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Biological Doctoral School

Leaf anatomical diversity of broad-leaved fescue (*Festuca* L.) taxa

PhD Thesis

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1. Introduction and Aims

The grass family (*Poaceae*) is one of the largest (ca. 10,000 species) and most diverse plant families among angiosperms. Grasses are useful in many different areas of life: they are major species in grasslands, meadows and pastures, backyards, parks, sport fields and roadsides, as well as essential food sources for several wild animals. Grasses are highly successful ecologically, in biomes such as steppes, savannahs, prairies and pampas they are the most dominant plant species, maintaining a whole food chain. Cereals (e.g. wheat, barley) are also members of the grass family, comprising one of our most important food even today. Under continuously changing environmental conditions, maintaining the species richness and diversity of grasses poses an important challenge for agriculture, rural development, environmental protection and the whole mankind.

The genus *Festuca* L. is among the largest ones within the grass family (*Poaceae, Pooideae, Loliineae*), comprising more than 450 species (CLAYTON & RENVOIZE 1986), which are dominant taxa in temperate zone grasslands (rocky grasslands, xerophilous and mezo-xerophilous grasslands, loess steppes, sedge meadows, wet meadows, hay meadows etc.). The word "Festuca" is of Latin origin, meaning weed, weedy grass, however, due to their significant economic value, the species of the fescue genus are not treated as weeds, rather as plants of high biological-ecological importance.

From broad-leaved *Festuca* L. taxa the Central European populations of *Festuca pratensis* agg. and related taxa are of particular interest as genetic reserve materials, since they occur not only as spontaneous flora elements, but serve as breeding material for certain local cultivars and cultivated varieties. Grass breeding programs resulted in marketing nearly hundred cultivars of high value. Their economic importance is supported by the fact that these *Festuca* taxa form the second largest group among cultivated grasses worldwide and also in Central European countries.

Although the genus *Festuca* as a whole has been thoroughly investigated from anatomical and phylogenetic aspects, there are few data available on the leaf anatomy (and molecular biology) of natural populations of broad-leaved taxa in Central Europe. Our leaf anatomical (micromorphological and micromorphometric) investigations and preliminary molecular biological analyses intend to make up for these shortcomings. Our research into the natural populations of Central European broad-leaved fescue (*Festuca* L.) taxa aimed at answering the following questions:

1. Which qualitative and quantitative leaf anatomical traits are characteristic to the taxa of broad-leaved genera ***Schedonorus*** and ***Drymanthele***?

