Volume 55(2):237-241, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Leaf epidermal investigations on *Festuca pratensis* Huds. subspecies

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ABSTRACT This work deals with the examination of the leaf epidermal anatomy of two subspecies of Meadow fescue: *Festuca pratensis* Huds.: subsp. *pratensis* and subsp. *apennina*. As a result of our studies carried out on Central European natural populations of different origin, it can be concluded that in the case of both microtaxa, in all the examined populations the upper and lower epidermis exhibits structural differences. The variation between the populations manifest in the differences in dimensions of certain cells of the epidermis, in the size and amount of silica cells, hairiness, and the size of the bristles. The difference of the two subspecies on the basis of epidermal structure is the length of stoma complexes, the number and dimension of bristles as well as the length of the epidermis cells. **Acta Biol Szeged 55(2):237-241 (2011)**

KEY WORDS

leaf epidermis
Festuca pratensis subsp. *pratensis*
F. pratensis subsp. *apennina*

The broad-leaved forage grass taxa from the temperate zones belong to the section *Bovinae* Fries. ap. Anders of the genus *Festuca* L. The populations and subspecies of *Festuca pratensis* Huds. (Meadow fescue) deserve special attention as genetic reserve materials (Borill et al.1977; Tyler 1988, Chapmann 1996). The diversity of populations of Meadow fescue is of scientific and economic importance, not only because they are constituents of the natural flora, but as germplasm sources or breeding materials for useful cultivars and biotypes as well (Kovács 1994).

In the line of natural biological materials, the genetically and ecologically stable subspecies (microtaxa) of Meadow fescue are the most important: *Festuca pratensis* Huds. subsp. *pratensis* and *F. pratensis* Huds. subsp. *apennina* (De Not) Hegi [Syn.: *Festuca apennina* Notaris, *F. pratensis* subsp. *apennina* (Notaris) Hackel ex Hegi]. The presence and separation of the two subspecies in Central Europe is taxonomically accepted (Markgraf-Dannenberg 1980). The Meadow fescue subspecies (microtaxa) differ morphologically mainly in the openness of sheaths, the width of the leaf lamina, the structure of the lemma, the presence or absence of teeth and arista (Aiken et al. 1991; Connert 1994; Ciocârlan 2009). The populations of the two subspecies show significant variations in chromosome number. Populations belonging to the basic type (*F. pratensis* subsp. *pratensis*) are usually diploids (2n = 14). They prefer the lower hilly collin-submontane landscapes. The other subspecies (*F. pratensis* subsp. *apennina*) has mostly tetraploid (2n = 28), rarely aneuploid (2n = 21, 25) populations, and usually exist in mountain and sub-alpine habitats (the Carpathians, the Alps, Apennines) (Borill et al. 1977; Kovács 1982; Tyler 1988). At the same time the populations' gene source research and plant anatomy studies concerns mostly the basic type (Toma et al. 1982; Kovács and Dani 1999). Major leaf anatomical and biological studies were published also regarding the related species of *F. arundinacea* populations (Cenci et al. 1990; Ueyama 1992; Gibson and Newman 1997).

As a continuation of our previous research (Kovács and Dani 1999; Dani and Kovács 2007), the aim of the present work is to study further populations, with particular regard to the finer details of the leaf epidermal structures (costal and intercostal cell rows, stoma complexes, trichomas, etc.), constant and changing properties and characteristics.

Materials and Methods

The individuals of the populations of the two subspecies (microtaxa) were collected in the years 2008 and 2010 during flowering period. The sample specimens of the populations were collected within the natural vegetation units of Central Europe (Eastern Alpes-Dolomites) and in the area of the Carpathian Mountains, usually 325-1600 m asl. (*F. pratensis* subsp. *apennina:* populations No.: 32, 33, 35, 36, 37, 39, 40, 41, 48 and *F. pratensis* subsp. *pratensis* No.: 42, 44, 46, 47, 49, 50, 56 (Table 1). The sampling was made from of the middle part of the flag leaf. The collected material was fixed in 70% alcohol and stored in a Flemming and Strasburger's (alcohol/glycerol/water, ratio = 1:1:1) preservative mixture. The epidermic peels for light microscope examinations of the epidermal tissue were prepared by manual technique, dyed with Erlich's acid haematoxylin stain solution and, after dehydration, were covered with Canada balsam (Metcalfe 1960; Mihalik et al. 1999; Rudall 2004). The epidermis peels were

Accepted Sept 17, 2011
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Table 1. The geographical origin of the samples and the habitat characteristics.

