MICROMORPHOLOGICAL STUDIES OF INDUMENTUM IN BROMUS L. (POACEAE) AS TAXONOMICAL EVIDENCE

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In the present study, 28 species of *Bromus* L. have been investigated based on micromorphological characters of abaxial surface. The lamina of grass leaf blades is divided into costal and intercostal zones. The results of this study showed that the costal zone includes short and long cells and probably hair and prickle and the intercostals zone includes long cells, stomata and hairs. They are similar and constant in all species. But indumentum types are different in species. Also the characters of indumentum have taxonomical value and can be used in distinguishing of species.

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مطالعات ریزساختاری پوشش کرکي در جنس .Bromus L (تیره گندم) به عنوان شاهد تاکسونومیکی

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در این تحقیق سطح زیرین برگ در ۲۸ گونه از جنس *Bromus* از نظر صفات ریزساختاری در دو ناحیه رگبرگی و بین رگبرگی مورد مطالعه قرار می‌گیرند. نتایج نشان داد که ناحیه رگبرگی از سلولهای بلند و کوتاه و در صورت وجود از کرک و خار تشکیل می شود و ناحیه بین رگبرگی شامل سلولهای بلند، روزنه‌ها و کرک است، که در همه گونه ها عمومیت دارد. ولی از نظر نوع کرک و تراکم آنها تفاوتهایی وجود دارد که می‌تواند دارای ارزش تاکسونومیکی باشد و در جدا سازی گونه‌ها حتی گونه‌های نزدیک مورد استفاده قرار گیرد.

Introduction

Bromus L. is a large genus of approximately 100 (Mabberley 1997) to 150 species which are found in the temperate regions of both hemispheres (Clayton and Renvoize, 1986; Pavlick 1995; Watson and Dallwitz 1992). The genus comprises annual and perennial grasses with wide geographical distribution (Smith 1970). Based on Flora Iranica (Bor 1970), the species of this genus are arranged in six sectoins. Altogether 40 species occur in Iran which belong to Sect. *Bromus* (15 spp.), Sect. *Genea* Dumort. (7 spp.), Sect. *Nevskiella* (V. Krecz. & Vved.) Bor (1 sp.), Sect. *Neobromus* (Shear) Hitchc. (1 sp.), Sect. *Pnigma* Dumort. (15 spp.) and Sect. *Ceratochloa* (P. Beauv.) Griseb. ex Ledeb (1 sp.). The species of Sect. *Pnigma* are perennial and other sections are annual or biennial species. *Bromus* L. is a large genus of *Poaceae* in Iran (Ghahreman and Attar, 1999). Abaxial epidermal of the leaves of some species of *Bromus* have been studied by Metcalfe 1960.

The aim of present study is to add more data of abaxial epidermis characters of the leaves for the genus and use of the characters which can be effected in taxonomical treatments.

Materials and Methods

After naming *Bromus* specimens based on morphological characters of Flora Iranica (Bor 1970) and Grasses of the Soviet Union (Tsvelev 1976) and

Table 1. *Bromus* specimens used for the study. The species arranged in alphabetical order in each section.