2. Which leaf anatomical characters reflect relatedness of investigated taxa?

3. Which leaf anatomical traits can differentiate between taxa and microtaxa?

4. What is the degree and character of leaf anatomical diversity among taxa and populations?

5. Is there a relationship between the anatomical characters of taxa investigated and their geographical origin?

6. Is there a relationship between anatomical and genetic diversity, based on our preliminary RAPD analysis?

7. Which populations are the most suitable for breeding programs based on their anatomical traits?

2. Materials and Methods

2.1. Investigated taxa and populations

Our investigations focused on broad-leaved *Festuca* taxa with diverse chromosome numbers, which occur in the natural vegetation of Central Europe, particularly in the Carpathian Basin. Samples were collected from the Eastern Carpathian Mountains to the Eastern Alps (Dolomites). Field observations and samplings were carried out from 2005 to 2011. The following were taken into consideration when selecting sampling sites: samples should be collected from natural habitats, different geographical areas and different types of habitats in the case of the same taxon. The 49 populations investigated represent 7 taxa of broad-leaved taxa in Central Europe (subgenus ***Drymanthele***: ***F. altissima***: Perőcsény-40/7, Hörmann-forrás-40/8, Ausztria-40/9; ***F. drymeja***: Sepsikőröspatak-21, Sepsibodok-22*, Szencsed-23*, Harangmező-47, Óház tető-43, Hörmann forrás-45, Hétforrás-46; subgenus ***Schedonorus***: section ***Schedonorus***: ***F. pratensis*** **subsp.** ***pratensis***: Zalaszántó-12*, Alsóverecke-14, Gyergyószentmiklós-16*, Kalibáskő-17, Veresvíz-19*, Lemhény-20*, Koloska-26*, Rugonfalva-29, Mezőpagocsa-27; ***F. pratensis*** **subsp.** ***apennina***: Sesto-2*, Sesto-3*, Comelico-5*, Croce-6*, Cortina d'Ampezzo-7*, Falzarego-9, Andraz-10, Arabba-11*, Borsa-18*; ***F. arundinacea*** **subsp.** ***arundinacea***: Koloska-13, Cegléd-25*, Bedellő-24*, Rugonfalva-28, Sesto-1, Cortina d'Ampezzo-7a*, Kede-31, Lukácsháza-33; ***F. arundinacea*** **subsp.** ***orientalis:*** Darvas-35, Mezőzáh-36, Mezőpagocsa-15*; section ***Plantynia*****:** ***F. gigantea***: Kápolnásfalu-81, Homoródalmás-82, Décsfalva-83, Havasgáld-84, Jádremete-85, Királykút-86, Nagy-hideg hegy-87, Hétforrás-88, Hörmann-forrás-89, Vasvár-90). The populations investigated were collected from altogether 41 study sites and 26 habitat types. Some individuals of the populations investigated were secured as herbarium material, and representatives of each population are being maintained in an experimental garden.

Anatomical studies and scanning electron microscopic investigations were conducted at the Faculty of Science, University of West Hungary; while molecular biological (RAPD-PCR) analyses were carried out at the Genetic Laboratory of the Hungarian Forest Research Institute, Sárvár Experimental Station.

Our molecular biological investigations do not cover the whole array of populations, since these analyses were intended as preliminary studies to complete the anatomical investigations. Our goal was to determine if the RAPD method is suitable for distinguishing between taxa and populations, and to gain insight into the possible relationship between anatomical and genetic diversity. The selected RAPD technique requires high purity, precision and the application of several primers, which would have meant substantially increased number of samples and time devoted to these experiments. Therefore, RAPD analyses were conducted only on 18 populations of 5 taxa (marked with *). Since *F. drymeja* from the subgenus *Drymanthele* separates well also genetically from the taxa in subgenus *Schedonorus* (HAND et al. 2010), this species was used as outgroup in the RAPD analysis of populations from subgenus *Schedonorus*.

2.2. Sampling for anatomical, scanning electron microscopic and molecular biological studies

For anatomical investigations the flag leaves were selected. Samples were taken from the widest middle part of healthy, fully developed leaves. Samples were fixed in 70% ethanol, and after 6-12 h placed into Strasburger-Flemming preservation solution (96% ethanol : 99.5% glycerol : distilled water 1:1:1) .

The scanning electron microscope used in our investigations allowed processing the same samples.

For RAPD-PCR young leaves were collected from 5 to 10 individuals per sampling site, which were kept frozen until further processing (HAJÓSNÉ 1999).

2.3. Anatomical investigations

Anatomical studies included the micromorphological and micromorphometric analysis of leaf transverse sections, as well as the investigation of both adaxial and abaxial leaf epidermis.

Leaf transverse sections were prepared manually with a razor blade. Sections were cleared, stained, dehydrated, and finally mounted in Canada balsam (SÁRKÁNY & SZALAY 1964, MIHALIK et al. 1999).

Twelve characters, discussed as prominent traits in the genus *Festuca* already by METCALFE (1960) and ELLIS (1976, 1979), were investigated in leaf transverse sections: the character of leaf ribs; the number of ribs (veins); structure of the mesophyll; the presence,

distribution and amount (μm^2/ transverse section) of sclerenchyma; the number of vascular bundles in the main rib; the type of the bundle sheath; the type and size of bulliform cells; the features of the leaf edge; the type of stomatal complexes (mesomorphic or xeromorphic); presence or absence of cuticle; presence of papillae.

