremopyrum confusum* and *Eremopyrum boneopartis* are not close species anatomically (Keshavarzi *et al.*, 2007). The cluster analysis of the tribe also supports this study that, the similarity of *E. confusum* and *E. boneopartis* is 0,870, which means that, these are the most distinc two species within the genus.

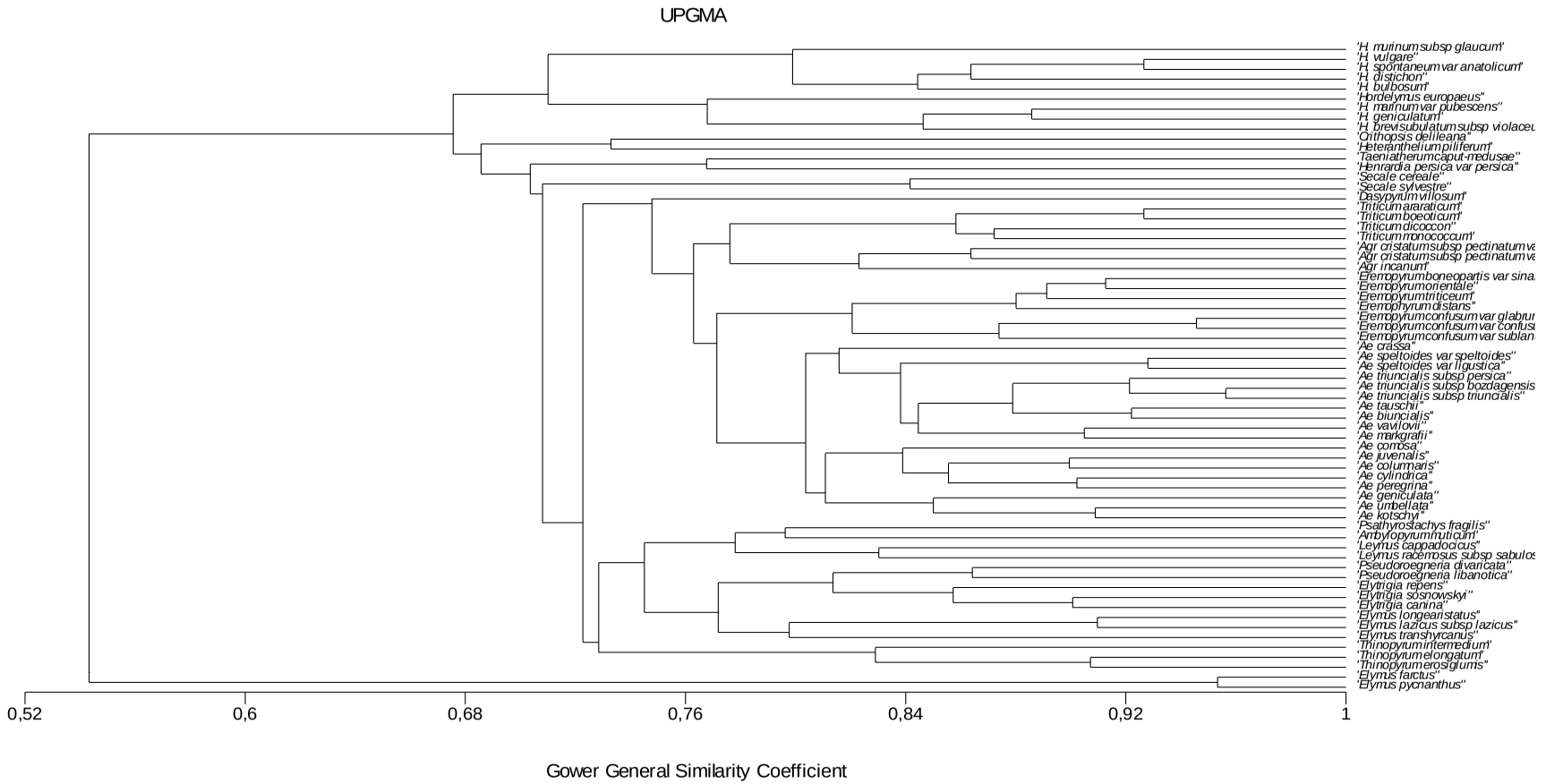

Figure 67. Phenogram constructed by means of UPGMA algorithm and Gower General Similarity Coefiicient

