TÜBİTAK

Research Article

Turk J Bot
35 (2011) 357-368

doi:10.3906/bot-1003-14

Comparative leaf anatomy of the genus Hordeum L. (Poaceae)

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Received: 10.03.2010
Accepted: 01.11.2010

Abstract: The genus *Hordeum* L., one of the most economically important cereal crops in the tribe *Triticeae*, has a worldwide distribution mainly in temperate and dry regions of the world. In Turkey, *Hordeum* is represented by 12 taxa, namely *Hordeum violaceum* Boiss. & Hohen., *H. geniculatum* All., *H. marinum* var. *marinum*, *H. marinum* Hudson var. *pubescent* (Guss.) Nevski, *H. murinum* L. subsp. *murinum*, *H. murinum* subsp. *glaucum* (Steudel) Tzvelev, *H. murinum* subsp. *leporinum* (Link) Arc. var. *leporinum*, *H. murinum* subsp. *leporinum* var. *simulans* Bowden, *H. bulbosum* L., *H. spontaneum* K.Koch, *H. distichon* L., and *H. vulgare* L. The main objective of this study is to assess the significance of anatomical characteristics of the leaves. For this purpose, dehydrated specimens were embedded into paraffin and transverse sections were gathered. Furthermore, the leaf surfaces were examined. Results of the study show that anatomical characteristics of the leaf blades vary between the taxa in both qualitative and quantitative values. Sclerenchymatic cells, silica bodies, and stomata, furrow and rib properties, the existence of midrib and bulliform cells, and indumentum properties such as arrangement, density, and length of macro hairs are all included in these diagnostic characteristics. Based on these anatomical characteristics, an identification key for the taxa is given for the first time.

Key words: *Hordeum*, leaf anatomy, Poaceae, *Triticeae*, Turkey

Hordeum L. (Poaceae) cinsinin karşılaştırmalı yaprak anatomisi

Özet: *Triticeae* oymağının ekonomik açıdan en önemli tahıl bitkilerinden olan *Hordeum* L. (Poaceae) cinsi, ılıman ve kuru bölgeler olmak üzere dünya çapında bir dağılım gösterir. *Hordeum* cinsi Türkiye' de, *Hordeum violaceum* Boiss. & Hohen., *H. geniculatum* All., *H. marinum* Hudson var. *marinum*, *H. marinum* var. *pubescent* (Guss.) Nevski, *H. murinum* L. subsp. *murinum*, *H. murinum* subsp. *glaucum* (Steudel) Tzvelev, *H. murinum* subsp. *leporinum* (Link) Arc. var. *leporinum*, *H. murinum* subsp. *leporinum* var. *simulans* Bowden, *H. bulbosum* L., *H. spontaneum* K.Koch, *H. distichon* L. ve *H. vulgare* L. olmak üzere 12 takson ile temsil edilir. Bu çalışmanın esas amacı, anatomik karakterlerin öneminin değerlendirilmesidir. Bu amaçla, dehidrasyonu yapılan örnekler parafine gömülerek, enine kesitleri alınmıştır. Ayrıca yaprakların yüzey görünümleri incelenmiştir. Çalışmanın sonucu, yapraktaki anatomik karakterlerin, taksonlar arasında hem nitel hem de nicel değerler olarak çeşitlendiğini göstermektedir. Sklerenkima hücreleri, silica hücreleri, stomalar; oluk ve damar özellikleri; midrib ve bulliform hücrelerinin varlığı; makro tüylerin dizilimi, yoğunluğu ya da uzunluğu gibi tüylülük özelliklerinin tamamı bu ayırt edici karakterler içindedir. Bu anatomik karakterlere dayalı olarak ilgili taksonlara ait teşhis anahtarı ilk kez verilmektedir.

Anahtar sözcükler: *Hordeum*, yaprak anatomisi, Poaceae, *Triticeae*, Türkiye

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Introduction

Including the cultivated barleys *Hordeum vulgare* L. and *H. distichon* L., the genus *Hordeum* L. consists of 31 species around the world (Baden & Bothmer, 1994; Bothmer et al., 1995). This genus is one of the genera of the tribe *Triticeae* Dumort. given in the *Flora of Turkey* and includes 8 species (Melderis, 1985). As well as morphological studies of the genus (Asfaw, 1989; Bao-rong, 1997; Sahebi et al., 2004; AL-Saghir et al., 2009), most of the research studies related to molecular (Nishikawa et al., 2002; Sun et al., 2009), genetic (Linde-Laursen et al., 1992; Monte et al., 1993; Blattner, 2004; Blattner, 2009), and anatomical structures (Islam et al., 2009) of the members of the genus, especially the cultivated species, *H. vulgare*.