In order to study the epidermis, epidermal peels were prepared with simple scraping technique, which yielded large surface of intact epidermis (mostly in the whole width of the leaf) in a short time. Similarly to leaf transverse sections, epidermal peels were mounted in Canada balsam, following steps of clearing, staining and dehydrating (SÁRKÁNY & SZALAY 1964, MIHALIK et al. 1999). The structure of the epidermis was studied following the suggestions of METCALFE (1960) and ELLIS (1976, 1979), regarding the diagnostic characters of epidermis in grasses. The following epidermal features were analysed both on the adaxial and abaxial surfaces, in the costal and intercostal zones:

a) Costal zone: width (number of cell rows) of costal zones; length (μm), width (μm) and outline of anticlinal walls of costal cells; frequency (number per mm^2) and shape of short cells (silica cell, cork cell); size of silica cells (length/width ratio);

b) Intercostal zone: width (number of cell rows) of intercostal zone; length (μm), width (μm), shape and outline of anticlinal walls of cells adjacent to costal zone; frequency (number per mm^2) and shape of short cells (silica cell, cork cell); length (μm), width (μm) and outline of anticlinal walls of cells between stomatal complexes; number of rows with stomatal complexes; type of stoma subsidiary cells; length (μm), width (μm) and frequency (number per mm^2) of stomatal complexes.

Preparations were investigated with NIKON LABOPHOT-2A type microscope, and digital images were taken with Nikon D3550 type camera. Micromorphometric analysis was done with Olympus DP-Soft 3.2 image analyser software.

The fine structure of the epidermal surface was investigated with scanning electron microscopy, using a TM-3000 type instrument. Samples were fixed in 70% ethanol and stored in 96% ethanol : glycerol : distilled water (1:1:1). Good quality micrographs could be taken with the scanning electron microscope specified above without gold sputter coating. Micrographs at 100 and 200 times magnification were taken on both the adaxial and abaxial surfaces of the central and peripheral part of the leaf (SNOW 1996, MIHALIK et al. 1999). The following characters were studied on both the adaxial and abaxial side of the epidermis: type, length (μm), width (μm) and frequency (number per mm^2) of trichomes.

2.4. RAPD - PCR analysis

DNA extraction, agarose gel electrophoresis etc. were performed with standard methods, and according to the manufacturer's protocol.

In order to select the most variable primers which at the same time provided stable patterns, 10 primers form the OPERON series (Eurofins MWG Operon, http://www.operon.com/) were screened: **A1, A2, A8, A10, B11, D5, E9, H2, N6, P3**. Screening was done with 5 samples for each primer. The optimized PCR mastermix comprised the following components for 15 µl final reaction volume: 5x buffer (Promega GoTaq Flexi) 2 µl; $MgCl_2$ 2,5 mM 0.6 µl; primer 10 pM 1.0 µl; dNTPmix (Promega 10 mM) 0.1 µl; polymerase enzyme (PromegaGoTaq Flexi) 0.4 Unit; DNA sample 1.2 µl (approx. 10 ng/µl). PCR reactions were performed in an Eppendorf Mastercycler Gradient thermocycler.

Fragments were separated with agarose gel electrophoresis, using 1.75% agarose gel (Roti®agarose NEEO, Roth GmbH), 1x TAE buffer, 120 V voltage for 3 h in a Sigma-Aldrich Midi tank. Fragment size was determined with the help of 100-5000 bp standard (100bp DNA Ladder, Roth GmbH). Gels were stained with GelRed (Biotium Inc.), then digital photos were taken under UV.

Further analysis was done with four primers (**A8, H2, E9, P3**) selected with the method above, on 5 to 10 samples per population.

Digital gel photos (fingerprints) were analysed with Kodak 1D software, applying a binary code for further genetic analysis.

