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MICROMORPHOLOGICAL AND ANATOMICAL FEATURES OF FOUR SPECIES OF *ELYTRIGIA* DESV. (POACEAE)

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Abstract

The micromorphological and anatomical characters of *Elytrigia caespitosa* (K. Koch) Nevski, *E. intermedia* (Host) Nevski × *E. elongata* (Host) Nevski, *E. intermedia* (Host) Nevski and *E. repens* (L.) Desv. *ex* Nevski have been studied using Scanning Electron Microscope (SEM) to determine interspecific variation. The results show that the root transverse section consists of epidermis, cortex and stele. Two rings of vascular bundles and a central pith cavity appear in stem morphology. The leaves of *E. caespitosa* have either single or twin, horseshoe-shaped short cells born along the costal zone of the upper epidermis, which lack prickle hairs and contain spherical or oblique-shaped papillae. In *E. intermedia,* the parallel subsidiary cells are distributed on the upper epidermis, and there are no short cells in the leaves. Dome-shaped subsidiary cells appear on the upper epidermis of *E. intermedia* × *E. elongata* and *E. repens*, but *E. intermedia* × *E. elongata* showes spot-shaped papillae, and its bulliform cells sank into the "hinge cells". *E. repens* has no papillae, and its bulliform cells are not sunken into the mesophyll. Therefore, the differences in micromorphological characters on the upper epidermis of the leaf could be useful in classifying and determining phylogenetic relationships among the species.

Introduction

Plant morphological feature is largely controlled by the genes of a species, but it can also be influenced by the environment (Sattler and Rutishauser, 1997; Liu, 2006). Therefore, the micromorphological and anatomical characters of root, stem and leaf can reflect the relationship between habitat and phylogenetics of plants (Liu, 2006). *Elytrigia* Desv. is a perennial rhizomatous grass of the family Poaceae. There are about 50 species of *Elytrigia* throughout the world (Chen and Jia, 2000; Lv *et al.,* 2007), and many of them are ecologically and economically important. *Elytrigia intermedia* is valued for its high quality forage, *E. repens* for stabilizing slopes and sandy soil (Chen and Jia, 2000), and *E. intermedia* and *E. elongata* for breeding distant hybrids of wheat (Lv *et al.,* 2007; Webb and Alrneida, 1990). Several authors have shown that the leaf epidermal morphology of the grass family has taxonomic significance because of the fine morphological structure (Chen *et al.,* 1993; Cai and Guo, 1995). The anatomy of roots, stems and leaves of *E. elongata* and *E. intermedia* were examined and analyzed using Scanning Electron Microscope (SEM) showing that the two species have significant differences in leaf epidermal micromorphology (Shi *et al.,* 2009), e.g. three to four rows of papillae are distributed along the costal zone of *E. elongata* leaves, but three to four rows of prickle hairs are distributed along the costal zone of *E. intermedia*. Therefore, the present study aims to contribute to the micromorphological and anatomical features of the roots, stems and leaves of the four species, *E. caespitosa,*

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E. intermedia × *E. elongata, E. intermedia* and *E. repens*, using Scanning Electron Microscopy (SEM), and to evaluate the differences of the micromorphological characters for systematic purposes.

Materials and Methods

Seeds of *Elytrigia caespitosa* (K. Koch) Nevski, *E. intermedia* (Host) Nevski × *E. elongata* (Host) Nevski, *E. intermedia* (Host) Nevski and *E. repens* (L.) Desv. *ex* Nevski were collected from the National Plant Germplasm System (NGPS) of USA in 2007; and planted at the experimental sites of the National Experiment Station of Precision Agriculture (NESPA), Xiao Tangshan, about 55 km far from Beijing (Lat. 39°34' N, Long. 116°28' E) in 2008. Twenty seedlings of each species were planted in 80 cm × 80 cm rows. Five healthy individuals for each of the four species were selected at the heading stage in May, 2009. Samples were collected by cutting 5 mm segments from the middle sections of the second functional leaves, mature roots, and between the stem stipe of each plant. There were two repetitions totaling 40 specimens. All specimens were pre-fixed for about 3-4 h in 3% glutaraldehyde, and then fixed for more than 12 h in 1% osmic acid (H_2OSO_4). The stationary liquid was formulated using a pH 7.2 phosphate buffer solution (PBS). All specimens were cleaned ultrasonically 3-5 times using PBS, then dehydrated in a 30%, 50%, 70%, 85%, 95% and 100% alcohol solution for 15 min each step by step, and laid in isoamyl acetate. The CO_2 critical point was obtained using a HCP-2 Critical Point Dryer (Hitachi Co. Ltd., Japan), and coated with gold by IB-5 Vacuum Ion Sputter (Eiko Engineering Co. Ltd., Japan).