Bothmer et al. (1986) divided *Hordeum* into four sections, as sect. *Hordeum*, sect. *Anisolepis*, sect. *Critesion*, and sect. *Stenostachys*. Furthermore, after the suggestion by Bothmer et al. (1995) of the genome types, Wang et al. (1996) changed the genome type names to I-genome (*H. vulgare* and *H. bulbosum* L.), X_a-genome (*H. marinum* Hudson), X_u-genome (*H. murinum* L.), and H-genome (all the remaining species). There have been some cytogenetic, molecular, karyological and ecogeographical research that discuss these phenomena (Popova, 1996; Blattner, 2006; Ueno et al., 2006; Kılıç et al., 2007; Morrell & Clegg, 2007). For example, Jana and Pietrzak (1988) clarified that the 2 species *H. spontaneum* K.Koch and *H. vulgare* are genetically related. Also in the study of Kochieva et al. (2001), the 2 species shared the same cluster based on the genetic distance between them. In another research study, Jaaska (1992) found that *H. bulbosum* was the most closely related species to *H. vulgare* and *H. spontaneum* by using isoenzyme data. However, *H. distichon* was not used in his study. Furthermore, according to Petersen and Seberg (2003), *H. bulbosum* and *H. vulgare* are related and the sister taxon of this clade is *H. murinum*.

In a study by Blattner (2004), the sister group relationship between *H. marinum* and the H-genome species was strongly supported by ITS analysis. However, the ITS sequence analysis of Sun et al. (2009) separated *H. marinum* from the H-genome species. Terzi et al. (2001) used RAPD and STS markers to display the distances of the genetic properties of the genus and suggested that all the subspecies of *H. murinum* were closely related, while *H. murinum* L. subsp. *glaucum* (Steudel) Tzvelev was the most divergent taxon within this group. Furthermore, El-Rabey et al. (2002) suggested that *H. murinum* subsp. *murinum* was more closely related to *H. murinum* subsp. *leporinum* (Link) Arc. than to *H. murinum* subsp. *glaucum*. All of these previous studies included many of the other species of the genus *Hordeum* from different phytogeographical regions (Bothmer et al., 1991; Jaaska, 1992; Bothmer et al., 1995; Komatsuda et al., 2001; El-Rabey, 2002; Sun et al., 2009).

Metcalfe (1960) studied the anatomy of the family Poaceae (Gramineae) and determined the diagnostic anatomical characteristics as epidermal cell type, stoma type, and the arrangement of the sclerenchymatic cells around the vascular bundles of the leaves. Also, some studies included the anatomy of some species of the family from Turkey. In these studies, taxonomic significance of some anatomical features, such as glumes, awns, and caryopsis cross-sections, were examined (Doğan, 1985, 1988, 1991a, 1991b, 1991c, 1997, 1999; Doğan & Tosunoğlu, 1992). Wenzel et al. (1997) studied the mutations of barley (*H. vulgare*) and found variations in leaf length associated with cell number, cell length, and cell type depending on leaf blade. In a more recent study, Islam et al. (2009) investigated the epidermal features of a rice cultivar leaf and described the leaf surfaces of the taxa of the family using commonly used anatomical diagnostic characteristics, such as stomatal aperture type and number, hair type and size, prickle density and size, long and short cell properties, and silica body density.

In spite of all these previous studies, there has hardly been any research on the comparative anatomical features of the genus *Hordeum*. Because it is the original and the most ancient centre of origin for the whole tribe *Triticeae* and one of the most important genetic centres of diversity for crop relatives in the world, Turkey is an important place for this tribe (Bothmer, 1996).

This is the sixth paper from the taxonomic revision series of *Triticeae* in Turkey. The first 5 papers dealt with the taxonomy, palynology, and anatomy of different genera found in *Triticeae* (Başer et al., 2009; Cabi & Doğan, 2009; Cabi et al., 2009; Özler et al., 2009; Cabi et al., 2010). The present study

reveals the diagnostic anatomical characteristics of leaves of the *Hordeum* taxa found in Turkey. The taxa are classified on the basis of both qualitative and quantitative characteristics using numeric taxonomic methods (Doğan et al., 1992). An identification key for the taxa and descriptions of the subgenera and the sections are also given.

Materials and methods

Both herbarium materials collected in Turkey in the past 3 years as well as freshly obtained samples were used in this study. The herbarium samples, collected as an initial part of a revisional study of the genus and kept at the Biological Sciences Department of Middle East Technical University, were first softened by boiling in distilled water. All the materials were fixed in formalin-acetic-alcohol solution for 48 h. Then the samples were dehydrated with increasing strengths of ethyl alcohol solutions. Xylene was used in order to embed the samples into paraffin (Johansen, 1944). Leaf blade transverse sections of 15 µm thickness were acquired by using a Leica RM2125RT Rotary Microtome. These sections were stained with Safranin and fixed with Entellan (Metcalfe, 1960; Doğan, 1985). Next, they were examined using a Euromex FE 2025 light microscope and photographed using a Euromex CMEX DC.1300 digital camera. Studied specimens with their collection location are given in Table 1.

In order to example their epidermal architecture, 10 slides for each taxa were prepared. Average number of silica bodies, length of macro hairs, prickles, hooks, and length and width of stomata were measured for 30 different 234 × 186 µm² samples of 4 leaves of each taxon. The micromorphology of the adaxial and abaxial surfaces of leaves were studied using a FEI QUANTA400F scanning electron microscope (SEM) in low vacuum mode (Doğan, 1988).