Species	Voucher specimen
Sect. ***Bromus*** *B. arvensis* L.	Azerbaijan: Jolfa, 790 m, Foroughi, 5210-TARI
B. brizifomis Fisch. & C. A. Mey.	Khorassan: Maraveh tappeh, 819 m, Moazzeni, 35988-TUH
B. gedrosianus Penzes	Hormozgan: Rudan, 150 m, Khatamsaz & Zaeifi, 73161-TARI
B. intermedius Guss.	Gilan: Kalaj Manjil, 1800 m, Sabeti, 10565-TARI
B. japonicus Thunb.	Lorestan: Khorram abad, 1100 m, Veiskarami, 23538- TUH
B. oxyodon Schrenk	Khorassan: Neyshabour, 1700-1800 m, Rafeii & Zangooii, 26928-FUMH
B. racemosus L.	Gilan: Deilaman, 1600 m, Attar & Columbus, 34830-TUH
B. scoparius L.	Lorestan: Khorram abad, 1100 m, Veiskarami, 23537-TUH
Sect. ***Genea*** Dumort. *B. fasciculatus* Presl	Khuzestan: Andimeshk, 580 m, Gheissari, 1290-TARI
B. madritensis L.	Kermanshah: Kuh- e Pyry, 1320-1520 m, Hamzeh'ee & Lashkarbolouki, 423-TARI
B. rubens L.	Hormozgan: Bandar- e Khamir, 10 m, Mozaffarian, 63662-TARI
B. sericeus Drobov	Esfahan: Khansar, 1900 m, Attar & Columbus, 33767-TUH
B. sterilis L.	Mazandaran: Lake of Shoormast, 890 m, Attar & Alemi, 35133-TUH
B. tectorum L.	Tehran: Chitgar, 1320 m, Attar, 15367- TUH
Sect. ***Neobromus*** (Shear) Hitchc. *B. danthoniae* Trin.	Tehran: Shahrak-e Motahari, 1320 m, Attar, 15354-TUH
Sect. ***Nevskiella*** (V. Krecz. & Vved.) Bor *B. gracillimus* Bunge.	Tehran: Damavand, 2150 m, Attar & Columbus, 34044-TUH
Sect. ***Pnigma*** Dumort. *B. Benekenii* (Lange) Trimen	Mazandaran: Nowshahr, 500 m, Assadi, 33467-TARI
B. biebersteinii Roemer & Schultes	Kuhgiloy-e and Boyer-Ahmad: Sisakht, 2800 m, Ghahreman, Attar & Mahdigholi, 25366-TUH
B. cappadocicus Boiss. & Bal.	Azerbaijan: Siah cheshmeh, 1600-2000 m, Assadi, 85221-TARI
B. frigidus Boiss. & Hausskn.	Chaharmahal e Bakhtiari: Khederabad, 2600-3200 m, Mozaffarian, 58116-TARI
B. inermis Leyss.	Tehran: Homand-e absard, 1950 m, Pabot, 30834-TARI
B. kopetdaghensis Drobov	Tehran: Shemshak, 2400-3400 m, Mozaffarian, 6749-TUH
B. ramosus Hudson	Mazandaran: Kheyrud kenar forest, 500-700 m, Assadi, 33791-TARI
B. riparius Rehman	Mazandaran: Pol-e Sefid, 664 m, Attar & Alemi, 35073-TUH
B. stenostachyus Boiss.	Mazandaran: Amol, 1000 m, Assadi, 73349-TARI
B. tomentellus Boiss.	Esfahan: Karkas Mts., 2400 m, Attar & Columbus, 33616-TUH
B. tomentosus Trin.	Mazandaran: Siah-Bisheh Mountains, 1700 m, Attar & Alemi, 35972-TUH
B. variegatus M. B.	Azerbaijan: Arasbaran protected area, 2500 m, Assadi & Sardabi, 23880-TARI

taxonomical survey of species, abaxial epidermis of leaves of twenty eight species of Iranian *Bromus* species were studied. Sixteen species are annual and twelve species are perennial. Annual species contain *B. arvensis*, *B. briziformis*, *B. gedrosianus*, *B. intermedius*, *B. japonicus*, *B. oxyodon*, *B. racemosus*, *B. scoparius*, *B. fasciculatus*, *B. madritensis*, *B. rubens*, *B. sericeus*, *B. sterilis*, *B. tectorum*, *B. danthoniae*, *B. gracillimus*, and perennial species contain *B. benekenii*, *B. biebersteinii*, *B. cappadocicus*, *B. frigidus*, *B. inermis*, *B. kopetdaghensis*, *B. ramosus*, *B. riparius*, *B. stenostachyus*, *B. tomentellus*, *B. tomentosus*, *B. variegatus*.