Mature leaves, roots and stems are examined with S-570 Scanning Electron Microscope (Hitachi Co. Ltd., Japan), and are analyzed with WD-5 Online Photo Management System for SEM (Analysis and Examination Center, Wuhan University of China). The characteristics of the epidermal micromorphology of the upper leaves, including the length of long cells, the shape, density and distribution of short cells and papillae, the density and distribution of prickle hairs and the stomata cell parameters, and the leaf transverse section parameters, such as leaf thickness and characteristics of bulliform cells are thoroughly examined and analyzed. Root anatomical features, including the number of vascular bundles, diameter of stele, thickness of cortex and diameter of metaxylem, and the stem anatomical features including the density of vascular bundles, thickness of cuticle and diameter of inner cycle vessel, are also examined and analyzed. Each index has 10 data points, measured using Photoshop, and means are calculated. Statistical analyses are conducted using SPSS, 13.0 version. The standard leaf micro-morphological terminology is mainly adopted from Chen *et al.* (1993), Cai and Guo (1995), Cai (2000), Kocsis *et al*. (2004) and Torre (2004), whereas terminology for the morphology of roots, stems and leaf transverse sections are adopted from Liu (2006).

Results and Discussion

Root micromorphological characters:

Generally, three parts including the epidermis, cortex and stele are found in the root transverse section of four *Elytrigia* species. The epidermis is made up of a layer of tightly packed cells with many epidermal hairs (root hairs). Under the epidermis is exodermis with larger 2-3 layered cortex cells, which has thicker cell walls and no intercellular spaces. The endodermis is a single layer of small, tightly packed cells, each of which has five thick sides and are the horse-shoe type with the passage cell. There are 2-3 layers of cells adjoining the endodermis to the thick cell wall and the radialized sequence, and the cell volume becomes larger from inner to outer layers. The stele includes the pericycle, vascular bundles and marrow. The pericycle is a layer of

parenchymatous cells adjoining the endodermis. The exarch vascular bundle is polyarch xylem, and the centre of the stele contains the marrow filled with parenchyma (Fig.1). The comparative result of root anatomical features of the four *Elytrigia* species is presented in Table 1.

Table 1. Comparison of root anatomical features of four *Elytrigia* species.

Species	No. of vascular bundles	Diameter of stele (μm)	Thickness of cortex (μm)	Diameter of metaxylem (μm)
E. caespitosa	13	466.57 ± 5.36 a	102.25 ± 2.56 a	36.13 ± 4.41 a
E. intermedia × *E. elongata*	10	442.43 ± 15.81 a	59.52 ± 7.17 c	31.03 ± 6.38 b
E. intermedia	9	456.97 ± 14.09 a	73.76 ± 11.53 b	35.83 ± 2.48 a
E. repens	5	323.69 ± 17.77 b	91.90 ± 3.92 ab	29.54 ± 3.61 b

Different small letters in the same column indicate significant differences at $P<0.05$.