To determine the phenetic similarity among the species, cluster analyses were carried out. The data were obtained from the characteristics of both the transverse sections and the abaxial and adaxial surfaces of the leaf samples. The cluster analyses were carried out to examine infrageneric grouping. The characteristics and their possible states used in the method are given in Table 2. The data consist of both qualitative and quantitative values. With the help of principle component analysis, overlapping characteristics were eliminated.

The cluster obtained from the mixed data was found to express the natural grouping best. This mixed data was analysed based on Gower's coefficient of similarity (Friedman & Meulman, 2004). MVSP was used to create the phenogram. In order to group the studied species based on the anatomical similarities of their leaves, the UPGMA (unweighted paired group with arithmetic average) clustering method was used (Sneath & Sokal, 1973).

Table 1. Specimens used for anatomical studies.

Taxa	Collector's number	Collection Areas
H. violaceum	*E.Cabi* 2437	Sivas: Kangal to Gürün, crossroad of Konakpınar, roadside and slopes, 1793 m, 16.06.2007
H. geniculatum	*E.Cabi* 2781	Hatay: İskenderun to Arsuz, around Madenli district, 67 m, 02.05.2008
H. marinum	*E.Cabi* 3199b	Edirne: Keşan, Enez to İpsala, 2 km N of Enez, on the border road, roadside, 31 m, 03.06.2008
H. murinum subsp. *glaucum*	*E.Cabi* 3215	Bolu: Düzce, 27 km from Hendek, 241 m, 06.06.2008
H. bulbosum	*E.Cabi* 3703	Yozgat: Yozgat to Sivas, roadside, 1064 m, 19.07.2008
H. spontaneum	*E.Cabi* 1932	Şanlıurfa Ceylanpınar Tarım Çiftliği, Sarnıçtepe region, 450 m, 24.04.2007
H. distichon	*E.Cabi* 2584	Afyon: Dazkırı to Çardak, close to Çardak, roadside, 844 m, 26.04.2008
H. vulgare	*E.Cabi* 2655	Antalya: Kalkan to Elmalı, junction of Bezirgan village, 740 m, 28.04. 2008

Table 2. Characteristics used in cluster analysis.

1. Shape of blade
   Flat (0)
   Curved and semicrescent form (1)

2. Furrows of adaxial surface
   With shallow furrows (0)
   With deep furrows (1)

3. Furrows of abaxial surface
   Absent (0)
   With shallow furrows (1)
   With deep furrows (2)

4. Mid-vein
   Uncertain (0)
   Certain (1)

5. Outer bundle sheath
   Without chloroplasts (0)
   With some chloroplasts (1)

6. Sclerenchyma
   Without abaxial strands (0)
   With abaxial strands (1)

7. Margins
   sclerenchymatic cells are 1 or 2 layered (0)
   sclerenchymatic cells are 3 or 4 layered (1)

8. Epidermal cells in cross section
   Regularly arranged uniformly shaped cells (0)
   Irregularly arranged differently shaped cells (1)

9. Bulliform cells
   Uncertain (0)
   Certain (1)

10. Pitted epidermal cell wall
    Absent (0)
    Present (1)

11. Hooks on adaxial surface
    Only costally located (0)
    Both costally and intercostally located (1)

12. Macro hairs on adaxial surface
    Absent (0)
    Present (1)

13. Macro hairs on abaxial surface
    Absent (0)
    Present (1)

14. Maximum line numbers of intercostal stomata
    2 lines (0)
    4 lines (1)

15. Number of interstomatal cell lines
    Maximum 3 lines (0)
    Maximum 6 lines (1)
    Maximum 9 lines (2)

16. Silica bodies
    Only costally located (0)
    Both costally and intercostally located (1)

17. Number of costal prickle lines
    Only 1 line (0)
    1 or 2 lines (1)
    3 or 4 lines (2)

18. Average density of stomata

19. Average density of silica bodies

20. Average length of stomata

21. Average breadth of stomata

22. Length/breadth ratio of stomata

Results

Leaf blades

It is clearly observed from the leaf transverse sections of the taxa that all leaves have single-layer vascular bundles parallel to the adaxial and abaxial surfaces in a homogenous mesophyll (Figure 1). The general appearance of the transverse sections indicates 2 types of leaf blades: a) flat (Doğan & Tosunoğlu, 1992), and b) V-shaped (Metcalfe, 1960). In the second type, the leaves are curved from both sides of the mid-vein and present a semicrescent form, as in *H. geniculatum* All., *H. violaceum* Boiss. & Hohen., and *H. distichon*. In the other studied taxa,

leaf blades are completely flattened. A projecting midrib gives the appearance of a V-shape in their middle region, as in *H. vulgare*, *H. murinum* subsp. *glaucum*, *H. bulbosum,* and *H. marinum*, which have expanded leaf blades.