Taxon name	Population name and number of sample	Altitude above sea level (m)	Habitat-types
Festuca pratensis subsp. *apennina*	Sesto-32	950-1000	Tall herb communities along the streams
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	Beluno-35	1350	Mesophilic mountain meadow
	Croce-36	1600	Marshes/wet meadows
	Cortina d'Ampezzo-37	1300	Marshes/wet meadows
	Falzarego-39	1700	Wet mountain meadow
	Cernadoi-40	1350	Wet mountain meadow
	Arabba-41	1600	Wet mountain meadow
	Borsa-48	1420	Upland marshes
Festuca pratensis subsp. *pratensis*	Zalaszántó-42	340	Wet meadow
	Verecke-44	820	Mountain meadow with red fescue
	Gyergyószentmiklós-46	730	Upland marshes
	Kalibáskő-47	910	Mountain meadow with red fescue
	Veresvíz-49	950	Mountain meadow with red fescue
	Lemhény-50	560	Mesophilic meadow
	Koloska-56	325	Wet meadow

Table 2. The size and the number of trichomes.

Taxon name	Number of sample	The size of trichomes (µm)				The number of trichomes (pcs/mm^2)	
		Adaxial epidermis		Abaxial epidermis			
		length	width	length	width	Adaxial epidermis	Abaxial epidermis
F. *pratensis* subsp. *pratensis*	42	27,21	10,17	33,50	15,35	29	6
	44	32,77	10,86	34,91	16,40	13	5
	46	27,91	11,65	25,49	10,97	8	5
	47	37,26	12,62	0,00	0,00	52	0
	49	34,41	12,98	29,41	13,02	15	8
	50	34,22	14,09	27,66	13,86	10	3
	56	27,77	10,18	27,71	11,69	9	13
F. *pratensis* subsp. *apennina*	32	38,19	13,81	44,99	19,72	51	8
	33	61,73	15,58	0,00	0,00	100	0
	35	38,98	12,50	0,00	0,00	24	0
	36	42,34	13,28	0,00	0,00	60	0
	37	41,47	12,80	49,02	22,88	63	22
	39	42,50	12,68	64,30	21,79	61	39
	40	35,00	10,67	0,00	0,00	29	0
	41	61,91	11,80	0,00	0,00	71	0
	48	61,91	25,52	78,61	22,51	16	22

Table 3. The size and the number of stoma complexes.

Taxon name	Epidermis side	Stomatal complexes length	Stomatal complexes width	The number of stoma complexes per 1mm^2
F. pratensis subsp. *pratensis*	Adaxial epidermis (µm)	24,21 – 37,17	13,14 – 18,20	32-94
	Abaxial epidermis (µm)	31,31 - 35,51	20,77 – 29,53	15-45
F. pratensis subsp. *apennina*	Adaxial epidermis (µm)	25,84 - 47,55	12,99 – 24,22	21-86
	Abaxial epidermis (µm)	32,19 – 49,05	22,01 – 33,28	4-36

examined and photographed with Labophot Nikon 2A optical microscope (Ellis 1976). The histological measurements (costal and intercostal cells' length, width, stoma complexes' length and width, stoma density/1mm^{2,} density of the hair cells/1mm^2) were carried out using an Olympus DP-Soft 3.1 type image processing system. Averages were calculated from