The varieties of *Ae. speltoides* are included another point of the cluster analysis with the similarity of 0,928. Generally, the taxa of the *Aegilops* are closely related to each other. For example, the similarity of *Ae. triuncialis* and *Ae. biuncialis* is 0,905. Samely, the similarity of *Ae. triuncialis* and *Ae. umbellata* is 0,850. Moreover, *Ae. umbellate* is most closely related with *Ae. kotchyii* with the similarity of 0,910. The most closely related taxon to this node is *Ae. geniculata* with the similarity of 0,850. All these taxa are included in section *Aegilops* (Cabi, 2010). On the other hand, *Ae. comosa* the only taxon of the section *Comopyrum*, seems a little distinct from the clade including *Ae. peregrina* and *Ae. columnaris* with the similarity of 0,839.

The similarity value of *Triticum boeticum* and *Triticum araraticum* is 0,926. The close node to this group includes *Triticum monococcum* and *Triticum dicoccon*, wild relatives of wheat, with the similarity of 0,858. The similarity value 0,864 is seen between the two var of *Agropyron cristatum* subsp *pectinatum* and *Agropyron incanum* is closely related to these two varieties with the similarity of 0,823.

The similarity of the taxa of the genus *Hordeum* according to leaf cross sections and surface micromorphology was discussed by Mavi *et al.* (2011a). In this comparative study, also the sectional delimitations of the genus were exposed and the results supported the most recent phylogenetic studies about *Hordeum*. When the root and the culm characters are added to these leaf properties, the infrageneric delimitation of the genus are not so much changed. With the addition of the vegetative characters of all the genera within the tribe, it is seen that *Hordelymus europeaus*, wood barley, is being the most closely related to the taxa of the genus *Hordeum*. The close relationship of these two genera is also proved in many of the recent genetic studies (Zhang and Sun, 2010).

From the phenogram, it can be observed that, the genera *Hordeum* and *Hordelymus* are included in a big clade. The anatomical resemble characters of these two genera can be summarized as: the leaves of the genera have all I-shaped sclerenchymatic girders through the adaxial epidermis, they have smooth outer frame of culms, the vascular bundles of the culms are not connected to eachother, but all the small vascular bundles are connected to epidermis and each other.

With the help of vegetative anatomical characters, the generic delimitation in the genus *Elymus* has also been evaluated. It is decided that the *Thinopyrum, Pseudoroegneria, Elyrtigia* should be separated from the genus. Moreover, *Elymus pycnanthus* and *Elymus farctus* should be separated as a subtribe. The similarity of *Pseudoroegneria libanotica* and *P. divaticata* is 0,864. The closest genus to *Pseudoroegneria* seems as *Elytrigia*. In Elytrigia, the most closely species are *E. canina* and *E. sosnowskyi*. The similarity of *E. repens* to this node is 0,853.

Terrel and Peterson (1993) studied the 35 species included in 18 genera recognized in tribe *Triticeae* and proposed that this tribe can be divided into three major subtribes as *Elymoid*, *Triticinoid* and *Henrardinoid*. According to this study, *Elymus*, *Elytrigia, Thinopyrum, Leymus* and *Psathyrostachys* are included in the first group. In the summary of this study, the authors pointed out the relations of the genera *Elytrigia, Thinopyrum, Elymus, Leymus* and *Psathyrotachys*. Moreover, they indicated that, the caryopsis of *Leymus* and *Psathyrostachys* are similar. Finally they suggested that *Elytrigia, Leymus,* and *Psathyrostachys* might be included in a broad genus *Elymus* (Terrel and Peterson, 1993). By using vegetative anatomical characters, the cluster analysis results of the tribe *Triticeae* also support this wide range study that, the genera *Psathyrostachys* and *Leymus*, both of which are closely related to *Elymus* sensu lato, are related genera with the similarity of 0,778. However, *Psathyrostachys* is more close to the genus *Ambylopyrum* than *Leymus*. Terrel and Peterson (1993) added the genus *Ambylopyrum* into the second subtribe, which includes also the genus *Aegilops*. However, in the final decition they agreed the distinction of *Ambylopyrum* and *Aegilops* (Terrel and Peterson, 1993). This discrimination is also supported by other studies (Bor, 1968; Tzvelev, 1976).