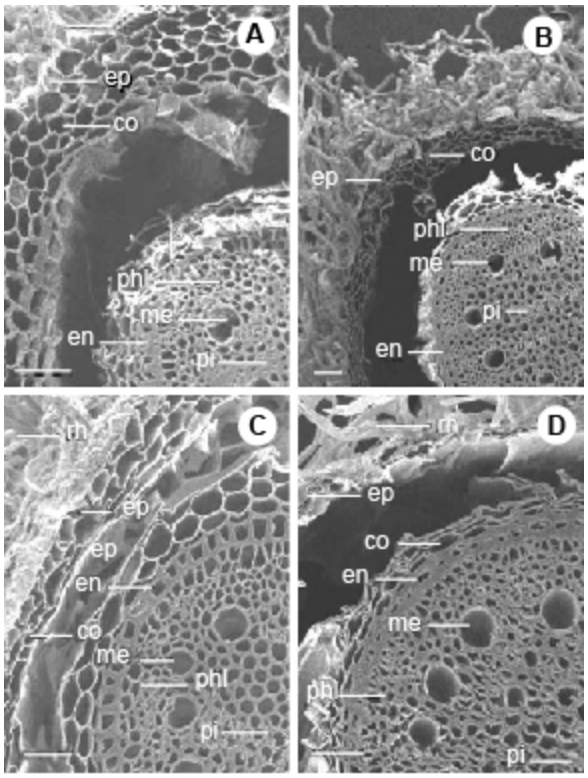

Fig. 1. Root transverse sections of four *Elytrigia* species. A) *E. repens*, B) *E. intermedia*, C) *E. intermedia* × *E. elongata* and D) *E. caespitosa*. rh: root hair; ep: epidenmis; co: cortex; phl: phloem; en: endodermis; me: metaxylem; pi: pith (A, B and D×200, C×150, scale bars = 50 μm).

There are approximately 13 vascular bundles in *E. caespitosa*, which is significantly different than *E. repens* ($P<0.05$), that has only 5 vascular bundles. *E. intermedia* × *E. elongata*, and *E. intermedia* have 10 and 9 vascular bundles, respectively, and do not differ significantly ($P>0.05$). However, the thickness of the cortex of *E. caespitosa* (about 102.25 μm) is 1.72 times than that of

E. intermedia × *E. elongata*. There are no significant differences among stele diameters of *E. caespitosa*, *E. intermedia* × *E. elongata* and *E. intermedia* ($P>0.05$), but diameters are significantly larger than those of *E. repens* ($P<0.05$), which is only 323.69 μm.

Table 2. Comparison of stem anatomical features of four *Elytrigia* species.

Species	Density of vascular bundles (number mm^{-2})	Thickness of cuticle (μm)	Diameter of inner cycle vessel (μm)
E. caespitosa	9 ± 0.21 ab	4.01 ± 0.09 a	34.47 ± 0.80 b
E. intermedia × *E. elongata*	8 ± 0.23 b	4.89 ± 0.14 a	35.08 ± 1.01 b
E. intermedia	8 ± 0.14 b	5.22 ± 0.09 a	51.67 ± 0.89 a
E. repens	10 ± 0.35 a	4.26 ± 0.15 a	31.15 ± 1.08 b

Different small letters in the same column indicate significant differences at $P<0.05$.