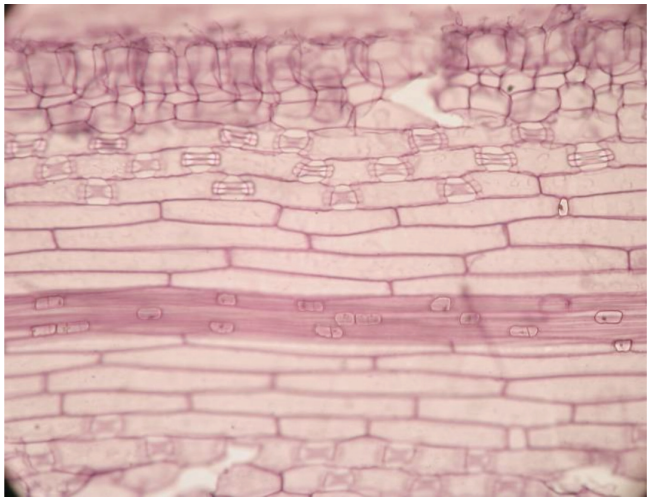

Figure 1. Microscopic photograph of the *F. pratensis* subsp. *pratensis*-46 adaxial epidermis (100x).

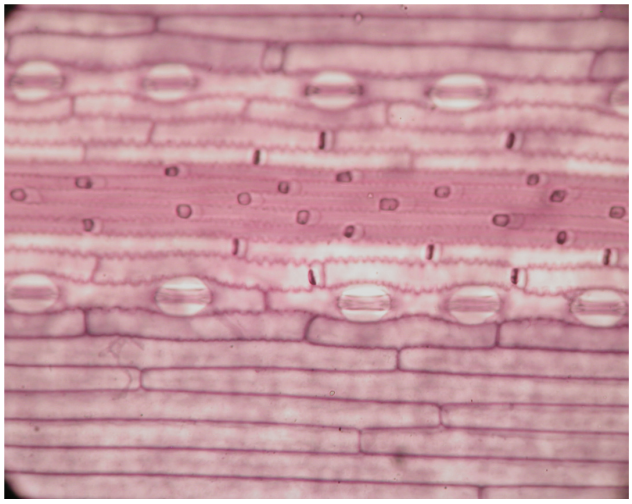

Figure 3. Microscopic photograph of the *F. pratensis* subsp. *pratensis*-48 abaxial epidermis (100x).

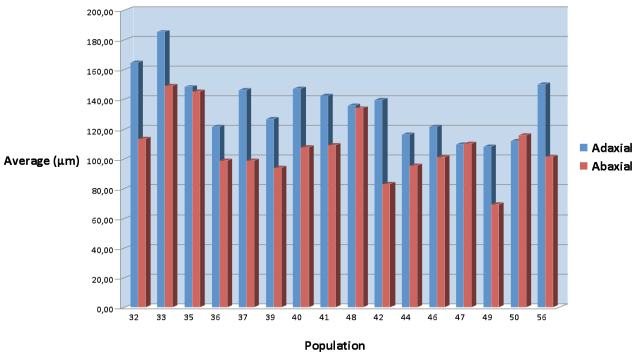

Figure 2. The length of the cells in the costal zone (µm).

50 measurements per population. The dataset made from the numerous photos were analysed with Microsoft Excel. The leaf epidermis' surface structures were examined in images prepared on a Tesla BS 300 scanning electron microscope form fresh, green leaf material.

Results and Discussion

In the examination of the Meadow fescue leaf epidermis, the histological features of the surface of the lamina, the constant and variable features and the characteristics of the epidermal structures were well defined. In each population, in the costal zone of the adaxial (upper) leaf epidermis, there are an average of 2-6 rows of elongated cells (the length being multiple of the width), their radial wall being straight. The cells are on average 108 to 185 µm long and 4.5 to 5, 9 µm wide. Between the long cells there are single silica cells, of mostly rectangular, elongated rectangular shape, rarely square (Fig. 1). The silica cell characteristics (length, width and distribution) will be studied later on.