It is clearly seen from Figure 67 that, *Elymus, Thinopyrum, Elytrigia, Pseudoroegneria, Leymus, Psathyrostachys* and *Ambylopyrum* are included in one big clade. The vegetative anatomical characters that are nearly similar in the genera are the regularly arranged epidermal cells in cross sections of their leaf blades, epidermal cell wall undulations, the absence of macrohairs on their abaxial leaf surfaces, both costally and intercostally located silica bodies on their abaxial surfaces of leaves, hollow culms, vascular bundle line numbers of their culms and

connection of all these vascular bundles to each other.

In a cladistic analysis of *Triticeae* based on the morphology, Kellogg (1989) emphasized that *Agropyron* and *Triticum* are closely related genera. Moreover, the genus *Dasypyrum* was also placed near the *Agropyron* and *Triticum* clade (Kellogg, 1989). However, more recent phlogenetic analysis of *Triticeae* based on morphology added also the genus *Secale* to these clades (Seberg and Frederiksen, 2001). Furthermore, Clayton and Renvoize (1986) suggested that the genus *Eremopyrum* is closely related to the genus *Agropyron* (*A. incanum* group) and grouped the genera *Aegilops, Eremopyrum, Triticum, Agropyron, Dasypyrum, Secale* and *Henrardia* in the same branch. The cluster analysis based on the vegetative anatomy of the tribe show that, the similarity of the genera *Agropyron* and *Triticum* is 0, 776. This clade is close to the *Aegilops* and *Eremopyrum* clade with the similarity of 0,763. *Dasypyrum* follows these clades with the similarity of 0,749. From the similarity matrix of the analysis, it is seen that the maximum similarity value of *Dasypyrum* is with *Ae. speltoides* as 0,818. As seen in the phenogram, *Aegilops, Eremopyrum, Triticum, Agropyron* and *Dasypyrum* are closely related genera. The vegetative anatomical characters that are nearly similar in all these genera are the I-shaped sclerenchymatic girders through the adaxial epidermis, vascular bundle line numbers of their culms, the connection of large vascular bundles to the epidermis with a little exception of *Triticum monococcum*.

Altough they are different species, *Elymus farctus* and *Elymus pycnanthus* are the second closely related taxa. The reason of this similarity may be because of their being the most different taxa from the others within the tribe. The similarity of these species is 0,953. Moreover, the two species are distinctly different from the other species of the taxa with the least similarity of 0,554. The main distinctive characters are the appearance of continuous sclerenchyma on their abaxial leaf surfaces, the 3 layers of sclerenchyma on the inner sides of the cortex of their roots and the solid piths, comprising vascular bundles and parenchyma cells of their internodes. There is not any species, having such different character states from the other species. Because of these differences, the two species are suggested to be a subtribe of

Triticeae and this suggestion is supported by the results of the PCO analysis (Figure 68).