2.5. Statistical analysis

Analysis of micromorphometric data was done with Past statistical software version 2.17b (HAMMER et al. 2001). The log-transformed micromorphometric data were compared with One-way-ANOVA with Tukey's pairwise comparisons. If the normality assumption was violated, we applied Kruskal-Wallis test with Mann-Whitney pairwise comparisons. The normality of data series was checked by using Shapiro-Wilk test.

Cluster analysis of populations based on the frequency and size of silica cells was done with the Ward-method. Correlation analysis was used for revealing the relationship between bulliform cells and the amount of sclerenchyma. For comparison of populations based on micromorphometric characters, multidimensional scaling was applied (Principal Coordinates Analysis, PCoA – Canberra similarity coefficient), using the software package SYNTAX (PODANI 2000).

3. Results and Discussion

The investigation of **leaf transverse sections** of broad-leaved *Festuca* taxa from the genera *Schedonorus* and *Drymanthele* revealed that each taxon is characterised by a heterogeneous **mesophyll**, and palisade parenchyma may occur next to spongy parenchyma both under the adaxial and abaxial epidermis. Palisade parenchyma can be observed under the abaxial epidermis in each taxon, however, under the adaxial epidermis it is characteristic only in some populations of *F. pratensis* subsp. *pratensis* and *F. pratensis* subsp. *apennina*. The appearance of palisade parenchyma is not uniform in each population, being more pronounced under the adaxial and abaxial parenchyma in populations 14, 16, 17 and 26 of *F. pratensis* subsp. *pratensis*, as well as in populations 5 and 11 of *F. pratensis* subsp. *apennina*. Such a mesophyll structure can be considered as an advantageous feature when using populations as genetic reserve materials.

Besides the subepidermal palisade parenchyma, some populations featured radially arranged, palisade-like cells around the vascular bundles. Such radially arranged cells have not been described previously in C_3 species, but our observations confirmed that they are unequivocally present in some populations of *F. pratensis* subsp. *pratensis* and *F. pratensis* subsp. *apennina*. In *F. arundinacea* populations these radially arranged palisade-like cells occur less frequently (only around a few bundles), whereas in *F. gigantea* and the species of subgenus *Drymanthele* (*F. altissima*, *F. drymeja*) they are not characteristic at all. The above two characters are in close relationship, i.e. palisade parenchyma under the adaxial epidermis occurs together with radially arranged palisade-like cells around the vascular bundles.

The mesophyll structure of subgenus *Schedonorus* and *Drymanthele* differs significantly, with regularly arranged *flat cells* bordering the bulliform cells from the mesophyll side in the species of subgenus *Drymanthele* (*F. altissima*, *F. drymeja*), while these flat cells cannot be observed in the species of subgenus *Schedonorus*. Such flat cells have not been reported previously, although this structure can be treated as one of the differentiating characters between the two subgenera.

The leaves of taxa in subgenus *Schedonorus* are **ribbed**, while the leaves of subgenus *Drymanthele* are rather slightly ribbed. The central vein comprises usually a single **vascular bundle**, only *F. gigantea* can be characterised by 3 (4-5) bundles. In the taxa investigated the vascular bundles are surrounded by double bundle sheath, the inner sclerenchymatic being contiguous, whereas the outer parenchymatic sheath can be contiguous or broken abaxially in the taxa of subgenus *Schedonorus*. In subgenus *Drymanthele* the parenchymatic bundle sheath is broken both adaxially and abaxially.

The presence and amount of **sclerenchyma** in the leaf is an important anatomical character and contributes to the good quality of leaf. Knowing the quantity of sclerenchyma is essential for breeding purposes, as well. The amount of sclerenchyma can be influenced by ecological factors such as drought and moisture. In the populations investigated no relationship was detected between the habitat and the amount of sclerenchyma. The largest amounts of sclerenchyma were detected in the populations of *F. arundinacea* subsp. *arundinacea* and *F. arundinacea* subsp. *orientalis*. When determining the quantity of sclerenchyma we suggest taking into account not only the number of sclerenchymatous ribs and bundles, but also the presence of so-called **colourless cells**, because these cells with non-sclerenchymatised cell walls can also contribute to the formation of seemingly wide, well-developed bundles of sclerenchyma (e.g. *F. arundinacea* or *F. gigantea* taxa).