Twelve species were not included because they are rare and the materials were not available. Samples were removed from herbarium specimens at TUH, TARI and FUMH (Ferdowsi University Mashhad Herbarium). A complete list of material used including of scientific names, exact localities, collectors and herbarium names are given in Table 1. For constancy of micromorphological characters, secondary leaf below the inflorescence was selected in all cases. Then mounted directly on 12.5 mm diameter stubs and

attached with sticky tape. The specimens were coated in a sputter coater with approximately 25 mm of Gold-Paladium. The specimens photographed by a Philips Scanning Electron Microscope, model XL30 (Holland). The micromorphological characters used for this study follow Metcalfe (1960) and Ellis (1979).

Results

The leaf blades are usually divided into longitudinal zones with the costal zones lying opposite the veins and the intercostal zones present between the veins (Metcalfe 1960).

Costal zone

Costal zone includes long cells, short cells, hairs and prickles in species of *Bromus*. In the zone length of long cells is slightly greater than width, these cells adjoin one another by single short cell. Hairs and prickles present between long cells.

Prickle hairs are short with swollen bases and barbs pointed sharply. Their bases are longer than stomata and their barbs are shorter than bases.

Intercostal zone

Intercostal zone includes long cells, stomatea and hairs in species of *Bromus*. In the zone length of long cells is 3x or more than 3x width. Stomata and hairs present between long cells. Stomatal complex are long and narrow with Parallel-sided subsidiary cells. There are one to three rows of stomata in intercostal zone. These rows separate by one to three file of long cells.

Indumentum types

Macro- and micro-hairs present in both zones. They are unicellular with swollen and superficial bases. Their density varies from low as *B. cappadocicus* (Fig. 4, A) to high as *B. tomentosus* (Fig. 5, C). Hairs are hard and stiff except *B. tomentosus* (Fig. 5, C) that hairs are soft and thin.

Indumentum types are different in species. The species are arranged based on indumentum types of abaxial surface in three groups:

Group 1. epidermal cells are glabrous in annual species: *B. arvensis* (Fig. 1, A), *B. scoparius* (Fig. 2, B), *B. rubens* (Fig. 2, E) and in perennial species: *B. benekenii* (Fig. 3, E).

Group 2. epidermal cells are hairy and are dividing to three subgroups:

I. epidermal cells have macro-hairs in annual species: *B. briziformis* (Fig. 1, B), *B. gedrosianus* (Fig. 1, C), *B. racemosus* (Fig. 2, A), *B. tectorum* (Fig. 3, B) and in perennial species: *B. frigidus* (Fig. 4, B), *B. tomentosus* (Fig. 5, C), *B. variegatus* (Fig. 5, D).

II. epidermal cells have only micro-hairs in perennial species: *B. cappadocicus* (Fig. 4, A).

III. epidermal cells have macro-hairs mixed with micro- unicellular hairs between them in annual species: *B. intermedius* (Fig. 1, D), *B. japonicus* (Fig. 1, E), *B. oxyodon* (Fig. 1, F), *B. fasciculatus* (Fig. 2, C), *B. madritensis* (Fig. 2, D), *B. sericeus* (Fig. 2, F), *B. sterilis* (Fig. 3, A), *B. danthoniae* (Fig. 3, C), *B. gracillimus* (Fig. 3, D) and in perennial species: *B. kopetdaghensis* (Fig. 4, D), *B. riparius* (Fig. 4, F), *B. stenostachyus* (Fig. 5, A) and *B. tomentellus* (Fig. 5, B).

Group 3. epidermal cells have only prickles in perennial species: *B. biebersteinii* (Fig. 3, F), *B. inermis* (Fig. 4, C) and *B. ramosus* (Fig. 4, E).