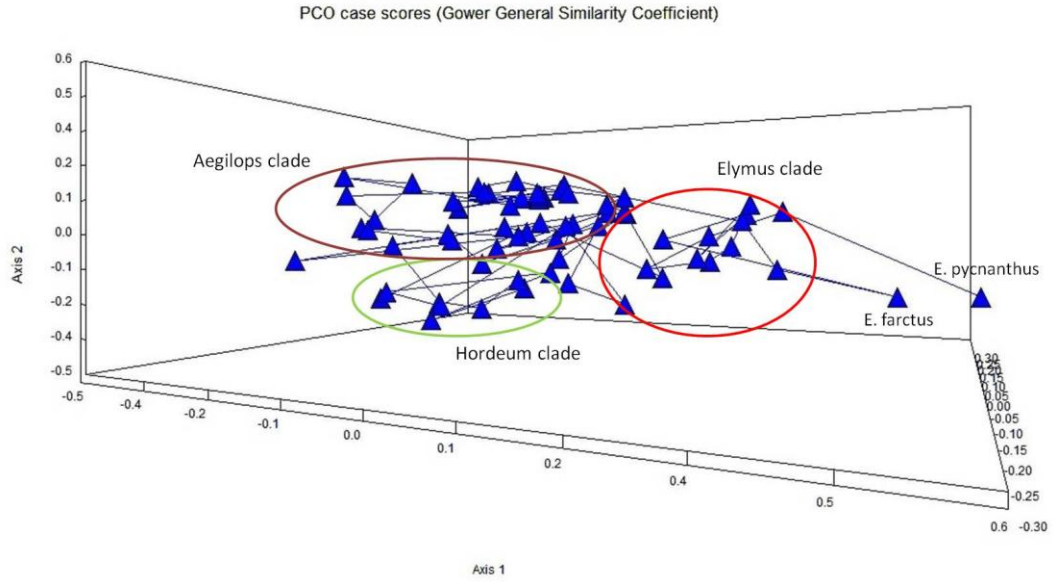

Figure 68. Three dimensional display of the first three principal axes from PCO analysis of all the taxa used in the cluster analysis.

CHAPTER 4

CONCLUSION

This comprehensive study on the tribe *Triticeae* showed the taxonomic significance of vegetative anatomy so as to clearify the systematics of the tribe and also supplied new information about the general vegetative anatomy of the Tribe. Moreover, with the help of these anatomical features, the diagnostic characters have been identified and a more natural classification of the tribe is achieved.

The root anatomy of the tribe resembles the general simple root anatomy of monocothyledons with a little variation. Under the epidermal layer, the cortex is irregularly arranged and formed by thin walled parenchymatic cells. The cortex may have wide or narrow intercellular cavities. There may be 1-3 sclerenchymatic rings on outer, inner or both sides of the cortex. U-shaped endodermis covers pericyle and vascular cylinder. Large metaxylem vessels are scattered and there isn't any large central metaxylem in the middle. The protoxylem is much smaller then the metaxylem elements and covered by pericyles.

The hair density of roots, the presence, position and the number of the sclerenchymatic layers seem to be important to distinguish the taxa. By considering these root characters, the most prominent species are *Elymus pycnanthus* and *Elymus farctus*, which have 3 sclerenchymatic rings on the inner parts of their cortex. The roots of the genera *Triticum* and *Secale* are in the same structure that, the taxa of them have dense hairs and 2 layers of sclerenchyma in the inner parts of the cortex. The same structure is seen in some of the taxa of the genera, as such *Eremopyrum* and *Aegilops*. All the taxa of *Agropyron* have dense hairs and 2 layers of sclerenchyma at inner part with only 1 layer of sclerenchyma at outer parts of the cortex. *Ae. vavilovii* is the only species that has the same structure. Moreover, all the

three taxa of *Elymus* and the genus *Crithopsis* have dense root hairs and 1 ring of sclerenchyma at the inner part of their cortex. However, the root surface of the genus *Leymus* has rarely arranged hairs and only 1 ring of sclerenchyma appears in the inner part of the cortex. Some of the taxa of *Aegilops* have the same structure. The absence of the root sclerenchyma is seen in the genera, *Ambylopyrum* and *Dasypyrum* with some taxa of *Hordeum* and *Aegilops*.

The nodes of the culms are found to have the same structure and parts of them also support the previous studies. There are 3 regions as lower, middle and upper region. The lower region comprises the beginning of vascular bundle appearance. In the middle region, the vascular bundles and the leaf sheath can be seen easily but the separation of the culm and sheath appears in the upper region.

