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Leaf anatomy of *Agropyron* Gaertn. (Gramineae) in Turkey

D. Özlem MAVİ*, Musa DOĞAN, Evren CABİ

Department of Biological Sciences, Middle East Technical University, 06530, Ankara - TURKEY

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Abstract: *Agropyron cristatum* (L.) Gaertn. is represented by 2 subspecies in Turkey, namely subsp. *incanum* (Nábělek) Melderis and subsp. *pectinatum* (M.Bieb.) Tzvelev. This study examines the taxonomic significance of the leaf properties of the taxa of *Agropyron* Gaertn., comprising diagnostic anatomical features obtained from leaf transverse sections as well as leaf surface micromorphology. Results of the study show that most of the characters, such as the shape of the leaf blades; the size and number of stomata; the density, arrangement, and length of macro hairs; and the presence or absence of midrib and bulliform cells are different in the studied samples of *Agropyron cristatum*.

Key words: *Agropyron*, anatomy, Gramineae, Turkey

Türkiye'deki *Agropyron* Gaertn. (Gramineae) cinsinin yaprak anatomisi

Özet: *Agropyron cristatum* (L.) Gaertn. türü subsp. *incanum* (Nábělek) Melderis ve subsp. *pectinatum* (M.Bieb.) Tzvelev olmak üzere Türkiye'de 2 alttür ile temsil edilir. Bu çalışma, *Agropyron* Gaertn. türlerinde yaprakların, enine kesitleri ve yüzey mikromorfolojilerinden elde edilen ayırt edici anatomik özellikleri incelemektedir. Çalışmanın sonuçları, yaprak ayası şekli; stomaların büyüklükleri ve sayıları; makro tüylerin yoğunluk, dizilim ve uzunlukları; bulliform hücrelerinin ve orta damarın bulunup bulunmaması gibi pek çok karakterin, *Agropyron cristatum*'un çalışılan örneklerinde farklılık gösterdiği görülmektedir.

Anahtar sözcükler: *Agropyron*, anatomi, Gramineae, Türkiye

Introduction

The genus *Agropyron* Gaertn. was once thought to be one of the largest genera in the tribe Triticeae Dumortier, comprising more than 100 species (Dewey, 1983). Nevski (1934) restricted *Agropyron* to perennial taxa with keeled glumes, a group of species referred to in English as the crested wheatgrasses. Subsequent work showed that members of *Agropyron* sensu Nevski are diploids, tetraploids, or hexaploids in which only the P genome is present (Dewey & Asay, 1975; Melderis, 1978; Dewey, 1983; Assadi, 1995; Jensen et al., 2006). This narrow concept of *Agropyron* is now followed in most taxonomic works (e.g. Tzvelev, 1976; Melderis et al., 1980; Melderis, 1985; Barkworth et al., 2007). It was also supported by intergeneric crossing experiments (Assadi &

* E-mail: mavi@metu.edu.tr

Runemark, 1995).

According to Tzvelev (1976), the genus *Agropyron* had 10 species, 9 subspecies of which were included in *Agropyron cristatum* (L.) Gaertn. *A. cristatum* is one of the well-known species of the "crested-wheatgrasses" complex (Knowles, 1955). In the *Flora of Turkey*, Melderis (1985) recognized only one species, *A. cristatum* s.l., in *Agropyron* and subdivided it into 2 subspecies (subsp. *incanum* and subsp. *pectinatum*). Subsp. *pectinatum* was further divided into 2 varieties, one of which was var. *imbricatum*, which has pilose spikelets, and the other one var. *pectinatum*, which has glabrous spikelets.

Löve (1984) recognized another species from Turkey, which he named *A. deweyi* A Löve. The seeds of this species were collected by J.R. Harlan in 1948 and cultivated in Evans Farm, Utah, USA. He noted that this species might be a variant of *A. cristatum* that arose under cultivated conditions far from its native habitat. Baum et al. (2008) neotypified the species.

