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Taxonomic importance of leaf anatomical characters for the genus *Alopecurus* L. (Poaceae)

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Taxonomic importance of leaf anatomical characters for the genus *Alopecurus* L. (Poaceae)

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Running title: LEAF ANATOMY OF *ALOPECURUS* SPECIES IN TURKEY

Abstract – Tiller leaf anatomical features of nine *Alopecurus* species collected from high mountainous areas in Turkey were evaluated in the present study. Detailed leaf anatomical descriptions of the studied taxa and an identification key generated using the anatomical features are given. Clustering and principal coordinate analysis (PCA) methods were applied based on a total of 14 leaf anatomical characters. Anatomical differences between species were detected and anatomical characters of taxonomic interest were identified in this genus. With clustering analyses, a UPGMA tree was obtained to show the relationship between the species studied. Based on the results of PCA, the arrangement of adaxial sclerenchyma cells, the number of adaxial ribs, the number of abaxial sclerenchyma strands, the size of epidermal cells and the number of vascular bundles are designated as the most reliable characters to separate the species.

Keywords: *Alopecurus*, leaf anatomy, numerical taxonomy, systematics

Introduction

The Poaceae Barnhart is one of the most diverse plant families, represented by about 12,000 species and 780 genera worldwide (Clayton and Renvoize 1986, Kellogg 2015, Christenhusz and Byng 2016, Soreng et al. 2017). In Turkey, Poaceae consists 658 species and infraspecific taxa within 146 genera (Cabi and Doğan 2012). *Alopecurus* L. (the foxtail genus) is a genus of the subtribe Alopecurinae with about 50 species all around the world, including many alpine species (Doğan 1988), and the centre of diversity of the genus is in southwest Asia (Boudko 2014). According to Cabi and Doğan (2012) and Cabi et al. (2017), the genus *Alopecurus* is represented by 27 taxa, seven of which are endemic to Turkey. The Turkish *Alopecurus* species occupy a wide variety of habitats ranging from sea level to high mountain steppes (Doğan 1985).

The grass family has highly specialized and reduced flowers and its fine morphological distinctions are often essential to define differences between taxa (Ellis 1976, 1986). Therefore, Ellis (1976) indicated that anatomical data are regarded as being of undoubted importance in the jigsaw of complete systematic evidence. Leaf anatomical characters in cross-section have been the main supplementary tools to add to the morphological features for characterizing some genera of difficult taxonomy within the grass family (e.g., Ellis 1976, 1986, López and Devesa

1991, Martínez-Sagarra et al. 2017, Aykurt et al. 2022) such as *Festuca* L. (e.g., Martínez-Sagarra et al. 2017).

According to Doğan (1985), the *Alopecurus* taxa found in Turkey were classified under four sections: Sect. *Alopecurus*, Sect. *Colobachne* P.Beauv., Sect. *Pseudophalaris* Tzvelev., and Sect. *Tozzettia* (Savi) Endl. Then, as a result of the numerical taxonomy on the genus, *Alopecurus* was divided into three different sections: *Alopecurus*, *Alopecurium* Dumort. and *Colobachne* (Doğan 1997). According to Doğan (1985, 1997) the most diverse section in Turkey is *Colobachne*, and it contains a total of 10 species if the recently described *A. goekyigitiana* Cabi & Soreng is counted (Cabi et al. 2017). All of the species in this section are mountainous caespitose perennials that grow in the subalpine or alpine zone (Doğan 1985, 1999, Cabi et al. 2017) with ovoid panicles and branches bearing 1–6 spikelets (Doğan 1985, Boudko 2014). The glumes gradually attenuate into long points (Doğan 1985) and are longer than the lemma. The palea of taxa in the Sect. *Colobachne* is generally present and rarely absent (Boudko 2014). The Turkish species included in this section, except for three species, which are *A. anatolicus* Doğan, *A. glacialis* K.Koch and *A. laguroides* Balansa, were evaluated within the scope of our study. In addition, *A. aequalis* Sobol. and *A. arundinaceus* Poir. were included in the study, because they also occur in the high mountain areas. They are included in the sections *Alopecurium* and *Alopecurus*, respectively. The sections *Alopecurium* and *Alopecurus* consist of both annual and perennial species and the palea of the species is absent (Doğan 1985, Boudko 2014). The panicles are cylindrical to oblong and with 1–5 spikelets in the section *Alopecurium*. The glumes are acute to obtuse and are longer than or equalling the lemma. The section *Alopecurus* has ovoid to cylindrical panicles with each branch bearing 1–10 spikelets. Glumes are acute, equal or longer than the lemma and connate in the lower half (Boudko 2014). Although numerous studies conducted on the morphology of the genus *Alopecurus* can be found (e.g., Doğan 1997, 1999, Soreng et al. 2007, Boudko 2014), there are limited studies conducted on the importance of leaf anatomical characters for *Alopecurus* species in the taxonomy of this genus.