Midrib or median vascular bundle

Except for *H. spontaneum*, all taxa have a solitary median vascular bundle. In 5 of the studied leaf samples, a midrib is conspicuously present. The median vascular bundles, forming a rib on the abaxial surface of the leaf, have I-shaped adaxial and triangular-shaped abaxial sclerenchyma. In *H. bulbosum* and *H. murinum* subsp. *glaucum,* the solitary median vascular bundles do not make ribs. However, they are clearly distinguishable from other vascular bundles of their leaves. A midrib or median vascular bundle is not readily distinguishable in *H. spontaneum*. This is a distinct characteristic usable for the identification key (Table 3).

Furrows and ribs

H. violaceum, *H. geniculatum*, *H. murinum* subsp. *glaucum*, and *H. distichon* have deep furrows between the veins on their upper surfaces (Figure 1). In contrast, the furrows on the upper surfaces of *H. bulbosum* and *H. vulgare* are found to be shallow (Figure 1), while *H. marinum* and *H. spontaneum* have deep furrows between the veins on both surfaces of their leaves (Figure 1). Rib lengths between 45.86 μm and 91.03 μm were measured on the surface of the leaves that had deep furrows, while the maximum length of the ribs in the leaves with shallow furrows was 32.57 μm. The average length of the ribs in *H. marinum* was measured as 25.67 ± 3.17 μm. However, the average width of leaves from adaxial to abaxial surface in *H. marinum* was 72.14 ± 4.02 μm.

Vascular bundle sheaths

According to Brown (1975) all non-Kranz grasses have both a mestome and a parenchyma sheath around all their leaf bundles. Vascular bundles of the taxa are found to have double sheaths on their surfaces. The sclerenchymatic inner sheath is complete and surrounded with an incomplete parenchymatic outer sheath. The presence of chloroplasts in the outer sheaths of the vascular bundles is also found to vary between the taxa. In the leaves of *H. bulbosum*, *H. spontaneum* and *H. vulgare*, the outer bundle sheaths contain small numbers of chloroplasts. However, the other taxa do not contain chloroplasts in their outer bundle sheaths.

Sclerenchyma

Sclerenchyma occurs in the leaf cross-sections as girders or strands (Metcalfe, 1960). All the studied taxa have I-shaped adaxial and/or abaxial girders in their leaves. However, there seems to be no sclerenchyma either on the adaxial or the abaxial side of the small veins. The median vascular bundles have triangular sclerenchyma on their abaxial side and I-shaped sclerenchyma on their adaxial side. Except for *H. geniculatum*, the leaves of all taxa have strands on the abaxial and/or adaxial sides of the vascular bundles. *H. geniculatum* is the only species that does not have strands on its abaxial side.

Moreover, there are also sclerenchymatic cells on the margins of the leaves. The density of this marginal sclerenchyma varies with regard to row numbers of the cells. Based on these marginal sclerenchymatic cells, the leaves can be classified into two main groups: a) having 1 or 2 layers, or b) having 3 or 4 layers of sclerenchymatic cells on their margins.

Bulliform cells

In the taxa studied, the bulliform cells uniformly have thin walls and are larger than the adjacent epidermal cells. There are usually 3-7 fan-shaped bulliform cells on the adaxial surfaces of the leaves and these control leaf folding. From the middle to the margins of the leaf of *H. vulgare*, adaxially arranged bulliform cells gradually get smaller and become the same size as the abaxial epidermal cells. The bulliform cells are found to be variable only in the leaves of *H. marinum*.

Epidermis

The epidermal characteristics of leaves play an important role in distinguishing the taxa of Poaceae (Metcalfe, 1960). Epidermal cells in transverse sections of the taxa have regular or irregular shapes with either uniform or varying sizes. *H. violaceum*, *H. bulbosum*, *H. geniculatum*, *H. distichon,* and *H. vulgare* have regularly arranged uniformly sized epidermal cells (Figure 1). However, both the shape and the arrangement of the epidermal cells of *H. marinum*, *H. murinum* subsp. *glaucum,* and *H. spontaneum* are irregular. Abaxially, epidermis is differentiated into long cells, stomata, hairs, and