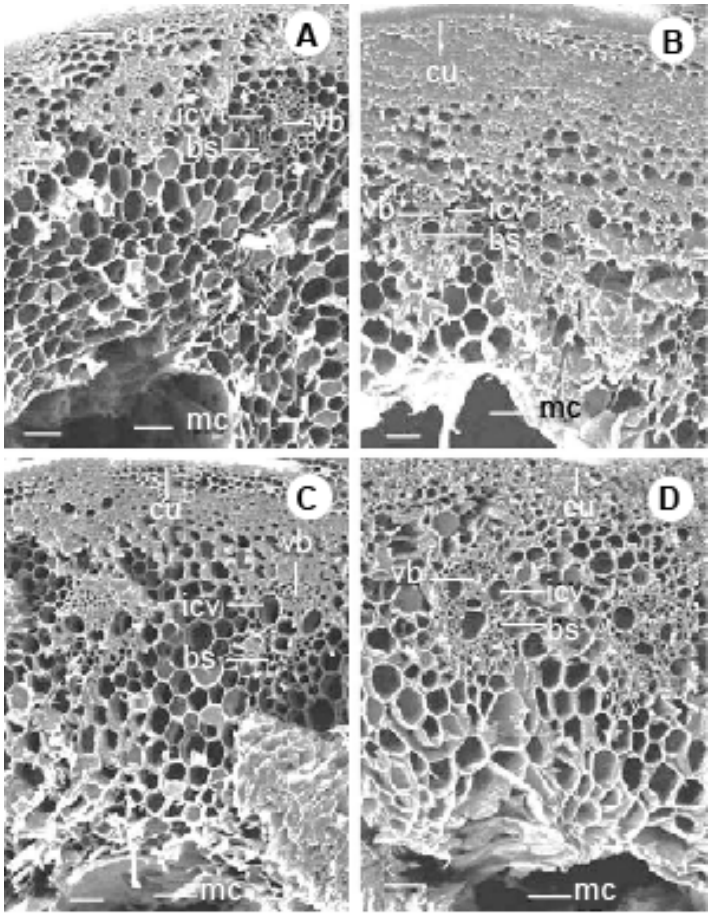

Fig. 2. Stem transverse sections of four *Elytrigia* species. A) *E. repens*, B) *E. intermedia*, C) *E. intermedia* × *E. elongata* and D) *E. caespitosa*. vb: vascular bundle; bs: bundle sheath; icv: inner cycle vessel; cu: cuticle; mc: medullary cavity (×100, scale bars = 50 μm).

Stem micromorphological characters:

Stem transverse sections of the four *Elytrigia* species consist of four parts: the epidermis, ground tissue, vascular bundles and the medullary cavity (Fig. 2). Cuticle thickness in the epidermis is only about 4.01-5.22 μm. Under the epidermis are two to three layers of sclerenchyma tissue (fiber) with thick cell walls, which are composed of parenchymatous cells. The collateral vascular bundle with sheath and no cambium are arranged in two rings. The outer ring contains small vascular bundles, which are embedded under the fiber and form the mechanical tissue, and parenchymatous cells are presented among the vascular bundles. The inner ring is composed of larger vascular bundles, and these are distributed among the parenchyma between the medullary cavity and the outer mechanical tissue (Fig. 2).

The cuticle thickness of four species do not differ significantly ($P>0.05$), but the density of the vascular bundles in *E. repens* differs from *E. intermedia* × *E. elongata* and *E. intermedia* ($P<0.05$). The diameter of the inner cycle vessel in *E. intermedia* is wider than that of *E. caespitosa*, *E. intermedia* × *E. elongata* and *E. repens*, but there are no significant differences in the inner cycle vessel diameters among the latter three species (Table 2).