The costal zone of the abaxial (lower) zone of the epidermis is formed by 3-10 rows of elongated rectangular-shaped cells that in contrast to the adaxial side's costal zone, are wavy (radial walled). Regarding their size, in the case of most populations, they are shorter (Fig. 2) and wider than the costal cells of the adaxial side. The cells are on average 70 to 185 µm long and 5 to -7, 9 µm wide. Compared to the adaxial epidermis, the number of silica cells is greater and they are typically occurring in pairs with phellem cells (Fig. 3).

The intercostal zones of the adaxial epidermis have 15 to 19 cell rows. Directly next to the vein zone, there are 3 to 5 cell rows, of rectangular and even more often having an elongated hexagonal shape, running straight, containing no stoma complexes. The cell lengths are 144-354 µm, the width 10 to 19 µm. Silica cells are rarely found between these cells (The population 36 is an exception to that). After these rows, there are 2 to 4 (rarely 5, e.g. population 50) cell rows of rectangular shape and straight-running, however shorter than in the foregoing (41 to 84 µm long and 9.5 to 16, 5 µm wide) accompany the articular (bulliform) cells on both sides. There are stoma complexes between the cells (Fig. 4.).

The abaxial epidermis, due to the lack of articular cells is more even than the upper epidermis, but the fields above the vein (costal) and between the veins (intercostal) are also well distinguishable. The intercostal zones are composed of 14 to 27 cell rows. Next to the costal zone, an average of 2 or 2 to 3 (rarely 1) cell rows, without stoma complexes, and, unlike the upper side, these cells are strongly wavy and of rectangular shape. The cells are all the same length or longer and in all populations they are unambiguously wider than the cells of the vein zone. Compared to the cells next to the upper side's

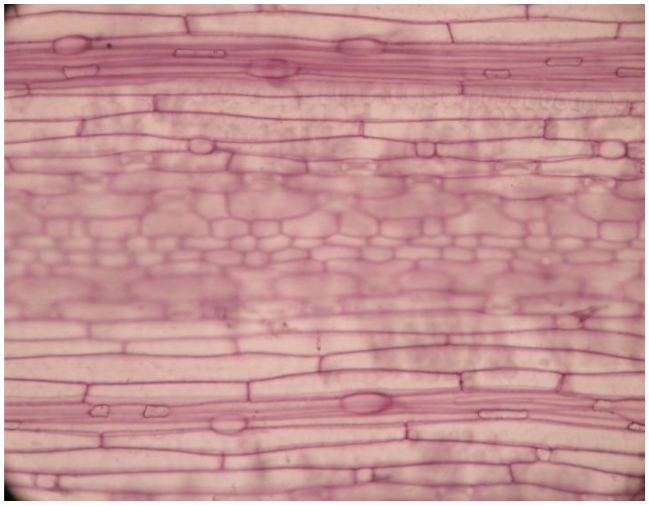

Figure 4. Microscopic photograph of the *F. pratensis* subsp. *apennina*-39 adaxial epidermis (100x).

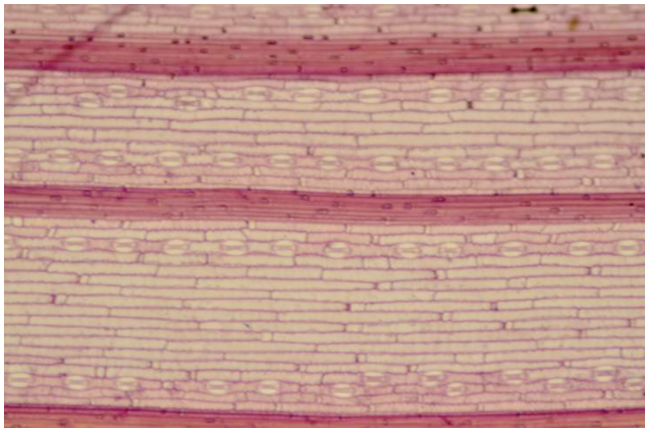

Figure 5. Microscopic photograph of the *F. pratensis* subsp. *apennina*-39 abaxial epidermis (100x).