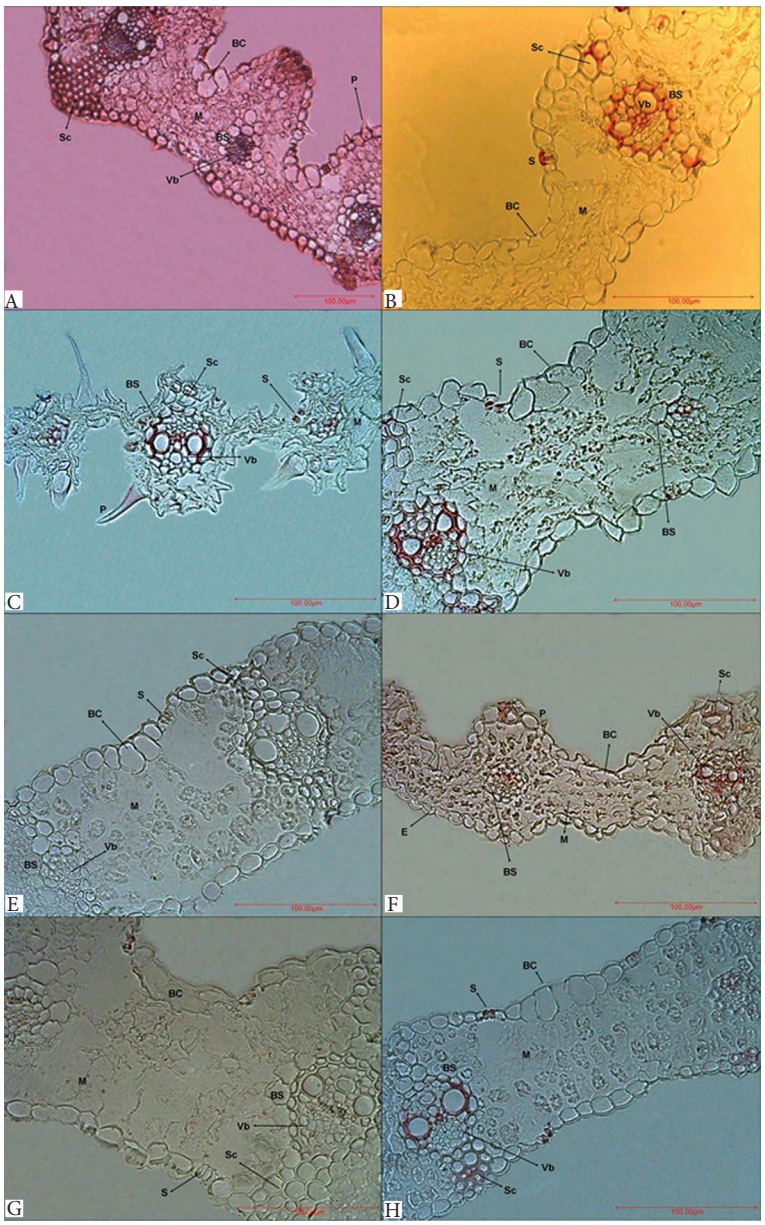

Figure 1. Transverse sections of leaves. A- *H. violaceum* (10×), B- *H. geniculatum* (40×), C- *H. marinum* (40×), D- *H. murinum* subsp. *glaucum* (40×), E- *H. bulbosum* (40×), F- *H. spontaneum* (40×), G- *H. distichon* (40×), H- *H. vulgare* (40×).
BC: Bulliform cell, BS: Bundle sheath, M: Mesophyll, S: Stoma, Sc: Sclerenchyma, Vb: Vascular bundle, E: Epidermis, P: Prickle

short cells such as silica bodies. On the adaxial surfaces of the leaves, in addition to these types of cells, epidermis also has bulliform cells. The type and the arrangement of bulliform cells are determined easily from transverse sections of leaves.

Long cells

These are elongated horizontally, parallel with the long axis of the leaf. The length of these cells shows variation within each taxon. However, the thickness as well as the structure of the walls is usually uniform

Table 3. Identification key based on leaf anatomical characteristics.

1. Mid-rib of leaves inconspicuous in cross section ..... ***H. spontaneum***
1. Mid-rib of leaves prominent in cross section
2. Bulliform cells inconspicuous; length/breadth ratio of stomata at least 3.0 ..... ***H. marinum***
2. Bulliform cells prominent; length/breadth ratio of stomata lower than 3.0
3. Leaf blades V-shaped
4. Pitted epidermal cell wall present..... ***H. violaceum***
4. Pitted epidermal cell wall absent
5. Vascular bundles of mid-vein without abaxial strand ..... ***H. geniculatum***
5. Vascular bundles of mid-vein with abaxial strand ..... ***H. distichon***
3. Leaf blades flat
6. Only adaxial surface of leaves sparsely covered with macro hair (up to 200 μm); epidermal cells irregularly arranged; interstomatal cell line number up to 6 ..... ***H. murinum* subsp. *glaucum***
6. Macro hair absent on both surfaces; epidermal cells regularly arranged; interstomatal cell line number up to 9
7. Furrows absent on abaxial surface; with hooks on adaxial surface, costally and intercostally located; intercostal stomata row number up to 2; costal prickle line number 1 or 2..... ***H. bulbosum***
7 . Furrows shallow on abaxial surface; with hooks on adaxial surface, only costally located; intercostal stomata row number up to 4; costal prickle line number only 1..... ***H. vulgare***

within all leaf samples. The lower surface of the leaves of *H. violaceum* has epidermal long cells with pitted walls, whereas all the other taxa have both adaxial and abaxial epidermal long cells with straight walls. In leaves of all taxa, the long cells in the intercostal zones are arranged between the stomatal rows. The taxa can be classified according to the maximum number of these long cell lines, having 3, 6, or 9 lines between the stomatal rows.

Silica Bodies

The cells smaller then the long cells are recognised as the short cells, which are nearly equidimensional. Silica bodies are a kind of short cell on the leaf surfaces (Metcalfe, 1960). All the taxa of *Hordeum* have costally arranged silica bodies on their leaf surfaces. These cells are usually located next to the prickles in the costal zones. However, *H. bulbosum*, *H. violaceum*, *H. geniculatum*, *H. marinum,* and *H. murinum* subsp. *glaucum* have silica bodies in the intercostal zones of their leaves as well. Costally located silica bodies are generally horizontally elongated, but they are equidimensional in intercostal zones (Figure 2).