In recent years, studies evaluating the phylogenetic relationships between the members of this genus have been conducted, and infrageneric and interspecific relationships have been interpreted by using both nuclear (ITS and ETS) and plastid markers (*trn*TLF, *rpo*B-*trn*C and *mat*K) (e.g., Soreng 2007, Boudko 2014, Cabi et al. 2017). As a result of the plastid (TF+*rpo*B+*mat*K) and combined (ETS+ITS+TF+*rpo*B+*mat*K) maximum parsimony analyses made by Boudko (2014), it was shown that the infrageneric classification of the genus accepted by Doğan (1999) (sect. *Alopecurus* incl. *A. myosuroides*, sect. *Alopecurium* and sect. *Colobachne*) is not monophyletic. Phylogenetic studies involving more species are very important in order to resolve the phylogenetic relationships in both infrageneric and interspecific classification of the genus *Alopecurus* (Boudko 2014).

A large number of specimens belonging to nine *Alopecurus* species were collected in the high mountainous zone of the Western Taurus (Antalya, Turkey) during our project on the diversity of the Poaceae, and their leaf anatomy (in cross-section view) was evaluated. There are some difficulties during the identification and separation of some *Alopecurus* species by using morphological characters. This study aimed to (i) determine the leaf anatomical features in the *Alopecurus* species studied; (ii) determine the useful anatomical characters to identify the species of the genus; (iii) evaluate the relationship between the taxa using the anatomical data.

Material and methods

The tiller leaf anatomical features of mountain *Alopecurus* species that occur in subalpine and alpine areas in Turkey were evaluated in the present study. We investigated a total of nine *Alopecurus* species, seven of which are included in the Sect. *Colobachne*, which are *A. aucheri*

Boiss., *A. davisii* Bor., *A. gerardii* (All.) Vill., *A. goekyigitiana, A. lanatus* Sm., *A. textilis* Boiss. and *A. vaginatus* (Willd.) Pall. ex Kunth. *Alopecurus goekyigitiana* and *A. lanatus* are endemic to Turkey. The other two species investigated, which are *A. aequalis* and *A. arundinaceus*, are common foxtails in different habitats including high mountainous regions. Most of the plant specimens examined were collected from natural populations during field trips between the years 2018 and 2020. We used herbarium materials deposited in AKDU and the collection data of the taxa used for our analyses are presented in Tab. 1. A total of 45 individuals, three individuals from each location, belonging to nine species were used for the anatomical measurements carried out within the scope of the study. At least three tiller leaves from each individual were analyzed.

Short pieces of tiller leaves taken from herbarium materials were kept in distilled water for c. five minutes. Then, cross-sections ± 0.05 mm thick were cut by free-hand from pieces of leaf fixed in styrofoam. This was done under a stereomicroscope using reflected light and the sections were stained in a drop of water with toluidine blue. After 1–3 minutes, depending on stainability, the sections were washed in distilled water and studied under a light microscope at a magnification of 10–40 × (general anatomical pattern) or 100 × (detailed shape of the epidermal and bundle sheath cells). The cross-sections taken from the tiller leaf blades of all species studied are shown in Figs. 1 and 2.

The leaf preparations were analyzed in detail and anatomical features were determined for each taxon. As a result, 14 anatomical characters that can be used for the genus *Alopecurus* were scored for each taxon and included in the numerical analysis. In this study, two different numerical analysis methods were used: clustering (UPGMA) and principal coordinate analysis (PCA) by using PAST Version 4.03 computer software. The anatomical characters determined for this study and evaluation of these characters for each taxon are presented in Tabs. 2 and 3, respectively. Besides, the scatter biplot diagram of the studied *Alopecurus* species as OTUs based on the leaf anatomical characters determined is presented in Fig. 3. Ellis (1976, 1979) was used as the main source to choose the leaf anatomical characters.

Results

The results of our study are presented in two parts, in which the general leaf anatomical features and anatomical characters of *Alopecurus* taxa are evaluated taxonomically with numerical analyses.

The tiller leaf anatomical characters determined within the scope of the study are generally related to the epidermis, sclerenchyma strands and girders, both abaxial and adaxial ribs, and vascular bundles. The first anatomical character was determined as the shape of the leaves in cross-section. Almost all species of Sect. *Colobachne* included in the study have a U-shaped tiller leaf cross-section, except for *A. vaginatus*, which has a U- to O-shaped cross-section. In contrast, leaf cross-sections of *A. aequalis* (sect. *Alopecurium*) and *A. arundinaceus* (sect. *Alopecurus*) are flat. Characteristics determined in relation to the epidermis are the size of epidermal cells (C13), the size of bulliform cells (C14), papillae on the epidermal cells (C3) and density of macro-hairs (C7). The epidermal cells of all *Alopecurus* species studied are single-layered and generally polygonal in shape. The size of epidermal cells was categorized under three different groups according to their cell length. Accordingly, the epidermal cells of the species included in sect. *Colobachne* are quite different in size. In addition, bulliform cells are prominent in all species studied except *A. aequalis*.