In general the structure of culm internodes of the tribe, there are 2 or 3 layers of vascular bundles under the epidermis. The sizes of the vascular bundles are different in different layers that, they are getting larger through the central part, the pith. These vascular bundles may be connected to each other and/or epidermis with sclerenchymatic cells. The number of diagnostic characters in culms is more than in roots. The hollowness and the outer frame of culms, the number of vascular bundle layers and connection properties of them, the diameters and the measurement of the cellular region up to the hollowed pith seem to have diagnostic value in the tribe.

Although in the internodular parts of the culms the central regions, piths, are generally hollowed, there are some species having solid culms. *Elymus pycnanthus* and *Elymus farctus* are the only two species having solid culms covered with scattered vascular bundles, which are surrounded by parenchymatic cells at the centre. Other solid culms are seen in *H. murinum, Ae. comosa* and the genus *Crithopsis*. However, in these taxa the central parts of the culms are covered with only parenchymatic cells. The rest of the taxa have hollowed culms.

The outer frame of the culms may be waved or smooth. Generally all of the taxa of the tribe have smooth frame. However, *Ae. comosa, Leymus racemosus* and *Ae. speltoides* have waved frames of culms.

Hordelymus europeaus and most of the taxa of *Hordeum* and the genus *Secale* have 3 layers of vascular bundles in which the smallest ones are all connected to epidermis. All the rest of the taxa have 2 layers of vascular bundles. Except the genera, *Hordelymus, Hordeum, Secale* and the taxa of *Leymus racemosus, Triticum monococcum,* all the vascular bundles of the culms are connected to each other and/or to epidermis.

Hordeum and *Hordelymus* have large bundles that are connected neither to the small ones nor to epidermis and the assimilatory tissue is mostly narrow. In *Aegilops*, *Eremopyrum* and *Agropyron*, all the taxa have large vascular bundles that are connected to the epidermis in their culms. In the culms of *Eremopyrum*, all vascular bundles are also connected to each other.

To generalize the culm diameters of the genera, the average values were also measured for each genus. According to these results, the longest diameters within the culms of the tribe were measured in *Elymus pycnanthus* and *Elymus farctus* as a little more than 2 mm. *Hordeum* and *Hordelymus* follow these measurements as 1, 5 and 1, 4 mm. The diameters of *Dasypyrum* and *Elymus* are nearly the same as 1, 2 mm. Also *Triticum* and *Leymus* are nearly the same as about 1-1, 1 mm. The rest of the taxa have culms, diameters of which are lower than 1000 μm. When we compare the cellular parts of the culms up to the pithes, the smallest measurement were found in *Taeniatherum, Heteranthelium, Henrardia, Eremopyrum and Aegilops*. However, it is obviously seen from the ratio of the diameters of culms and the cellular parts of them that, *Hordeum* has large culms, but also has large hollowed pithes. Some taxa of it have 3 layers of vascular bundles in a very small cellular region. Moreover, the assimilatory tissue mostly seems narrow.

The leaves of the tribe *Triticeae*, have the most numerous diagnostic characters. In a general appearance of leaf cross sections, simply a leaf of the tribe has homogenous mesoderm between the adaxial and abaxial sides. Epidermises of both sides are arranged in a single layer that includes bulliform cells only adaxially. The mesoderm comprises a single layer of vascular bundles and sclerenchymatic cells connecting the bundles to the epidermises. All vascular bundles have two bundle

sheaths, the inner of which completely sorruounds the bundle and made by sclerenchymatic cells. However, the outer sheaths are composed of parenchymatic cells and surround half of the bundle.

The variations of the cross sections are seen in the blade shapes, the adaxial and abaxial furrows, sclerenchymatic cell arrangements and types, the midvein properties, the chloroplast contents of the outer bundle sheaths, the shape and arrangement differences of epidermal cells and bulliform cells.

Except *Ae. umbellata* and *Ae. speltoides var speltoides*, there is no furrow seen abaxially in the leaves of all the *Aegilops* species. *Hordelymus* and *Ae. crassa* are the two taxa which don't have adaxially or abaxially furrows. However, *Heterathelium piliferum*, *H. spontaneum* var *anatolicum*, *Triticum dicoccon* and *H. marinum* var *pubescens* have deep furrows on both adaxial and abaxial sides.