In the populations of *F. arundinacea* and *F. drymeja* strong correlation was found between the size (length, width) of **bulliform cells** and the amount of sclerenchyma (μm^2/leaf transverse section): for the length of bulliform cells R^2=0.6624 and R^2=0.9342, for the width of bulliform cells R^2 =0.7171 and R^2 =0.9477 in populations of *F. arundinacea* and *F. drymeja*, respectively.

● Our investigation of the **epidermis** confirmed that – in contrast with some earlier data (METCALFE 1960, WATSON & DALLWITZ 1992) – it is equally important to study not only the abaxial, but also the adaxial epidermis. Several characters that are suitable for differentiating between taxa can be observed on the adaxial epidermis, such as the frequency, shape and size of silica cells in the costal zone.

Our detailed micromorphological and micromorphometric analyses of the adaxial and abaxial epidermis revealed that the taxa investigated can be differentiated based on both qualitative (the presence or absence, the appearance and shape of various long cells and short cells) and quantitative (size and frequency of given cell types) characters, and the structure of the adaxial and abaxial epidermis differs significantly, too. Regarding the quantitative traits, the variability of taxa and populations can be observed in each case. Several of the taxonomically and anatomically important characters (e.g. the shape and type of silica bodies, the type of stoma subsidiary cells) show high diversity in various populations.

Concerning the structure of the costal zone, the highest variability among taxa and populations was found in the appearance (alone or in pairs with cork cells), shape and size of **silica cells**. The silica cells are suitable for differentiating not only the two subgenera, but also the taxa within each subgenus. The taxa can be easily distinguished by taking into consideration the appearance, shape and size of silica cells.

Regarding the **intercostal zones** we concluded that the number of cell rows adjacent to the costal zone lacking stomatal complexes, the shape and the outline of the anticlinal cell

walls can differentiate taxa within subgenus *Schedonorus* and can be used also to distinguish taxa from the members of subgenus *Drymanthele*.

The adaxial epidermis of the leaves is covered with **trichomes** in each taxon, while it is less characteristic for the abaxial epidermis. Confirming the earlier observations of BURR & TURNER (1933) (cit. in METCALFE 1960), but contrasting those of METCALFE (1960), we found that the trichomes of the taxa investigated are the so-called prickles. In the taxa of subgenus *Schedonorus* two types of prickles can be observed, the "robust" and the "slender" type, whereas in subgenus *Drymanthele* only the robust type prickles are present. The frequency of trichomes shows great diversity in different populations of the same taxon. Taxa can be differentiated from each other by the length of the prickles in the costal zone of the adaxial epidermis.

There are only few reports on **papillae** in the genus *Festuca*. In contrast to some earlier data (METCALFE 1960, WATSON & DALLWITZ 1992), and completing the data of others (CONERT 1998, ZARINKAMAR & JOUYANDEH 2011), we observed that papillae were present on both the adaxial and abaxial surface in *F. pratensis* taxa, mostly on the adaxial surface in *F. arundinacea* and *F. gigantea*, and they were absent from the taxa *F. drymeja* and *F. altissima*.

● Regarding the occurrence of **stomata**, the leaves of *F. pratensis* and *F. arundinacea* were found to represent the amfistomatic type, while the leaves of *F. gigantea* and the species of subgenus *Drymanthele* (*F. altissima, F. drymeja*) belong rather to the epistomatic type. Earlier reports (METCALFE 1960, NYAKAS 2003) described the leaves of *F. pratensis* and *F. arundinacea* as epistomatic. The type of stomata (mesomorphic or xeromorphic) is also a differentiating character between the two subgenera, being xeromorphic on the adaxial and mesomorphic on the abaxial epidermis in subgenus *Schedonorus*, while both epidermal surfaces bear mesomorphic stomata in subgenus *Drymanthele*.