In the species studied, the arrangement of adaxial sclerenchyma strands (C4) can be scattered as few groups, regular groups at the level of the vascular bundles, extended along the lobes or T-shaped. The adaxial sclerenchyma strands are as: regular groups at the level of the vascular bundles in *A. aequalis* and *A. arundinaceus*; scattered few groups in *A. vaginatus, A.*

gerardii and *A. goekyigitana*; and extended along the lobes in *A. aucheri* and *A. textilis*. Only in *A. davisii*, the adaxial sclerenchyma strands are T-shaped. The abaxial sclerenchyma strands (C 10) are as: very small or small strands; big strands like a cap; or with well-developed girder. Abaxial sclerenchyma strands with well-developed girders appear only in *A. arundinaceous*. In *A. textilis* and *A. aucheri*, the abaxial strands are big like a cap. The rest of the species studied are with very small or small abaxial sclerenchyma strands. The anatomical characters determined within the scope of the study are given in Tab. 2.

Based on the PCA results, the variance value of the first two components is 59.58%, and the variance value of the first four components is 91.06%. The eigenvalue and the percentage of eigenvalue of the components, and the eigen vector value of the components are given in the Appendixes, respectively. According to the results of the numerical analysis PC1, the first five most reliable characters are C4 (arrangement of adaxial sclerenchyma strands), C9 (the number of adaxial ribs), C5 (The number of abaxial sclerenchyma strands), C13 (the size of epidermal cells) and C11 (the number of vascular bundles), respectively; based on PC2, it is seen that they are C12 (the connection of midrib with epidermal layer), C10 (the number adaxial ribs), C14 (the size of bulliform cells), C3 (Papillae on the epidermal cells) and C2 (the ratio of midrib size to the laterals).

The obtained UPGMA dendrogram shows that *A. aequalis* (Sect. *Alopecurium*) is separated from all other species studied (Fig. 2). *Alopecurus arundinaceus* is located close to the cluster of the Sect. *Colobachne* species. *Alopecurus* Sect. *Colobachne* species are grouped together into two main branches. *Alopecurus vaginatus*, *A. gerardii* and *A. goekyigitiana* are grouped together and close to *A. textilis* (first group), whereas *A. lanatus* and *A. davisii* are grouped in a different branch close to *A. aucheri* (second group). The most useful anatomical characters to separate these two species groups are C9 (the number of adaxial ribs) and C11 (the number of vascular bundles). The number of adaxial ribs is between 6–9 in the first group, whereas they are between 12–20 in the second group. The adaxial ribs are between 17–20 in *Alopecurus aucheri* and are between 12–16 in *A. davisii* and A. *lanatus*. While the number of vascular bundles varies between 6–10 in the first group; in the second group, it varies between 11–18. Detailed evaluations and measurements of the species studied are also given in Tab. 3.

The abaxial ribs (C8) are very prominent in only two of the species studied, which are *A. textilis* and *A. aucheri*. The abaxial ribs are slightly lobed in *A. aequalis* and are absent in the other species studied. The number of vascular bundles was evaluated under two categories, and this character is very useful to separate the species groups in the sect. *Colobachne*.

The depth of adaxial furrows is more than one half of the leaf thickness (C6), and thin-walled wide papillae are easily visible as scattered throughout the epidermis (C3) in *A. aequalis.* With these characters, *A. aequalis* can be easily distinguished from other species. *Alopecurus arundinaceus* is the only species having a midrib connected to both sides (C12). The sclerenchyma bands around the mature vascular bundles of this species are elongated to both abaxial and adaxial surfaces.

Results from the PCA analysis showed that the least reliable characters are C6 (Depth of adaxial furrows in comparison to the leaf thickness) and C7 (Density of macro-hairs) according to PC1.

Identification key for *Alopecurus* taxa studied

1. Leaves flat in cross-section ……...……...…..……2
1. Leaves U- or O-shaped in cross-section ……..……..……3
2. Abaxial and adaxial surfaces connected with sclerenchyma cells; depth of adaxial furrows quarter to one half the leaf thickness; outer walls of epidermal cells arched but not papillose…….…… *A. arundinaceus*

2. Abaxial and adaxial surfaces not connected with sclerenchyma cells; depth of adaxial furrows more than one half the leaf thickness; thin-walled wide papillae scattered throughout the epidermis ……..……. *A. aequalis*
3. The number of vascular bundles 6–10…….……4
3. The number of vascular bundles 11–18 ……. 7
4. Adaxial sclerenchyma strands scattered as few groups…….……..5
4. Adaxial sclerenchyma strands extended along the lobes …….…….. *A. textilis*
5. Outer walls of epidermal cells arched but not papillose …….. *A. goekyigitiana*
5. Entire or major part of epidermis composed of thin-walled wide papillae …….…….. 6
6. Epidermal cells up to 12.5 µm long …… *A. gerardii*
6. Epidermal cells up to 17.99 µm long …… *A. vaginatus*
7. Abaxial ribs distinct …… *A. aucheri*
7. Abaxial ribs absent……..… 8
8. Adaxial sclerenchyma bands T-shaped; epidermal cells up to 17.99 µm long ……..……..…….. *A. davisii*
8. Adaxial sclerenchyma bands not T-shaped; epidermal cells up to 12.5 µm long …….……...…..……...…… *A. lanatus*