Generally, the tribe has I-shaped sclerenchyma connecting the vascular bundles and adaxial sides of the leaves. However, *Psathyrostachys*, *Pseudoroegneria*, *Leymus racemosus*, *Elymus pycnanthus, Elymus farctus* and the most of the taxa of *Thinopyrum* have T-shaped sclerenchymatic girders through the adaxial sides. However, there are also taxa which do not have sclerenchymatic cells around some of their vascular bundles of leaves. Especially, all the taxa of *Eremopyrum* and most of the taxa of *Agropyron*, *Aegilops* and *Triticum* comprise vascular bundles that are not connected to epidermis with sclerenchyma. Moreover, in *Heteranthelium*, *Henrardia*, *Hordelymus* and most of the taxa in *Elymus*, sclerenchyma around the vascular bundles are both connect to adaxial and abaxial sides.

Generally, sclerenchyma occurs around the vascular bundles of the leaves. However, *Elymus pycnanthus* and *Elymus farctus* have also continuous sclerenchyma on the abaxial side of their leaves. Also in cross sections of leaves, the epidermal cells may be regular or irregularly arranged. Most of the taxa of the tribe have regularly arranged epidermal cells. However, *Crithopsis, Ae. markgrafii, Ae. juvenalis* with *Hordeum murinum* and *H. marinum* var *pubescens* have irregularly arranged epidermal cells in their leaf cross sections.

There are five different bulliform cell shapes on the adaxial sides of the leaves. Moreover, in some taxa, these cells are not certain. For example the genera *Psathyrostachys*, *Secale* and *Triticum* with the taxa *Thinopyrum intermedium* and *Agropyron cristatum* subsp *pectinatum* var *puberulum* have regularly arranged bulliform cells in same sizes. Only *Elymus longearistatus*, *Leymus racemosus, Eremopyrum confusum* var *confusum* and var *glabrum* have fan shaped bulliform cells.

Surface micromorphology of leaves comprises epidermal long cells, macrohairs, prickles, hooks, stomata and silica bodies. These may all seen on both adaxial and abaxial sides of the leaves. However, stomata are only located on intercostal zones. Also, because of the continuous sclerenchyma on the abaxial surface of the leaves of *Elymus pycnanthus* and *Elymus farctus*, stomata could not be seen on the abaxial surfaces.

If a leaf has abaxial macrohairs, this leaf always has macrohairs on its adaxial surface. *Heteranthelium piliferum* is the only species that lacks hooks or prickles, but has macrohairs on both adaxial and abaxial sides of the leaves. Moreover, *Hordeum geniculatum* is the only species having macrohairs both in costal and intercostals zones on both surfaces.

With the help of all these anatomical characters, it is seen from the cluster analysis that, there are 3 general big clades within the tribe. *Elymus, Thinopyrum, Elytrigia, Pseudoroegneria, Leymus, Psathyrostachys* and *Ambylopyrum* are closely related. Moreover, *Aegilops, Eremopyrum, Triticum, Agropyron*, *Dasypyrum* and *Secale* are closely related in another branch. The third group comprises *Hordeum* and *Hordelymus*. The rest of the taxa seem to be close both to the *Hordeum* group and *Aegilops* group.

The taxa *Elymus pycnanthus* and *Elymus farctus* are quite different from the other taxa of the tribe according to all leaf, root and culm anatomy they posses. Because of these large differences, the two taxa should be assigned as a subtribe.

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APPENDIX A

PHOTOGRAPHS OF THE GENERA

Aegilops juvenalis (Cabi, 2010)

Hordeum bulbosum (Cabi, 2010)

Agropyron cristatum (Cabi, 2010)

Elymus transhyrcanus (Cabi, 2010)

Crithopsis delileana (Cabi, 2010)

Dasypyrum villosum (Cabi, 2010)

Psathyrostachys fragilis (Cabi, 2010)

Thynopyrum elongatum (Cabi, 2010)

Henrardia persica (Cabi, 2010)

Heteranthelium piliferum (Cabi, 2010)

Secale sylvestre (Cabi, 2010)

Triticum araraticum (Cabi, 2010)