Vukolov (1929) first showed the arrangement of sclerenchyma around the vascular bundles diagrammatically. There have been several studies including the anatomy of the grasses (Andulov, 1931; Prat, 1932; Stebbins, 1956; Doğan, 1985, 1988, 1991a, 1991b, 1991c, 1997, 1999; Doğan & Tosunoğlu, 1992). For example, Schwendener (1890) studied the sclerenchyma in the leaves and emphasized that the presence, shape, and type of the sclerenchyma on the upper or lower surfaces had systematic importance. Moreover, the anatomy of the family Poaceae (Gramineae) was studied by Metcalfe (1960) and epidermal cell types, stoma types, and the arrangement of the sclerenchymatic cells around the vascular bundles were indicated to be the diagnostic anatomical characters of the leaves. Most recent studies have dealt with the taxonomy, anatomy, and palynology of the genera found in the tribe Triticeae (Başer et al., 2009; Cabi et al., 2009; Cabi & Doğan, 2009; Özler et al., 2009; Cabi et al., 2010).

In this study, the diagnostic value of the leaf anatomy of *A. cristatum* was evaluated. The 3 taxa of the genus *Agropyron* found in Turkey were discussed because the leaf anatomy was important for the classification of the family Poaceae (Metcalfe, 1960). This study aims to clarify the taxonomic statuses of the taxa already recognized in Turkey.

Materials and methods

All of the samples used in this study were freshly obtained and collected in Turkey within the past 3 years as an initial part of a revisional study of the genus. Table 1 provides information on the localities from which the samples were gathered. Mature samples were selected from upper parts of the second

Table 1. Descriptions of the specimens used in the study.

Taxon	Collectors	Collector number	Location
Agropyron cristatum subsp. *incanum*	Evren Cabi Birol Başer	*E. Cabi.* 2545	A8 Erzurum, Aşkale-Bayburt, Kop Mountain, 2401 m, 40°01′38.0″N, 40°31′20.4″E, 9 July 2007
Agropyron cristatum subsp. *pectinatum* var. *imbricatum*	Evren Cabi Ersin Karabacak	*E. Cabi.* 2258	A9 Kars, Kuyucak village, around Kuyucuk Lake, 1642 m, 40°43′40.9″N, 43°25′29.9″E, 8 July 2007
Agropyron cristatum subsp. *pectinatum* var. *pectinatum*	Evren Cabi Ersin Karabacak	*E. Cabi.* 2422	B6 Sivas, Ulaş to Kangal, near Tecel village, roadside, dry meadows, 1405 m, 39°24′25.7″N, 37°04′50.3″E, 16 June 2007

nodes for each taxon and fixed in a formalin-acetic-alcohol (FAA) solution for 48 h. The dehydrated specimens were then embedded in paraffin with the help of xylene (Johansen, 1944). The 10-µm thickness of the leaf blade transverse sections was achieved by using a Leica RM2125RT model rotary microtome. These sections were stained with safranin and fixed with Entellan. All of the slides were studied using a Euromex FE 2025 light microscope and photographed with a Euromex CMEX DC.1300 camera.

To indicate the surface view, both scanning electron microscopy (SEM) and light microscopy (LM) photographs were used. For LM observations, 10 slides, which were not stained, were prepared from both adaxial and abaxial surfaces. All the quantitative results were gathered for 20 different 234 × 186 µm^2 sections of leaf surfaces. For SEM observations, leaf samples were placed directly on aluminium stubs and coated with gold using a gold-coating apparatus (Doğan, 1988). For photographs, a FEI QUANTA400F model microscope was used in the low vacuum mode.

Results and discussion

The leaf transverse sections (Figure 1) show that the shapes of the blades are grouped into 3 types: U-shaped (Metcalfe, 1960), V-shaped, and convolute (Doğan & Tosunoğlu, 1992). *A. cristatum* subsp. *incanum* has U-shaped and *A. cristatum* subsp. *pectinatum* var. *pectinatum* has V-shaped leaf blades, in which the mid-vein of the blade makes a rib towards the abaxial surface of the leaf. However, *A. cristatum* subsp. *pectinatum* var. *imbricatum* has convolute leaves (Figure 1).