Stomata

The intercostal zones of all the taxa include stomata, which occur in well-defined horizontal bands. Moreover, each band consists of 1-4 rows of stomata. The stomata are arranged regularly in these rows. In the genus, the number of rows of stomata in each intercostal zone varies not only from one species to another, but also in different areas of a single blade or in leaves taken from different levels of the same plant. However, each species has a maximum of 2 or 4 lines of stomata in the intercostal zones. Moreover, the rows can easily be seen in the SEM photos (Figure 2).

According to Metcalfe (1960), grass stomata can be classified in terms of the shape of their subsidiary cells and they can be used for diagnostic

and taxonomic purposes. From this point of view, the type of the stomata in the genus *Hordeum* can be referred to as having parallel-sided subsidiary cells. However, different taxa exhibit different stomata with great variation in their sizes (Raschke, 1979). Measurements of the length and breadth of the stomata for the taxa are given in Table 4. According to these results, the shortest and widest stomata are found in *H. bulbosum*, which means this species has the smallest stomata length/breadth ratio.

Although stomatal density is affected by environmental factors, its genetic background is certainly evident (Hetherington & Woodward, 2003). Comparing the stomatal densities of the taxa (Table 4), *H. vulgare* has the highest value.

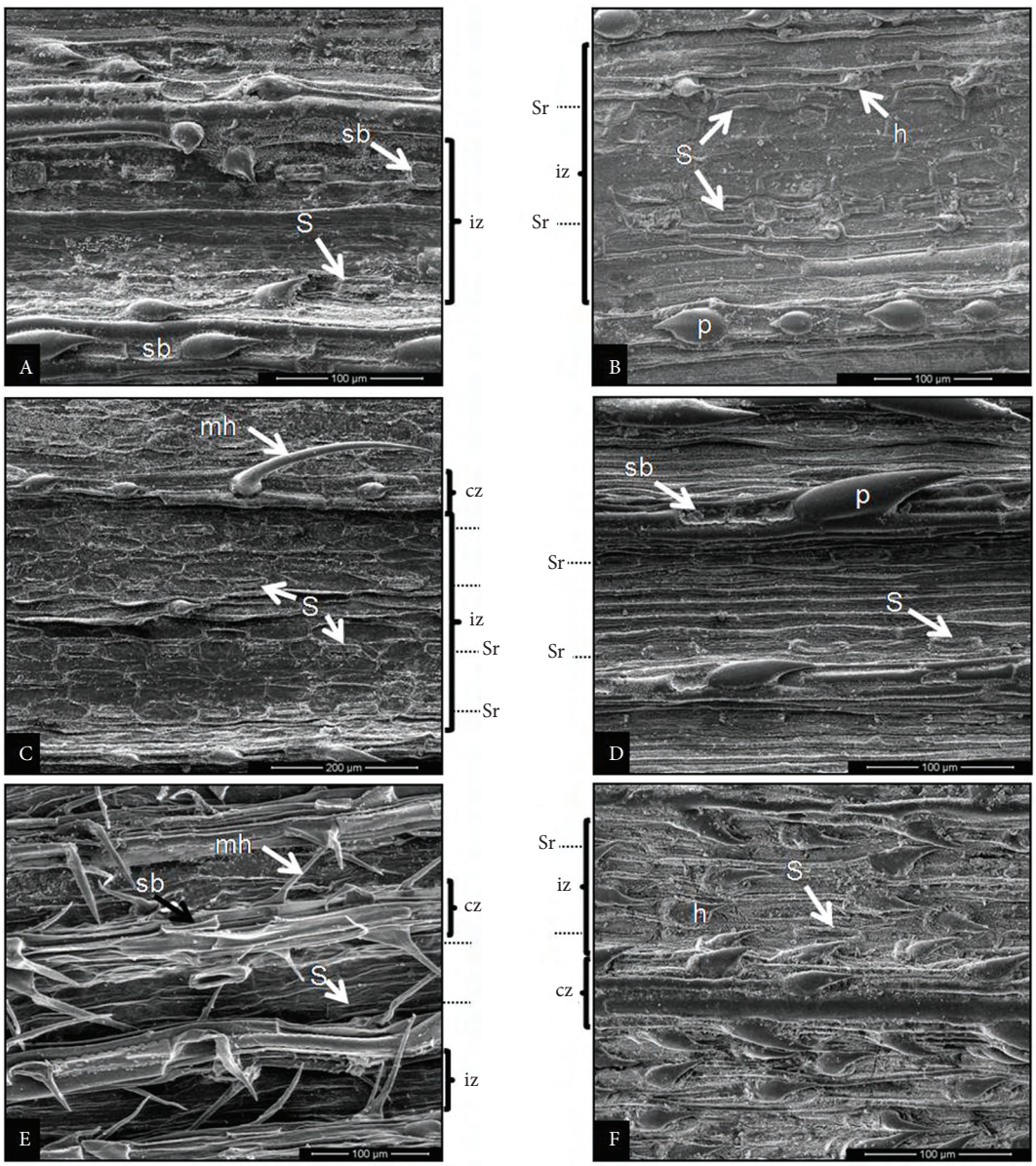

Figure 2. SEM observations of leaf surfaces. A- Abaxial surface of *H. bulbosum*, B- Adaxial surface of *H. distichon*, C- Adaxial surface of *H. murinum* subsp. *glaucum*, D- Abaxial surface of *H. spontaneum*, E- Adaxial surface of *H. marinum*, F- Abaxial surface of *H. violaceum*.
cz: costal zone, h: hook, iz: intercostal zone, mh: macro hair, p: prickle, sb: silica body, S: stoma, Sr: stomatal row.

Indumentum properties

All the studied taxa have prickles and hooks on their adaxial and abaxial surfaces. The average lengths of the prickles and hooks, 39.33 ± 2.6 μm and 25.27 ± 2.1 μm respectively, are nearly the same for all the taxa. Prickles and hooks are usually arranged in the costal zones of the leaves, whereas in most of the taxa there are intercostally located hooks as well.

Moreover, *H. violaceum*, *H. geniculatum*, *H. murinum* subsp. *Glaucum,* and *H. marinum* also have macro hairs on their leaf surfaces. However, the lengths of these macro hairs as well as the leaf surfaces where they appear vary greatly within the taxa. For example, they all have macro hairs on their adaxial leaf surfaces, while macro hairs are not found on the abaxial leaves of *H. violaceum* and *H. murinum* subsp. *glaucum*. Moreover, the longest macro hairs (350 μm) are found on the leaf surfaces of *H. violaceum* and *H. geniculatum*.