Discussion

We focused on the tiller leaf anatomical characters of the high mountain *Alopecurus* species in Turkey in the present study. All *Alopecurus* species adapted to subalpine and alpine mountainous areas in Turkey are caespitose perennials classified in the Sect. *Colobachne* (Doğan 1985, 1997, 1999, Cabi et. al. 2017). The tiller leaves of these morphologically similar species are filiform, narrowly lanceolate and generally convolute. The mountain *Alopecurus* species studied exhibit similar leaf anatomical characters to high mountain *Festuca* species. Abaxial sclerenchyma patterns, number of vascular bundles and number of ribs are among the most distinctive morphological characters for *Festuca* (Martínez-Sagarra et al. 2017). Considering the results obtained from our study, it is seen that these characters are similar to the most important characters in PC1 in the high mountain species of the genus *Alopecurus* (C4, C9, C5 and C8).

The anatomical characters analyzed within the scope of our study can be easily observed in leaf cross-sections that can be taken from herbarium materials. Since dry samples were used, some distinguishing anatomical characters specified by Ellis (1976, 1979) could not be used, such as vascular bundle, vascular bundle sheath, and leaf mesophyll (chlorenchyma and colourless parenchyma cells) in the present study. The results obtained showed that especially the number of both adaxial and abaxial ribs, and features of sclerenchyma strands such as number, arrangement and girders are very important useful anatomical characters. Metcalfe (1960) stated that the ribs are generally characteristic of and more fully developed on the adaxial than the abaxial surface. Adaxial ribs are distinct in all of the species evaluated within the scope of the study, while abaxial ribs are distinct only in *A. textilis* included in the first group and *A. aucheri* included in the second group. According to Ellis (1976), ribs and furrows may vary in depth, transverse shape, spacing and location in different species. In *A. aequalis* the adaxial furrows are distinct and much deeper than in the other species studied, which have medium furrows. In the PCA results obtained, the length of epidermal cells is one of the most significant anatomical characters. *Alopecurus textilis*, *A. gerardii* and *A. goekyigitiana* have the smallest epidermal cells, in contrast to *A. arundinaceus* and *A. aucheri*, which have the biggest epidermal cells.

The least significant leaf anatomical characters according to the PC1 results are depth of adaxial furrows (C6), density of macro hairs (C7), size of bulliform cells (C14) and papillae on

the epidermal cells (C3), respectively. We evaluated C7 as scattered and dense; accordingly, *A. davisii* and *A. lanatus*, which have densely hairy leaves, were scored differently from the rest of the species studied. In the obtained dendrogram, the relationships between the taxa are similar to the results obtained previously by different researchers, both from morphological and phylogenetic studies (e.g., Doğan 1997, 1999, Cabi et al. 2017). This is an indication that anatomical characters are useful for the taxonomy of the genus *Alopecurus*. *Alopecurus aequalis* was separated into a different branch from all other species. Among the studied species, *A. aequalis*, which is an annual and grows in damp places and marshy habitats, has distinct anatomical differences from the others in terms of the characters C3, C6, C8 and C14. Although *A. arundinaceus* is close to other species studied, it is separated into a different branch. It differs markedly from the other studied species in that the midrib is connected to both the abaxial and adaxial surfaces via sclerenchyma cells, and its leaves are flat in cross-section. Doğan (1985) stated that *A. arundinaceus* is rare in South Anatolia. However, we observed that this species is widespread in the high mountainous areas of southern Anatolia during the field studies.

The section *Colobachne* is divided into two groups in the dendrogram according to the number of adaxial ribs (C9), the number of vascular bundles (C11) and the size of midrib in comparison to the laterals (C2). In the first group composed of *A. vaginatus, A. gerardii, A. goekyigitiana* and *A. textilis*, the number of vascular bundles is between 6–10, the number of adaxial ribs is between 6–9 and the midvein is larger than the laterals. In the second group, which includes *A. aucheri, A. davisii* and *A. lanatus*, the number of vascular bundles is between 11–18, the number of adaxial ribs is between 12–20 and the midvein is not larger than the laterals.