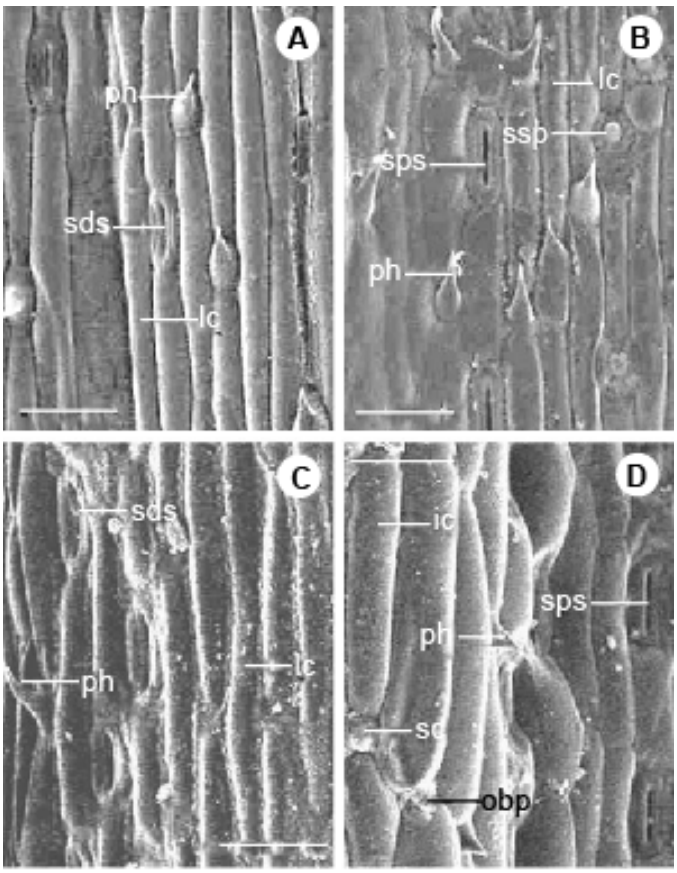

Fig. 3. Upper leaf epidermis of four *Elytrigia* species. A) *E. repens*, B) *E. intermedia*, C) *E. intermedia* × *E. elongata* and D) *E. caespitosa*. lc: long cell; sc: short cell; ph: prickle hair; obp: oblique papillae; ssp: spot-shaped papillae; sds: stomata with dome-shaped subsidiary cell; sps: stomata with parallel subsidiary cell (×200, scale bars = 50 μm).

Table 3. Comparison of upper leaf epidermal micromorphology of four *Elytrigia* species.

Features	Species			
	E. caespitosa	*E. intermedia* × *E. elongata*	*E. intermedia*	*E. repens*
Long cells				
Length (μm)	128.89 ± 2.98 d	183.89 ± 5.31 b	160.00 ± 2.77 c	233.33 ± 8.08 a
Cell wall	Thin	Thick	Relatively thick	Thin
Density (number mm^{-2})	78 ± 41	0[d]	0	0
Short cells				
Length (μm)	31.11 ± 4.81	0	0	0
Shape and distribution	Saddle-shaped, single or twin born in costal zone, absent in intercostal zone	0	0	0
Stomata cells				
Distribution	Absent in costal zone, 3 rows born in intercostal zone	2 rows born in each sides of the rib in costal zone, separated from prickle hair and absent in intercostal zone	1-2 rows on each side of the rib in costal zone, alternated with prickle hairs and absent in intercostal zone	1-2 rows along each side of the rib in costal zone, absent in intercostal zone
Density (number mm^{-2})	84 ± 1.94 b	96 ± 2.77 a	66±1.14 c	74 ± 2.56 c
Length (μm)	53.33 ± 1.23 ab	49.44 ± 1.43 b	52.78 ± 0.91 ab	57.78 ± 2.00 a
Width (μm)	25.00 ± 0.58 a	16.67 ± 0.48 c	19.44 ± 0.34 b	17.22 ± 0.60 bc
Subsidiary cells	Parallel	Dome-shaped	Parallel	Dome-shaped
Papillae				
Density (number mm^{-2})	132 ± 3.05 a	18 ± 0.52 c	99 ± 1.71 b	0
Shape and distribution	Spherical or oblique papillae distributed along the sides of costal rib alternating with long cells	Spot-shaped papillae scattered in costal zone and absent in intercostal zone	Spot-shaped papillae with 4-6 cluster born in costal zone and absent in intercostal zone	0
Prickle hair				
Density (number mm^{-2})	0	27 ± 0.78 c	130 ± 2.25 a	73 ± 2.53 b
Distribution	0	2-3 rows of prickle hair in costal zone, or 1 row born in each side of rib, and absent in intercostal zone	1-2 rows in each side of rib, and absent in intercostal zone	3-5 rows in costal zone, and absent in intercostal zone

Different small letters in the same row indicate significant differences at $P<0.05$. [d]represents that the indices are absent in this species. 0 indicates absent.