By using the UPGMA clustering method, 2 major clusters, separated from each other at the 0.54 similarity level, are observed in the phenogram (Figure 3). Each member of this subgroup is treated as a subgenus. According to the phenogram, *H. spontaneum* and *H. vulgare* are the most similar species and *H. distichon* is the next closest neighbour to this first cluster. There is a sister group relationship between these 3 species and *H. bulbosum* with a 0.65 similarity level. However, the similarity level of *H. bulbosum* and *H. murinum* subsp. *glaucum* is 0.57 within this first major cluster. These 5 taxa are all members of this cluster, which is treated as the subgenus *Hordeum* (Figure 3).

The second cluster, treated as the subgenus *Hordeastrum* (Doell) Rouy, has a group comprised of *H. marinum* and *H. geniculatum* with a 0.72 similarity level and the only neighbour of this clade, *H. violaceum*.

Discussion

Recent cytological (Blattner, 2009) and genetic (Petersen & Seberg, 2003) studies indicated that there are 2 subgenera and 4 sections with 4 different genotypes within *Hordeum*. Our results support these studies in that we identified 2 distinct clusters that may be referred to as the subgenus *Hordeum* and the subgenus *Hordeastrum* (Figure 3). The most prominent difference between these 2 subgenera is the indumentum property. In the subgenus *Hordeastrum*, the length of the macro hairs can be up to 350 μm and they are densely located on the adaxial and/or abaxial surface of the leaves. However, in the subgenus *Hordeum*, the abaxial surface does not contain macro hairs and only one taxon, *H. murinum* subsp. *glaucum*, has a few short macro hairs (up to 200 μm) on its adaxial surface.

Table 4. The measurements of stomata from 30 different 234 × 186 μm² samples of leaf surfaces, mean ± SD.

Taxa	Average Length of Stomata (μm)	Average Breadth of Stomata (μm)	Length/ Breadth Ratio	Average Density
H. violaceum	31.50 ± 1.0	13.30 ± 0.7	2.3	7.70 ± 0.5
H. geniculatum	38.12 ± 1.5	13.01 ± 0.8	2.9	7.30 ± 0.5
H. marinum	40.00 ± 2.0	13.30 ± 1.0	3.0	7.10 ± 0.5
H. murinum subsp. *glaucum*	35.00 ± 1.7	15.90 ± 1.2	2.2	6.10 ± 0.5
H. bulbosum	29.58 ± 1.3	17.71 ± 0.9	1.7	5.30 ± 0.1
H. spontaneum	33.67 ± 1.7	13.77 ± 1.0	2.4	6.30 ± 0.5
H. distichon	31.85 ± 1.9	15.23 ± 0.7	2.1	5.30 ± 0.5
H. vulgare	41.67 ± 2.1	16.35 ± 1.1	2.5	6.30 ± 0.6