The subsidiary cells of **stomatal complexes** show huge diversity both for taxa and populations. Our studies contribute to earlier monographic data (METCALFE 1960, WATSON & DALLWITZ 1992) with pointing out that the genus *Festuca* is characterised by parallel, as well as low dome-shaped and high dome-shaped subsidiary cells both on the adaxial and abaxial epidermis. The taxa of subgenus *Schedonorus* and subgenus *Drymanthele* can be distinguished from each other by the frequency of stomatal complexes on the adaxial epidermis. Stomata in the taxa of subgenus *Drymanthele* are significantly longer (196-358 μm) compared to the stomata in subgenus *Schedonorus* (24-193 μm).

- **Possible applications of results**

From the species and populations investigated the following **genetic resource materials** were found to have the most advantageous anatomical traits:

- *F. pratensis* subsp. *pratensis*: populations Kalibáskő-17, Veresvíz-19, Mezőpagocsa-27 – the presence of palisade parenchyma is pronounced, there is less sclerenchyma
- *F. pratensis* subsp. *apennina:* populations Sesto-2, Borsa-18 – pronounced palisade parenchyma, less sclerenchyma, fewer silica cells. Population Arabba-11– very pronounced palisade parenchyma (both on adaxial and abaxial side) and less sclerenchyma.
- *F. arundinacea* subsp. *arundinacea*: populations Cegléd-25, Cortina d'Ampezzo-7 – presence of palisade parenchyma, less sclerenchyma and fewer silica cells in the costal zones.
- *F. arundinacea* subsp. *orientalis*: population Mezőzáh-36 – presence of palisade parenchyma and little sclerenchyma.
- *F. gigantea*: population Kápolnásfalu-81 – presence of palisade parenchyma and little sclerenchyma.
- *F. altissima*: populations Hörmann-40/8, Ausztria 40/7 – pronounced palisade parenchyma and fewer silica bodies (in costal zones).
- *F. drymeja*: population Hörmann-45 – presence of palisade parenchyma and little sclerenchyma.

The molecular biological studies revealed that – similarly to earlier data (CHEN et al. 1998, FJELLHEIM & ROGNLI 2005, LISZTES-SZABÓ et al. 2009) – the genetic polymorphism of the taxa investigated is high (86-92%), particularly in *F. pratensis* microtaxa. The RAPD-PCR analyses allowed genetic differentiation between taxa, in accordance with previous reports (CATALÁN et al. 2004, 2007), and the taxa could be distinguished at the molecular level.

The taxa investigated segregated also at the population level: subgenus ***Schedonorus*** and subgenus ***Drymanthele*** separated at higher genetic distance (11%), while the populations of *F. pratensis* and *F. arundinacea*, being the taxa within subgenus ***Schedonorus*** section ***Schedonorus*** with different chromosome numbers, segregated at lower genetic dissimilarity (3.5%). The populations of the taxonomically related diploid *F. pratensis* subsp. *pratensis* and tetraploid *F. pratensis* subsp. *apennina* mikrotaxa could not be differentiated genetically at the population level. When analysing the relationship of genetic and anatomical distance, the frequency and length/width ratio of **silica bodies** in the costal zone of the adaxial epidermis was found to correlate with the genetic distance of the populations.

The results of these preliminary studies and previous reports emphasizing the differentiating role of silica bodies (NAMAGANDA et al. 2008, ORTÚÑEZ & DE LA FUENTE 2010) led us to the conclusion that the frequency and size of silica cells in the costal zones of the adaxial epidermis are genetically determined, constant features of the epidermis, which are less influenced by changing environmental conditions. This observation necessitates the more detailed investigation of the relationship between anatomical characters of silica bodies and their molecular biological background.