Leaf micromorphological characters:

The micromorphology and anatomy of the leaves in the four *Elytrigia* species comprise three layers: epidermis, mesophyll and leaf veins. The epidermis is composed of long cells, subsidiary cells and stomatal and bulliform cells. Quadrate long cells with thin or thick cell walls are arranged in parallel along the length of the costal and intercostal zones, and many of them show slight wave bending pattern (Fig. 3). The cells of *E. repens* are the longest (233.33 μm), whereas those of *E. caespitosa* are the shortest (128.89 μm). The short cells only appear in the epidermis of *E. caespitosa* at a density of about 78 mm^{-2}. They are saddle shaped, single or twin, and born along the costal zone, but absent in the intercostal zone. Three rows of stomatal cells are present in the intercostal zone of the upper epidermis of *E. caespitosa*, and 1-2 rows appear along each side of the ribs in the costal zone for *E. intermedia* × *E. elongata*, *E. intermedia* and *E. repens*. The density, length and width of the stomatal cells of the four species are expressed differently, and the subsidiary cells of *E. caespitosa* and *E. intermedia* are in parallel, but *E. intermedia* × *E. elongata* and *E. repens* are dome-shaped. The papillae of *E. caespitosa* are spherical or oblique in shape and are distributed along the sides of the costal rib alternating with the long cells, at a density of about 132 mm^{-2}. *E. intermedia* × *E. elongata* and *E. intermedia* show spot-shaped papillae scattered throughout the costal zone. The density of papillae in *E. intermedia* × *E. elongata* is 18 mm^{-2} but papillae are absent in *E. repens*. The density of prickle hairs in *E. intermedia* is 130 mm^{-2}, which is significantly different than that of *E. repens* (73 mm^{-2}) and *E. intermedia* × *E. elongata* (27 mm^{-2}) (P<0.05) (Table 3).

The transverse section of leaves consists of epidermis, vascular bundles, mesophyll cells and fiber, i.e., sclerenchyma tissue (Fig. 4). Bulliform cells are present in the intercostal zones of all four species. However, those of *E. intermedia* × *E. elongata* are sunken into the mesophyll and form "hinge cells", whereas those of the other species do not show this pattern. The ratio of leaf thickness of intercostal zones to thickness of bulliform cells of the four species is approximately 17.8-28.8%, and that of *E. intermedia* × *E. elongata* and *E. repens* are significantly different (P<0.05) compared with *E. caespitosa* and *E. intermedia*. The average costal and intercostal thicknesses of *E. caespitosa* and *E. intermedia* × *E. elongata* are greater than those of *E. intermedia* and *E. repens* (Table 4) and the veins are hump shaped. There are two layers of vascular bundles in the veins: smaller, thick cell walls in the inner layer, and larger, thin cell walls in the outer layer. Fiber is distributed along each side of the vascular bundles. The mesophyll tissue is made up of parenchymatous cells with large intercellular spaces, and there is no cellular differentiation between the palisade and spongy tissues (Fig. 4).

Table 4. Comparison of leaf transverse section of four *Elytrigia* species.

Species	Leaf thickness			Bulliform cell		
	Costal zones (μm)	Intercostal zones (μm)	Average (μm)	Characters	Thickness (μm)	Ratio of bulliform cell (%)[d]
E. caespitosa	250.33 ± 5.78 b	166.48 ± 3.84 a	208.41 ± 4.81 a	Not sunken	29.67 ± 0.69 b	17.8 ± 0.41 b
E. intermedia × *E. elongata*	280.04 ± 8.08 a	154.63 ± 4.46 a	217.33 ± 6.27 a	Sunken into mesophyll and form 'hinge cell'	41.04 ± 1.18 a	26.5 ± 0.76 a
E. intermedia	186.74 ± 3.23 c	122.67 ± 2.12 b	154.70 ± 2.68 b	Not sunken	23.63 ± 0.41 c	19.3 ± 0.33 b
E. repens	197.37 ± 6.84 c	100.41 ± 3.48 c	126.89 ± 4.40 c	Not sunken	28.93 ± 1.00 b	28.8 ± 1.00 a

Different small letters in the same column indicate significant differences at P<0.05.
[d] means that the ratio of bulliform cell thickness to leaf thickness.