Figure 6. Scanning EM photograph of the *F. pratensis* subsp. *apennina*-33 adaxial epidermis (100x).

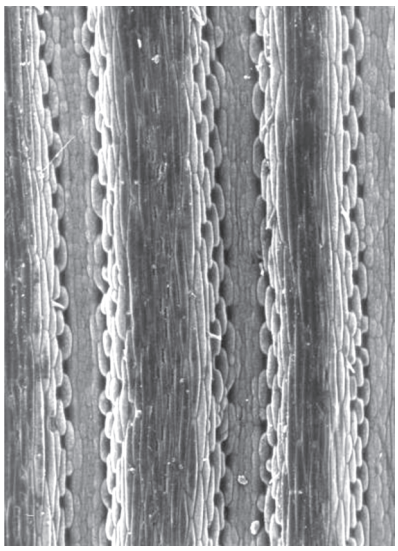

Figure 7. Scanning EM photograph of the *F. pratensis* subsp. *pratensis*-44 adaxial epidermis (100x).

costal zone, these are shorter (90 to 250 µm) and in most of the populations are narrower (7 to 15 µm) as well.

The number of the rows containing stomata in the lower epidermis intervein zone is mostly 1-1, more rarely 1-2 or 2-2. The cells between the stoma complexes are rectangular, their radial wall is also wavy. The length of the cells is 46 to 96 µm, and compared to the upper side's cells between the stomata, they are varied among the populations (longer or shorter), but their width in most populations is greater (12 to 17 µm). In most populations, in the abaxial epidermis we could observe 1 or 2 wavy cell rows containing stoma complexes in the intervein zone. In all cases the silica cells occur in pairs with the phellem cells, in the rows free of stomata cells and in those contaning stomata as well (Fig. 5.). In the middle of the intercostal zone, between the rows containing stomas, in each population, there are straight-running, rectangular cells – these are the longest cells in the zone.

The dumbbell-shaped guard cells of the 'Graminea' type stoma complexes are surrounded by slightly arched side cells, therefore the stoma complex has a flat bun shape. However, on the abaxial epidermis the stomatal cells are domed, more arched than on the adaxial side and the stoma complexes are more round. The length of the stoma complexes is similar on the lower and upper sides, their width is in all cases larger on

the lower side than those on the upper side. The *F. pratensis* subsp. *apennina* populations' stoma comlexes are in average longer both on upper and lower side (except for populations 37 and 39), than those of *F. pratensis* subsp. *pratensis*. The number of stoma complexes per 1mm^2 in all populations is greater in the upper epidermis than the number on the lower epidermis, the number varying from 21 to 94 pcs/mm^2 .

Related to the epidermal micromorphology, the scanning electron microscopy studies carried out on fresh epidermal segments reveal finer details in the structure of the epidermis. These are less detectable in the traditional epidermis peels. Bristles occur on the upper epidermis in both the costal and intercostal zones. The upper side of all populations is hairy. On the lower side we did not find any trichomas in six populations (population 33, 35, 36, 40, 41, 47). The populations of subspecies *F.. pratensis* subsp. *apennina* from the Dolomites region (Alpes) are on average more hairy. The bristles of *F. pratensis* subsp. *apennina* are longer than in the populations of *F. pratensis* subsp. *pratensis* that is anyway less hairy.

In conclusion we can remark that in all the examined populations the upper and lower side epidermis exhibits structural differences. The variation between the populations manifest in the differences in dimensions of certain cells of the epidermis, in the size and amount of silica cells, hairiness, and the size of the bristles. The differentiation of the two subspecies (*F. pratensis* subsp. *pratensis* and subsp. *apennina*) on the basis of epidermal structure is done according to the length of stoma complexes, the number and dimension of bristles as well as the length of the epidermis cells.

Acknowledgements

This work was supported by the TAMOP4.2.1-B of the University of Western Hungary, Research Fund grant No 06524/825.

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