Our results confirmed earlier reports (NYAKAS 2003), according to which the amount of sclerenchyma is strongly influenced by environmental factors. No correlation was found between the genetic distance of the populations and the quantity of sclerenchymatous tissue. However, comparison of the cluster diagrams based on the amount of sclerenchyma and dendrograms of genetic distance between the taxa *F. pratensis* subsp. *pratensis* and *F. arundinacea* suggests that lower amounts of sclerenchyma in *F. pratensis* and higher amounts of this tissue in *F. arundinacea* might be genetically determined to some extent.

High genetic polymorphism (P=76-91%) of the populations was accompanied by significant anatomical diversity. The statistical analysis of the dissimilarity of populations (PCoA method, Jaccard coefficient) based on highly variable quantitative (micromorphometric) characters revealed that the populations investigated segregated according to their taxonomic classification (even at the subgenus level) and their genetic distance.

4. Short Summary of Novel Results

- The thesis provides the first in-depth description of the leaf anatomical (micromorphological and micromorphometric) traits in Central European fescue taxa from the genus *Festuca*, subgenera *Schedonorus* and *Drymanthele*, including the analysis of both leaf transverse sections and leaf epidermis.
- In subgenera *Schedonorus* and *Drymanthele* the occurrence of palisade parenchyma under the abaxial epidermis can be regularly observed, and its appearance, contiguous or broken character is a taxonomically differentiating feature in broad-leaved fescue taxa.
- In *F. p.* subsp. *pratensis* and *F. p.* subsp. *apennina* there are radially arranged palisade-like cells around the vascular bundles. The occurrence of these cells shows strong correlation with the presence of palisade parenchyma under the adaxial epidermis.
- The taxa in subgenus *Drymanthele* can be distinguished from the taxa in subgenus *Schedonorus* by regularly arranged *flat cells* bordering the bulliform cells from the mesophyll side.
- The amfistomatic or epistomatic character of the leaf was used for the first time as a differentiating character between the subgenera *Schedonorus* and *Drymanthele*. The position of stoma guard cells related to epidermal cells was analysed for the first time in broad-leaved taxa: in subgenus *Schedonorus* stomata are xeromorphic on the adaxial side

and mesomorphic on the abaxial side; while in subgenus *Drymanthele* they are mesomorphic both on the adaxial and abaxial side.

- In *F. arundinacea* and *F. drymeja* there is strong positive correlation between the size (length, width) of bulliform cells and the amount of sclerenchyma.
- The presence of papillae was confirmed on both the adaxial and abaxial epidermis in *F. pratensis*, only on the adaxial epidermis in *F. arundinacea* and *F. gigantea*; while they are missing in *F. drymeja* and *F. altissima*.
- The length of long cells in the costal zone differentiates the species *F. gigantea* from other members in subgenus *Schedonorus* and from subgenus *Drymanthele*.
- The frequency and width of stomatal complexes on the adaxial epidermis was confirmed to be a differentiating character within subgenus *Schedonorus*, and between the subgenera *Schedonorus* and *Drymanthele*.
- In broad-leaved taxa trichomes on the adaxial epidermis were found to be most abundant in *F. p.* subsp. *apennina*.
- The features of silica cells (appearance, shape, size) in the costal zones of the epidermis can differentiate between subgenera *Schedonorus* and *Drymanthele*, as well as between taxa within each subgenus.
- The genetic distance of populations was found to be related to the frequency and size of silica cells in the costal zones of the epidermis, however, further studies are needed to confirm this observation.
- The following populations are suggested for use as genetic resources in breeding programs, based on their advantageous anatomical features (presence of palisade parenchyma, less sclerenchyma): *F. p.* subsp. *pratensis*: Kalibáskő-17, Veresvíz-19, Mezőpagocsa-27; *F. p.* subsp. *apennina:* Sesto-2, Borsa-18, Arabba-11; *F. arundinacea* subsp. *arundinacea*: Cegléd-25, Cortina d'Ampezzo-7; *F. a.* subsp. *orientalis*: Mezőzáh-36; *F. gigantea*: Kápolnásfalu-81; *F. altissima*: Hörmann-40/8, Ausztria 40/7; *F. drymeja*: Hörmann-45.

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