Alopecurus goekyigitiana as described by Cabi et al. (2017) was evaluated as closely related to *A. gerardii* and *A. vaginatus*. According to our results, it is similar to these species in terms of its leaf anatomical features. Unlike these species, however, the outer walls of the epidermal cells in *A. goekyigitiana* are arched but not papillose (C3). The entire or major part of the epidermis in *A. gerardii* and *A. vaginatus* is composed of thin-walled wide papillae. It was also noted by Codignola et al. (1987) that *A. gerardii* has a bulbous epidermis. When evaluated in terms of this character (C3), *A. goekyigitiana* resembles *A. lanatus* and *A. davisii*. *Alopecurus davisii* and *A. lanatus*, indicated as closely related species by Doğan (1985), have some differences in terms of their leaf anatomical characters such as C4, C13 and C14 (Tab. 3). The most distinctive anatomical feature that can be used to distinguish between these two species is the arrangement of the adaxial sclerenchyma strands (C4); the adaxial sclerenchyma bands are T-shaped in *A. davisii*, whereas the sclerenchyma strands are extended along the lobes in *A. lanatus*.

Among the studied species, only *A. aucheri* and *A. textilis* have prominent abaxial ribs. Although these two species are similar in many anatomical characters, they differ in the most reliable characters, C9 and C13. This has caused these species to be included in separate groups.

The results obtained show that the leaf anatomical characters we determined within the scope of the study are very useful in grouping close species and separating species from each other for the genus *Alopecurus*. The tiller leaf anatomical features of the species groups and of the species should be clarified by further analyzing the anatomical features by using fresh materials for this genus. With this study, it has been shown that tiller leaf anatomical characters will contribute greatly to the systematics of the genus.

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Author contribution

SG: Preparing the leaf anatomical slides, measuring and evaluating the anatomical characters. CA: Designing the study, determining the anatomical characters. Both authors made the numerical analyses and wrote the manuscript.

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Tab. 1. Studied *Alopecurus* taxa and collection information of the specimens.

OTU		Locality data of specimens studied
OTU1	*A. aequalis*	Isparta, Gölcük Nature Park, 1390 m, 10.07.2013, *C. Aykurt 3397*.
OTU2	*A. arundinaceus*	Antalya, Kaş, Gömbe, Ak Mountain, around Yeşilgöl, 1825 m, 29.07.2018, *C. Aykurt 4779*.
		Antalya, Gündoğmuş, between Eğrigöl and Söbüçimen Plateau, 2087 m, 20.06.2019, *C. Aykurt 5345*.
OTU3	*A. vaginatus*	Antalya, Serik, Bozburun Mountain, 1855 m, 24.05.2019, *C. Aykurt 4940a*.
		Antalya, Alanya, Ak Mountain, 1985 m, 18.06.2019, *C. Aykurt 5245*.
OTU4	*A. textilis* subsp. textilis	Antalya, Akseki, Gidengelmez Mountains, 1950 m, 13.06.2012, *C. Aykurt 3224*.
		Antalya, Kumluca, Sarıkaya Wildlife Development Area, Bey Mountains, 2754 m, 28.06.2021, *SWDA 97-1-12*.
OTU5	*A. gerardii* var. *gerardii*	Antalya, Alanya, Başyayla environs, 1579 m, 17.06.2019, *C. Aykurt 5153*.
		Antalya, Alanya, Ak Mountain, 2044 m, 16.07.2019, *C. Aykurt 5514*.
OTU6	*A. goekyigitiana*	Antalya, Gündoğmuş, Eğrigöl to Hadim, 2205 m, 20.06.2019, *C. Aykurt 5327*.
		Antalya, Gündoğmuş, between Eğrigöl and Söbüçimen Plateau, 2087 m, 20.06.2019, *C. Aykurt 5344*.
OTU7	*A. aucheri*	Bitlis, Tatvan, Nemrut Mountain, 2480 m, 28.05.2019, *L.Y. Konuralp*.
OTU8	*A. davisii*	Izmir: Kemalpaşa, Mahmut Mountain, 1250-1368 m, 28.04.1992, *A. Aksoy 744*.
OTU9	*A. lanatus*	Antalya, Kumluca, Sarıkaya Wildlife Development Area, Bey Mountains, 2754 m, 28.06.2021, *SWDA 97-1-11*.
		Antalya, İbradı, Toka Plateau, 1514 m, 24.06.2020, *C. Aykurt 5624*.

Tab. 2. Tiller leaf anatomical characters for the genus *Alopecurus* and their scorings determined within the scope of the study and used in the numerical analyses.