The leaves of all the taxa have furrows and ribs on their adaxial surfaces. The lengths of these furrows are different, however. The maximum length of the furrows in *A. cristatum* subsp. *incanum* was 83.65 µm, while in subsp. *pectinatum* var. *imbricatum* and subsp. *pectinatum* var. *pectinatum*, the maximum length was found to be 58.50 and 30.35 µm, respectively. Therefore, it can be seen that subsp. *incanum* has the deepest furrows.

The properties of the median vascular bundle, such as its size, presence, or absence, and the sclerenchymatic arrangement around it are diagnostic anatomical characters of the leaves in Gramineae (Poaceae) (Metcalfe, 1960). From this point of view, the solitary mid-veins of the taxa are all certain. In subsp. *pectinatum* var. *imbricatum*, although the large mid-vein is clearly distinguishable from the lateral vascular bundles, it does not make a rib towards the abaxial surface. In subsp. *pectinatum*, the abaxial sides of the median vascular bundle contain I-shaped sclerenchyma, while in subsp. *incanum* the sclerenchyma is arranged in a T-shape on the abaxial surface (Figure 1). Moreover, all of the taxa have I-shaped sclerenchyma on the adaxial sides of their large vascular bundles. On the other hand, the adaxial sides of the vascular bundles feature I-shaped sclerenchyma in subsp. *pectinatum* and triangular-shaped sclerenchyma in subsp. *incanum* (Figure 1).

A single layer of vascular bundles is arranged parallel to the adaxial and abaxial surfaces in homogenous mesophyll. There are 11 vascular bundles in subsp. *incanum* and 31 vascular bundles in subsp. *pectinatum*. In subsp. *incanum* and subsp. *pectinatum* var. *imbricatum*, the midrib has 2 neighbour vascular bundles without sclerenchyma towards the margins. The neighbour vascular bundles of the mid-vein of subsp. *pectinatum* var. *pectinatum* have I-shaped girders on their abaxial sides, however (Figure 1). Vascular bundles close to the leaf margins of subsp. *incanum* and subsp. *pectinatum* var. *imbricatum* do not have sclerenchyma, while the margins of subsp. *pectinatum* var. *pectinatum* have vascular bundles with I-shaped girders on their abaxial sides.

Vascular bundles of the taxa have double sheaths on their surfaces. The complete inner sheath is sclerenchymatic and surrounded by an incomplete outer sheath (Metcalfe, 1960; Brown, 1975). In the leaves of subsp. *pectinatum* var. *pectinatum* and subsp. *incanum*, the outer bundle sheaths contain a small amount of chloroplast, unlike subsp. *pectinatum* var. *imbricatum*, which does not contain chloroplast in its outer bundle sheaths.

The bulliform cells, having thin walls, are generally larger than the adjacent epidermal cells and control the leaf folding (Metcalfe, 1960). In subsp. *pectinatum* var. *pectinatum*, there are 3-7 fan-shaped bulliform cells on the adaxial surface of the leaves. However, in subsp. *pectinatum* var. *imbricatum*, the bulliform cells are not larger than the adjacent epidermal cells,