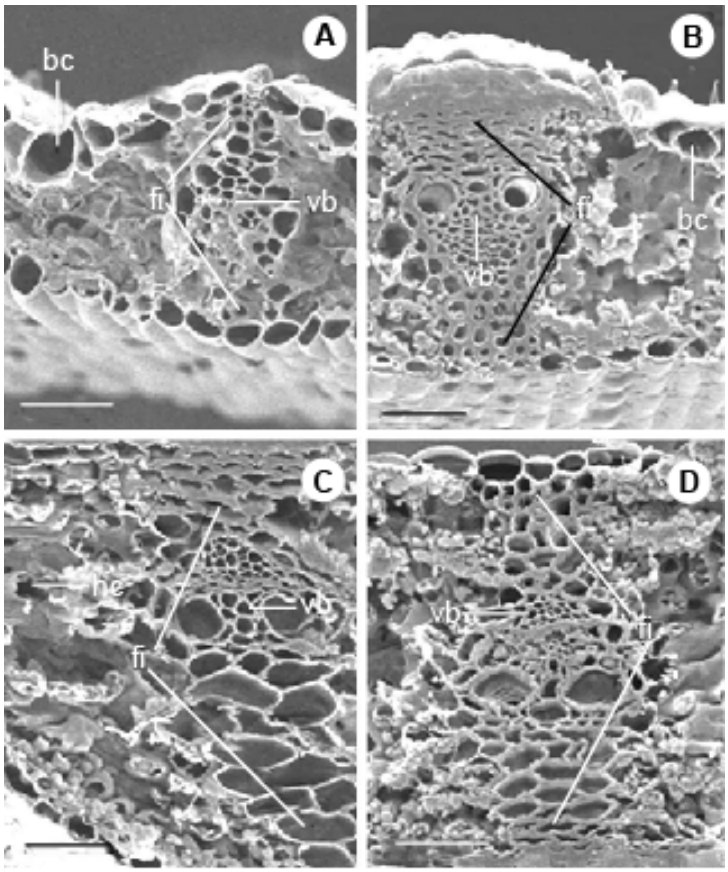

Fig. 4. Leaf transverse sections of four *Elytrigia* species. A) *E. repens*, B) *E. intermedia*, C) *E. intermedia* × *E. elongata* and D) *E. caespitosa.* bc: bulliform cell; hc: hinge cell; fi: fibre; vb: vascular bundle (×300, scale bars = 50 μm).

Leaf micromorphological and anatomical characters are useful tool for plant identification and bears taxonomic significance (Cai and Guo, 1995; Shi *et al.*, 2009; Cai and Zhang, 2006; Kahraman *et al.*, 2010). The present investigation of the leaf anatomy of four *Elytrigia* species agree with those in previous studies of *E. intermedia* and *E. elongata* (Shi *et al.*, 2009).

Based on leaf micromorphological and anatomical characters of four *Elytrigia* species a dichotomous key is presented below:

1. Single or twin short cells born along the costal zone, no prickle hairs on the upper epidermis. *E. caespitosa*
– No short cells, but prickle hairs on the upper epidermis. 2
2. Subsidiary cells on the upper epidermis are parallel. *E. intermedia*
– Subsidiary cells on the upper epidermis are dome-shaped. 3
3. Long cells thick-walled; papillae spot-shaped; bulliform cells sunken into mesophyll forming “hinge cell”. *E. intermedia* × *E. elongata*
– Long cells thin-walled; papillae absent; bulliform cells not sunken into mesophyll. *E. repens*