C1	The shape of the tiller leaves in cross-section: flat (0) / U- to O-shaped (1)
C2	The midrib larger than the laterals (1) / not larger (0)
C3	Papillae on the epidermal cells: Outer walls of epidermal cells arched but not papillose (0) / Entire or major part of epidermis composed of thin-walled wide papillae (1) / Thin-walled wide papillae scattered throughout the epidermis (2)
C4	Arrangement of adaxial sclerenchyma strands as: scattered few groups (0) / regular groups at the level of the vascular bundles (1) / extended along the lobes (2) / T-shaped (3)
C5	The number of abaxial sclerenchyma strands: 5–14 (0) / 17–21 (1) / 24–28 (2)
C6	Depth of adaxial furrows in comparison to the leaf thickness: Medium furrows; quarter to one half the leaf thickness (0) / Deep furrows; more than one half the leaf thickness (1)
C7	Density of macro hairs: few and scattered (0) / dense (1)

C8	Abaxial ribs: absent (0) / slightly lobed (1) / distinct 14–21 (2)
C9	The number of adaxial ribs: 6–9 (0) / 12–16 (1) / 17–20 (2)
C10	Abaxial sclerenchyma strands as: very small or small strands (0) / big strands like a cap (1) / with well-developed girder (2)
C11	The number of vascular bundles: 6–10 (0) / 11–18(1)
C12	The connection of midrib with epidermal layer: midrib not connected (0) / midrib connected with abaxial side (1) / midrib connected to both sides (2)
C13	The size of epidermal cells: up to 12.5 μm long (0) / up to 17.99 μm long (1) / up to 24 μm long (2)
C14	The size of bulliform cells: not distinct (0) / 16–26 μm (1) / 35–45 μm (2)

Tab. 3. Evaluation, measurements and scoring of the characters used in numerical analyses according to the tiller leaf anatomical features of *Alopecurus* species studied.

Leaf anatomical characters	*A. aequalis*	*A. arundinaceus*	*A. vaginatus*	*A. textilis*	*A. gerardii*
The shape of the tiller leaves in cross-section (C1)	Flat (0)	Flat (0)	U- to O-shaped (1)	U-shaped (1)	U-shaped (1)
The midrib larger than the laterals or not (C2)	Not larger than the laterals (0)	Larger than the laterals (1)	Larger than the laterals (1)	Larger than the laterals (1)	Larger than the laterals (1)
Papillae on the epidermal cells (C3)	Thin-walled wide papillae scattered throughout the epidermis (2)	Outer walls of epidermal cells arched but not papillose (0)	Entire or major part of epidermis composed of thin-walled wide papillae (1)	Outer walls of epidermal cells arched but not papillose (0)	Entire or major part of epidermis composed of thin-walled wide papillae (1)
Arrangement of adaxial sclerenchyma strands (C4)	Regular groups at the level of the vascular bundles (1)	Regular groups at the level of the vascular bundles (1)	Scattered few groups (0)	Extended along the lobes (2)	Scattered few groups (0)
The number of abaxial sclerenchyma strands (C5)	24-26 (2)	24-28 (2)	5–7 (0)	17–18 (1)	9–11 (0)
Depth of adaxial furrows in comparison to the leaf thickness (C6)	Deep furrows (1)	Medium furrows (0)	Medium furrows (0)	Medium furrows (0)	Medium furrows (0)
Density of macro-hairs (C7)	Few and scattered (0)	Few and scattered (0)	Few and scattered (0)	Few and scattered (0)	Few and scattered (0)
The number of abaxial ribs (C8)	Slightly lobed (1)	Absent (0)	Absent (0)	16–19 (2)	Absent (0)
The number of adaxial ribs	15–20 (2)	15–16 (1)	6–8 (0)	7–9 (0)	6–7 (0)

(C9)					
Abaxial sclerenchyma strands (C10)	Very small or small strands (0)	With well-developed girder (2)	Very small or small strands (0)	Big strands like a cap (1)	Very small or small strands (0)
The number of vascular bundles (C11)	12–20 (1)	17–20(1)	7–8 (0)	7–10 (0)	6–7 (0)
The connection of midrib with epidermal layers (C12)	Midrib not connected (0)	Midrib connected to both sides (2)	Midrib not connected (0)	Midrib connected with abaxial side (1)	Midrib not connected (0)
The size of epidermal cells (μm × μm) (C13)	11.24–14.79 × 8.57–8.87 (1)	15.31–20.33 × 8.67–10.11 (2)	9.36–17.57 × 7.57–12.42 (1)	10.11–11.90× 5.65–8.33 (0)	11.51–17.87 × 7.87–9.39 (0)
The size of bulliform cells (μm × μm) (C14)	Not distinct (0)	40.46–44.79 × 15.02–26.58 (2)	19.09–20.90 × 8.48–10.28 (1)	16.07–22.6× 9.31–16.45 (1)	16.36–19.39 × 12.12–15.75 (1)