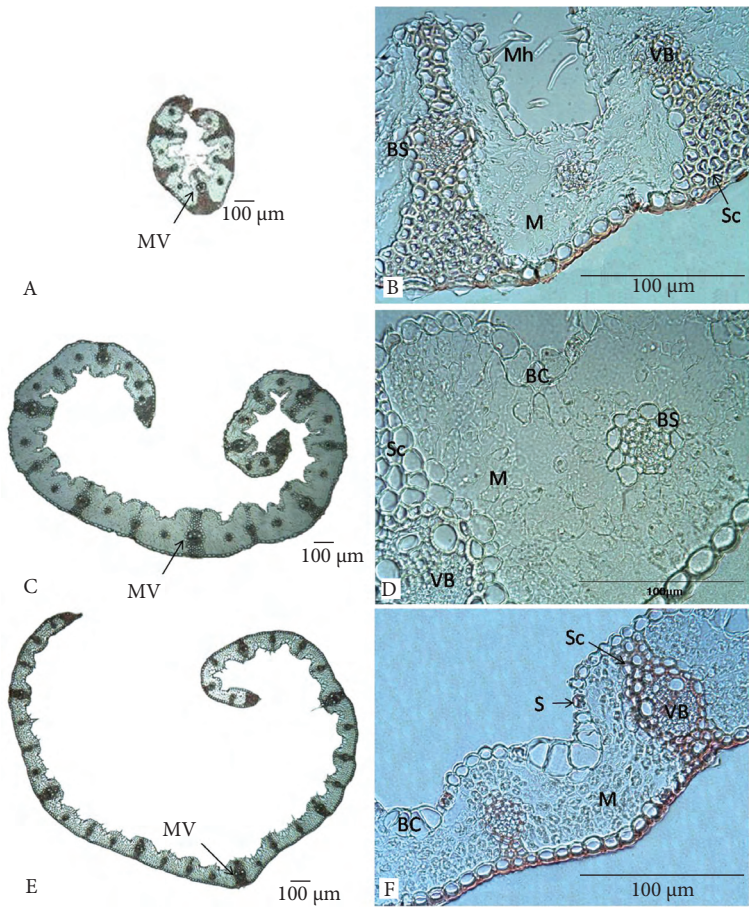

Figure 1. Transverse sections of leaves: A- *Agropyron cristatum* subsp. *incanum* (4×), B- *A. cristatum* subsp. *incanum* (40×), C- *A. cristatum* subsp. *pectinatum* var. imbricatum (4×), D- *A. cristatum* subsp. *pectinatum* var. *imbricatum* (40×), E- *A. cristatum* subsp. *pectinatum* var. *pectinatum* (4×), F- *A. cristatum* subsp. *pectinatum* var. *pectinatum* (40×).
BC: bulliform cell; BS: bundle sheath; M: mesophyll; Mh: macro hair; MV: mid-vein; S: stoma; Sc: sclerenchyma; VB: vascular bundle

as they are in var. *pectinatum*. On the adaxial surface of leaves of subsp. *incanum*, the bulliform cells are found to be uncertain (Figure 1).

The uniformly-sized epidermal cells of the studied taxa have regular shapes and the epidermis is differentiated into long cells, stomata, hairs, prickles, hooks, and short cells, both adaxially and abaxially. Horizontally elongated long cells are parallel to the long axis of the leaves (Metcalfe, 1960). Short cells are differentiated as being only single silica cells, macro hairs, prickles, or hooks between 2 long cells (Figure 2). However, the number of undifferentiated short cells between 2 long cells may be 1 or 2. All of the taxa of *Agropyron* have horizontally or equidimensionally elongated silica bodies on their leaf surfaces. These cells are usually located next to the prickles, hooks, and macro hairs in the costal and intercostal zones.

There are 3 or 4 long cells located between stomata rows of the leaves in subsp. *incanum* and subsp. *pectinatum* var. *imbricatum*. However, in subsp. *pectinatum* var. *pectinatum*, the maximum number of long cells was found to be 9. The length of the long cells shows variation between 52.95 and 155.11 µm within the taxa. The smallest length was measured