Bulliform cells are unique characteristics of the leaves of endemic xerophytic graminaceous plants (Cai, 2000). These cells are also known as motor cells, which can cause the leaf blades to curve or spread through the leaf during periods of water loss and absorption. Usually, the bulliform cells appear in groups along the interveins in the upper epidermis of grasses. Wang and Wang (1989) found that the bulliform cells sunk into the mesophyll and formed "hinge cells" on the upper epidermis of *Bouteloua breviseta*, increasing leaf curvature. However, Qiang *et al.* (2007) in *Carex orbicularis* and Guo *et al.* (2007) in *Blysmus sinocompressus* showed that bulliform cells with a little and smaller morphology appeared above the main vein of the leaves. In our study bulliform cells appear in all four *Elytrigia* species, but they are only embedded in the mesophyll into the "hinge cell" in *E. intermedia* × *E. elongata*. The parallel or dome-shaped subsidiary cells in the leaves of grasses exhibit plant adaptation to drought and cold (Cai and Guo, 1995). Therefore, having parallel subsidiary cells in *E. caespitosa* and *E. intermedia* and dome-shaped subsidiary cells in *E. intermedia* × *E. elongata*, and *E. repens* confirm that these species are capable to resist heavy drought and cold.

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References

Cai, L.B. 2000. Leaf epidermical characters of some species of *Roegneria* and their taxonomic significance. Bull. Bot. Res. **20**(4): 372-378.

Cai, L.B. and Guo, Y.K. 1995. Studies on constituent cells of leaf epidermis, systematics and phylogenetic path of the family Poaceae. Acta Botanica Boreali-Occidentalia Sinica **15**(4): 323-335.

Cai, L.B. and Zhang, T.L. 2006. Genetic relationship between *Leymus* and its related taxa in terms of the anatomical characteristics of their leaves. Acta Botanica Boreali-Occidentalia Sinica **26**(3): 537-543.

Chen, M.J. and Jia, S.X. 2000. Forage Plants in China. China Agriculture Press, Beijing, China, pp. 125-128.

Chen, S.L., Jin, Y.X. and Wu, Z.J. 1993. Leaf epidermal characteristic atlas of the family Poaceae. Jiangsu Science and Technology Press, Nanjing, China, pp. 59-63.

Guo, M., Qiang, K.B. and Zhang, X.Q. 2007. Study on morphological anatomy of leaf in *Blysmus sinocompressus*. J. Gansu Agri. Univ. **42**(1): 82-87.

Kahraman, A., Celep, F. and Dogen, M. 2010. Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae). S. Afri. J. Bot. **76**(2): 187-195.

Kocsis, M., Darok, J. and Borhid, A. 2004. Comparative leaf anatomy and morphology of some neotropical *Rondeletia* (Rubiaceae) species. Plant Syst. Evol. **248**: 205-218.

Liu, M. 2006. Morphology and Anatomy of Seed Plant (3rd edition). Science Press, Beijing, China, pp.121-268.

Lv, W.D., Xu, P.B. and Pu, X. 2007. Summary of the situation for applying genetic resources from *Elytrigia* in *Triticum aestivum* breeding. Acta Prataculturae Sinica **16**(6): 36-140.

Qiang, K.B., Guo, M. and Zhang, X.Q. 2007. Studies on the morphology and anatomy of leaves of *Carex orbicularis*. Acta Prataculturae Sinica **16**(2): 76-83.

Sattler, R. and Rutishauser, R. 1997. The fundamental relevance of morphology and morphogenesis to plant research. Annals Bot. **80**(5): 571-582.

Shi, G.D., Mao, P.C., Zhang, G.F., Meng, L. and Zhang, D.G. 2009. SEM observation on anatomical structure of *Elytrigia elongata* (Host) Nevski and *E. intermedia* (Host) Nevski. Prataculturae Science **26**(8): 52-56.

Torre, S. 2004. Morphology and Anatomy: Leaves. *In:* Roberts, A.V. (Ed.), Encyclopedia of Rose Science. Academic Press, London. Pp. 497-504.

Wang, X.L. and Wang, J. 1989. Plant Morphological Structure and Environment. Lanzhou University Press, Lanzhou, China, pp. 65-148.

Wang, Y.J. 2005. Bulliform cell of grass family. Biology Education **30**(11): 7-9.

Webb, M.E. and Alrneida, M.T. 1990. Micromorphology of the leaf epidermis in the taxa of the *Agropyron - Elymus* complex (Poaceae). Bot. J. Linn. Soc. **103**: 153-158.

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