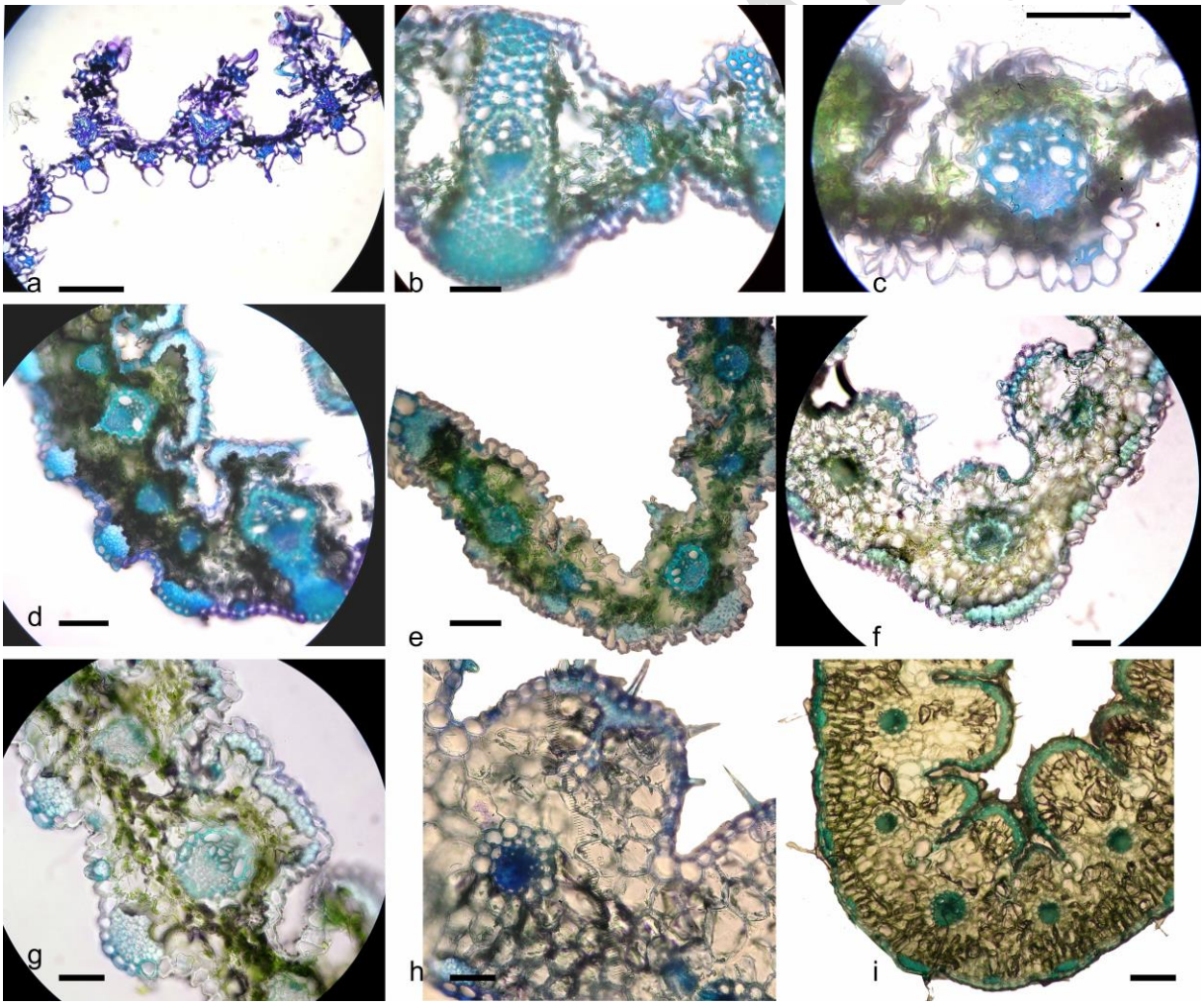

Fig. 1. Tiller leaf cross-sections of *Alopecurus* taxa studied. a – *A. aequalis* (from *C. Aykurt 3397*), b – *A. arundinaceus* (from *C. Aykurt 5345*), c – *A. vaginatus* (from *C. Aykurt 4940a*); d –: *A. textilis* subsp. *textilis* (from *C. Aykurt 3224*), e – *A. gerardii* var. *gerardii* (from *C. Aykurt 5514*), f – *A. goekyigitiana* (from *C. Aykurt 5327*), g – *A. aucheri* (from *L.Y. Konuralp*); h – *A. davisii* (from *A. Aksoy 744*), i – *A. lanatus* (from *C. Aykurt 5624*). Scale bars: 50 μm.