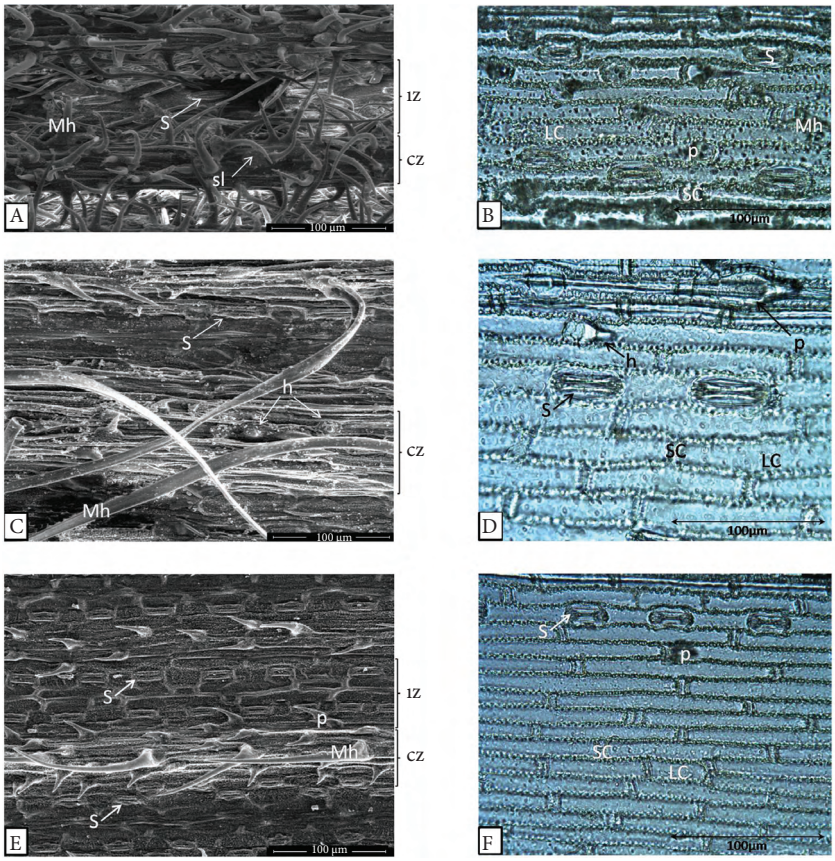

Figure 2. Surface view of leaves: A- Adaxial surface of *A. cristatum* subsp. *incanum*, B- abaxial surface of *A. cristatum* subsp. *incanum* (40×), C- adaxial surface of *A. cristatum* subsp. *pectinatum* var. *imbricatum*, D- abaxial surface of *A. cristatum* subsp. *pectinatum* var. *imbricatum* (40×), E- adaxial surface of *A. cristatum* subsp. *pectinatum* var. *pectinatum*, F- abaxial surface of *A. cristatum* subsp. *pectinatum* var. *pectinatum* (40×).
CZ: costal zone; H: hook; IZ: intercostal zone; LC: long cell; Mh: macro hair; p: prickle; S: stoma; SC: short cell; SL: silica cell

in subsp. *incanum*, while the latter number was measured in subsp. *pectinatum* var. *imbricatum*. The width of the long cells also shows variation. In subsp. *incanum* and subsp. *pectinatum* var. *pectinatum*, the average width of the long cells was 8.79 ± 1.3 and 8.35 ± 1.0 µm, respectively; in subsp. *pectinatum* var. *imbricatum*, it was 17.85 ± 1.1 µm (Table 2).

In the intercostal zones, the stomata are regularly arranged in a maximum of 3 rows, although the density of stomata varies between the leaves. The average number of stomata is 5.75 ± 0.9, 3.50 ± 0.5, and 4.16 ± 0.9 in subsp. *incanum*, subsp. *pectinatum* var. *imbricatum*, and subsp. *pectinatum* var. *pectinatum*, respectively. The stomata have parallel-sided subsidiary cells (Metcalfe, 1960). All of the qualitative and quantitative properties of the stomata for the taxa, including length and breadth, are given in Table 2. According to these results, subsp. *pectinatum* var. *imbricatum* has the longest and widest stomata.

All of the taxa have macro hairs, prickles, and hooks on their adaxial surfaces. The average lengths of these features are nearly the same for all the taxa, with prickles being 32.21 ± 3.7 µm and hooks measuring 17.45 ± 3.6 µm. In subsp. *pectinatum*, 1 or 2 layers of macro hairs are costally located. However, in subsp. *incanum* there are dense and irregularly located macro hairs found in the costal and intercostal zones on both surfaces (Figure 2). The length of the

Table 2. List of the characters and states for each taxon.