Pseudoroegneria divaricata (Cabi, 2010)

Eremopyrum orientale (Cabi, 2010)

Leymus cappadocicus (Cabi, 2010)

Hordelymus europeaus

Elytrigia sosnowskyi (Cabi, 2010)

Elymus pycnanthus (Cabi, 2010)

Taeniatherum caput medusae

Elymus farctus (Cabi, 2010)

APPENDIX B

CHEMICALS

Chemicals	Chemical Suppliers
Ethyl alcohol	Sigma
Paraffin	Leica
Xylene	Sigma
Safranine	Merck
Fast green	Merck
Alcian blue	Merck
Toluidine blue	Sigma
Glycerin	Sigma
Sodium salicylate	Sigma
Entellan	Merck
Technovit 7100 liquid	TAAB
JB-4	Polyscience
Catalyst C	Polyscience
Dpx	Sigma

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EDUCATION

Degree	Institution	Year of Graduation
MS	ANKARA, Department of Biology	2003
MS	ANKARA, Faculty of Education	2003
BS	ANKARA, Department of Biology	2001
High School	ANTALYA, Karatay High School	1997

WORK EXPERIENCE

Year	Place	Enrollment
2003-present	METU Department of Biological Sciences	Research Assistant
March- June 2011	USA, California, University of CA, Riverside	Visiting Scientist
December 2010-March 2011	UK, London, Jodrell Laboratory, Royal Botanic Gardens, KEW	Visiting Scientist

FOREIGN LANGUAGES

English

INTERNATIONAL PUBLICATIONS

1- **Mavi Ö.**, Doğan M., Cabi E. (2011). Comparative leaf anatomy of *Hordeum* L. (Poaceae) in Turkey. Turk J Bot 35: 357-368. **(SCI)**

2- **Mavi Ö.**, Doğan M., Cabi E. (2011). Leaf anatomy of *Agropyron* Gaertn. (Gramineae) in Turkey. Turk J Bot 35: 527-534. **(SCI)**

3- **Mavi Ö.**, Doğan M., Baser B., Pehlivan S., Cabi E., Akaydin G. (2011). Anatomy and pollen morphology of *Leymus racemosus* (Lam.) Tzvelev subsp. *sabulosus* (Bieb.) Tzvelev and *Leymus cappadocicus* (Boiss. & Bal.) Melderis. Bangladesh J. Plant Taxon. 18(1): 27-38. **(SCI)**

4- Cabi E., Doğan M., **Mavi Ö.**, Karabacak E., Başer B. (2010). *Elymus sosnowskyi* (Hackel) Melderis (Poaceae), a rare endemic species in Turkey. Turk J Bot 34(2): 105-114. **(SCI)**

5- Adıgüzel N., Bani B., **Mavi, Ö.** (2011). Rediscovery of *Dichoropetalum aureum* (Umbelliferae) in South Anatolia (Turkey). Phyton (Horn, Austria) 50 (2): 221-230. **(SCI)**

6- Cabi E., Doğan M., **Mavi Ö.** (2010). Morphological and Anatomical properties of the genus *Crithopsis* (Poaceae) in Turkey. Biodicon 3/2: 42-48.

7- Bani B., Adıgüzel N., **Mavi Ö.** (2011). "Morphological and anatomical notes on a local endemic species: *Grammosciadium confertum* Hub.-Mor. & Lamond (Umbelliferae)" Biological Diversity and Conservation 4 (1): 1-6.

INTERNATIONAL POSTER PRESENTATIONS

1- **Mavi, Ö.**, Doğan M. 2008. Anatomical studies on the genus *Limonium* Miller (Plumbaginaceae) in Turkey. XVI. Congress of the Federation of European Societies of Plant Biology (16th FESPB2008). 17-22 August, Tampere/ Finland.

2- **Mavi, Ö**., Cabi, E., Doğan M. 2008. Anatomy of *Leymus* Hochst. (Gramineae: Triticeae). First International Congress Documenting, Analysing and Managing Biodiversity in the Middle East. 20-23 October, Aqaba/ Jordan.