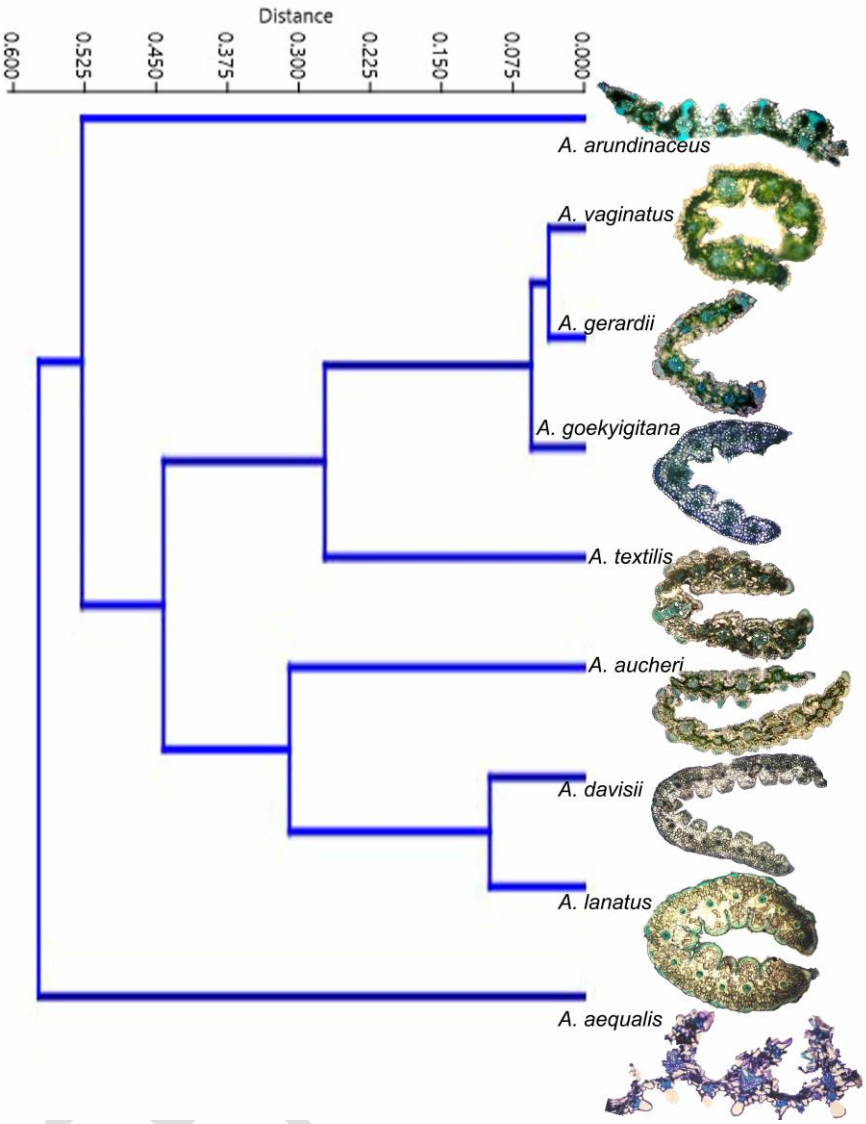

Fig. 2. UPGMA dendrogram created according to the Gower similarity index by using anatomical character matrix of *Alopecurus* species studied.

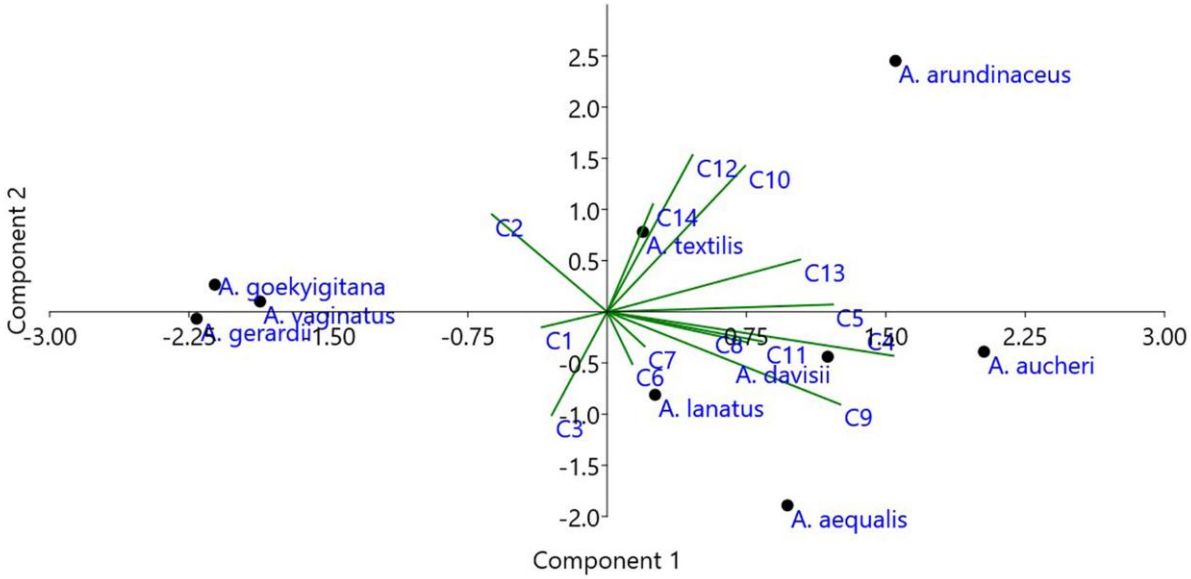

Fig. 3. PCA biplot scatter diagram of studied *Alopecurus* species as OTUs along PC1 and PC2 axes based on 14 leaf anatomical characters.