Taxon	LB	Abaxial side of MV	Bundle without girder or strand	BC	Adaxial Mh length (μm)	Costal Mh line number	Sc type of VB close to the margins	Shape of Sc on abaxial MV	Ch in the outer BS	Average breadth of inter-stomatal LC (Mean ± SD)
subsp. *incanum*	U-shaped	Ribbed	Present	Uncertain	Up to 200	>2	Absent	Triangle	present	8.79 ± 1.3
subsp. *pectinatum* var. *imbricatum*	Convolute	Unribbed	Present	Certain, small	Up to 1000	1 or 2	Absent	I-shaped	absent	17.85 ± 1.1
subsp. *pectinatum* var. *pectinatum*	V-shaped	Ribbed	Absent	Certain, large	Up to 150	1	I-shaped (abaxial)	I-shaped	present	8.35 ± 1.0

Taxon	Max. number of Mh line in ICZ	Max. number of ICZ prickle line	Max. number of inter-stomatal cell line	Average length of stomata (Mean ± SD)	Average breadth of stomata (Mean ± SD)	Length / Breadth ratio of stomata	Number of VB	Max length of furrows on adaxial surfaces (μm)	VB close to margins	Average Number of stomata
subsp. *incanum*	8	6	4	31.60 ± 2.1	13.15 ± 1.2	2.4	11	83.65	Without Sc	5.75 ± 0.9
subsp. *pectinatum* var. *imbricatum*	0	2	4	50.20 ± 2.3	19.6 ± 1.7	2.6	31	62.73	Without Sc	3.50 ± 0.5
subsp. *pectinatum* var. *pectinatum*	0	3	9	26.30 ± 2.1	12.3 ± 1.6	2.1	31	30.35	With Sc	4.16 ± 0.9

BC: bulliform cell; BS: bundle sheath; Ch: chloroplast; ICZ: intercostal zone; LB: leaf blade; LC: long cell; Mh: macro hair; MV: mid-vein; Sc: sclerenchyma; VB: vascular bundle

macro hairs can vary widely between the taxa, with measurements ranging from 150 to 1000 μm. *A. cristatum* subsp. *pectinatum* var. *imbricatum* has macro hairs of up to 1000 μm, while the same feature reaches lengths of 150 and 200 μm in subsp. *incanum* and subsp. *pectinatum* var. *pectinatum*, respectively (Table 2).

There are 20 different anatomical characters considered to be diagnostic for the taxa in the genus *Agropyron* L. (Table 2). Some of these properties showed close relationships between the varieties of the subspecies *pectinatum*; the number of vascular bundles, the absence of macro hairs in the intercostal zones, and the sclerenchyma type of the mid-vein are all properties that underline the similarities within the varieties of subsp. *pectinatum*. However, there are also some other characters, such as the maximum number of interstomatal cell lines, the absence of sclerenchyma on the sides of the vascular bundles closest to the margins, and the absence of bundles

without sclerenchyma, which indicate that the closest relationship between subsp. *pectinatum* var. *imbricatum* and subsp. *incanum* is seen. On the other hand, most of the properties show that each taxon has unique characteristics for each character. For example, the shape of the leaf blades, the maximum number of intercostal prickle lines, the length-to-breadth ratio of stomata, the average number of stomata, the lengths of adaxial furrows, the number of macro hair lines in the costal zones, and the presence or size of bulliform cells are all different for each taxon. Due to the lack of chloroplast in its outer bundle sheath, its unribbed mid-vein, the occurrence of macro hairs of up to 1000 μm in length, and the presence of the widest (19.6 ± 1.7) and the longest (50.20 ± 2.3 μm) stomata and the widest long cells (17.85 ± 1.1 μm), subsp. *pectinatum* var. *imbricatum* is distinctly separated from the other taxa.

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