On the basis of the anatomical features of the leaves, 2 species, *H. vulgare* and *H. spontaneum*, share the same clade with the highest similarity level (0.78) within the studied taxa. Because they are in the same cluster of the phenogram, our results support the close relationship between *H. bulbosum* and the *H. vulgare-H. spontaneum* clade. In the phenogram, *H. distichon* and *H. bulbosum* are both close neighbours of the *H. vulgare-H. spontaneum* clade with similarity levels of 0.71 and 0.65 respectively and *H. murinum* subsp. *glaucum* is a sister taxon of these species. This first major cluster includes the members of the subgenus *Hordeum* (Blattner, 2009). Within this subgenus, there are 2 sections, namely *Hordeum* and *Trichostachys* Dum. *H. murinum* subsp. *glaucum* is included in the latter. These 2 sections can be distinguished by indumentum properties, marginal sclerenchymatic line numbers, and the maximum number of long cell lines between the rows of intercostal stomata. *H. murinum* subsp. *glaucum* has adaxially located short hairs, 1 or 2 lines of marginal sclerenchymatic cells and a maximum of 6 cell lines between the stomatal rows. While the species within the section *Hordeum* do not contain macro hairs either on the adaxial or abaxial surfaces of their leaves, they have 3 or 4 layered marginal sclerenchyma and a maximum of 9 cell lines between stomatal rows.

In the subgenus *Hordeastrum*, the most similar species are *H. marinum* and *H. geniculatum*, as was also recognised by Baum and Johnson (1998). These 2 species are members of the section *Marina* (Nevski) Jaaska (Petersen & Seberg, 2003). *H. violaceum*, included in the section *Stenostachys* Nevski, is a neighbour of the *H. marinum-H. geniculatum* clade with a 0.64 similarity level. The main differences between these two sections are based on indumentum and epidermal cell wall properties. In the section *Stenostachys* (*H. violaceum*), macro hairs are dense and only adaxially located and the epidermal cell walls on adaxial and/or abaxial surfaces are pitted, whereas in the section *Marina*, there are dense macro hairs on both adaxial and abaxial surfaces and epidermal cell walls are straight. Descriptions of the subgenera and sections are as follows:

Subgenus *Hordeum*

Leaves having shallow (up to 32.57 μm) or deep (up to 91.03 μm) furrows on their adaxial surfaces. Costal prickle line number up to 2. Intercostal stomata row number up to 4. Macro hair absent on abaxial surface. Sclerenchymatic cell number on margins up to 4. Outer bundle is a sheath of large bundles with or without chloroplast. Macro hairs up to 200 μm long.

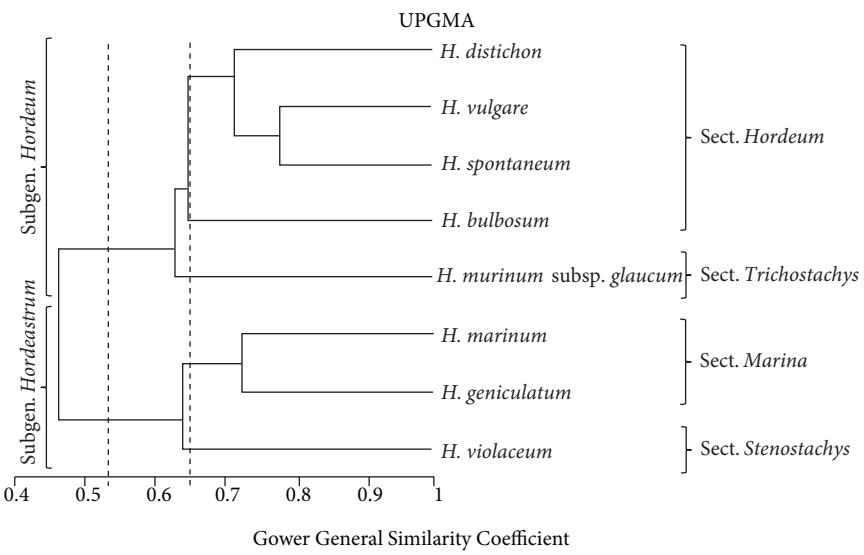

Figure 3. UPGMA phenogram showing the relationships within the genus *Hordeum*.

1. Sect. *Hordeum*

Interstomatal cell line count up to 9. Macro hairs absent. Epidermal cells regularly arranged.

2. Sect. *Trichostachys* Dum

Interstomatal cell line count up to 6. Adaxial surface of leaves sparsely covered with macro hair (up to 200 µm). Epidermal cells irregularly arranged.

Subgenus *Hordeastrum* (Doell) Rouy

Shallow (up to 32.57 µm) furrows, absent on adaxial surfaces of leaves. Costal prickle line number up to 4. Intercostal stomata row number up to 2. Macro hair present on adaxial surface only. Sclerenchymatic cell number on margins up to 2. Outer bundle is a sheath of large bundles without chloroplast. Macro hairs up to 350 µm long.

1. Sect. *Marina* (Nevski) Jaaska

Macro hairs present on abaxial surfaces of leaves. Epidermal cells straight. Length/ breadth ratio of stomata higher than 2.5.

2. Sect. *Stenostachys* Nevski

Macro hairs absent on abaxial surfaces of leaves. Epidermal cells pitted. Length/ breadth ratio of stomata lower than 2.5.

Acknowledgements

We wish to thank the Scientific and Technological Research Council of Turkey (TÜBİTAK-TBAG-105 T 171) for their financial support.

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