Discussion

In this study abaxial epidermis of the leaves of 28 species of Iranian *Bromus* species were examined. Of this number, 16 annual species belong to four sections and 12 perennial species belong to one section (Table 1). Macro- and micro-hairs, prickles, stomata, long and short cells have been investigated in costal and intercostal zones. The results are shown that characters of stomata, long and short cells and distribution of them in two zones are similar and constant in all species and have not taxonomical value. But indumentum types are different in species. The species are arranged based on indumentum types of abaxial surface in three groups. Also the characters of indumentum have not taxonomical value in grouping in section level but can be used in distinguishing of species (Table 2). For example, *B. japonicus* and *B. gedrosianus* (*B. rechingeri* in Flora Iranica) which are annual and morphologically very close by having ovate spikelets with bilateral arrangment of them, can be separated by indumentum type. Epidermal cells have macro-hairs with micro- unicellular hairs between them in *B. japonicus* (Fig. 1, E) but epidermal cells have macro-hairs in *B. gedrosianus* (Fig. 1, C). In *B. fasciculatus* and *B. rubens* which are annual morphologically closed together, based on the cuneate spikelets and bilateral arrangment of spikelets may be separated on the basis of indumentum type. Epidermal cells have macro-hairs mixed with micro- unicellular hairs between them in *B. fasciculatus* (Fig. 2, C) but glabrous in *B. rubens* (Fig. 2, E). *B. sericeus* and *B. tectorum* are morphologically very close by having cuneate spikelets with unilateral arrangment of them, can be separated by indumentum type. In *B. sericeus* (Fig. 2, F) epidermal cells have macro-hairs mixed with micro- unicellular between them and in *B. tectorum* (Fig. 3, B) epidermal cells have macro-hairs. In other species such as *B. cappadocicus* and *B. tomentellus* which are closed by having characters such as perennial habit, reticulated fibrous sheath in the culm base and long awn can be

Table 2. Distinghushing characters of indumentum inter-species

Species	Indumentum type
Sect. *Bromus* *B. arvensis* L.	Glabrous
B. brizifomis Fisch.& C. A. Mey.	Macro-hairs
B. gedrosianus Penzes	Macro-hairs
B. intermedius Guss.	Macro-hairs with mixed micro- unicellular between them
B. japonicus Thunb.	Macro-hairs with mixed micro- unicellular between them
B. oxyodon Schrenk	Macro-hairs with mixed micro- unicellular between them
B. racemosus L.	Macro-hairs
B. scoparius L.	Glabrous
Sect. *Genea* Dumort. *B. fasciculatus* Presl	Macro-hairs with mixed micro- unicellular hairs between them
B. madritensis L.	Macro-hairs with mixed micro- unicellular hairs between them
B. rubens L.	Glabrous
B. sericeus Drobov	Macro-hairs with mixed micro- unicellular hairs between them
B. sterilis L.	Macro-hairs with mixed micro- unicellular hairs between them
B. tectorum L.	Macro-hairs
Sect. *Neobromus* (Shear) Hitchc. *B. danthoniae* Trin.	Macro-hairs mixed with micro- unicellular hairs between them
Sect. *Nevskiella* (V. Krecz. & Vved.) Bor *B. gracillimus* Bunge.	Macro-hairs mixed with micro- unicellular hairs between them
Sect. *Pnigma* Dumort. *B. benekenii* (Lange) Trimen	Glabrous
B. biebersteinii Roemer & Schultes	Prickles
B. cappadocicus Boiss. & Bal.	Micro-hairs
B. frigidus Boiss. & Hausskn.	Macro-hairs
B. inermis Leyss.	Prickles
B. kopetdaghensis Drobov	Macro-hairs mixed with micro- unicellular hairs between them
B. ramosus Hudson	Prickles
B. riparius Rehman	Macro-hairs mixed with micro- unicellular hairs between them
B. stenostachyus Boiss.	Macro-hairs mixed with micro- unicellular hairs between them
B. tomentellus Boiss.	Macro-hairs mixed with micro- unicellular hairs between them
B. tomentosus Trin.	Macro-hairs
B. variegatus M. B.	Macro-hairs

separated by indumentum type. Epidermal cells have only micro-hairs in *B. cappadocicus* (Fig. 4, A) but epidermal cells have macro-hairs mixed with micro-unicellular hairs between them but in *B. tomentellus* (Fig. 5, B). Morphologically species of *B. benekenii* and *B. ramosus* are very close by having similar characters such as perennial habit, short rhizome, auricle and not reticulated fibrous sheath in the culm base but can be separated on the basis of indumentum type. As in *B. benekenii* (Fig. 3, E) epidermal cells are glabrous and in *B. ramosus* (Fig. 4, E) epidermal cells have only prickles.

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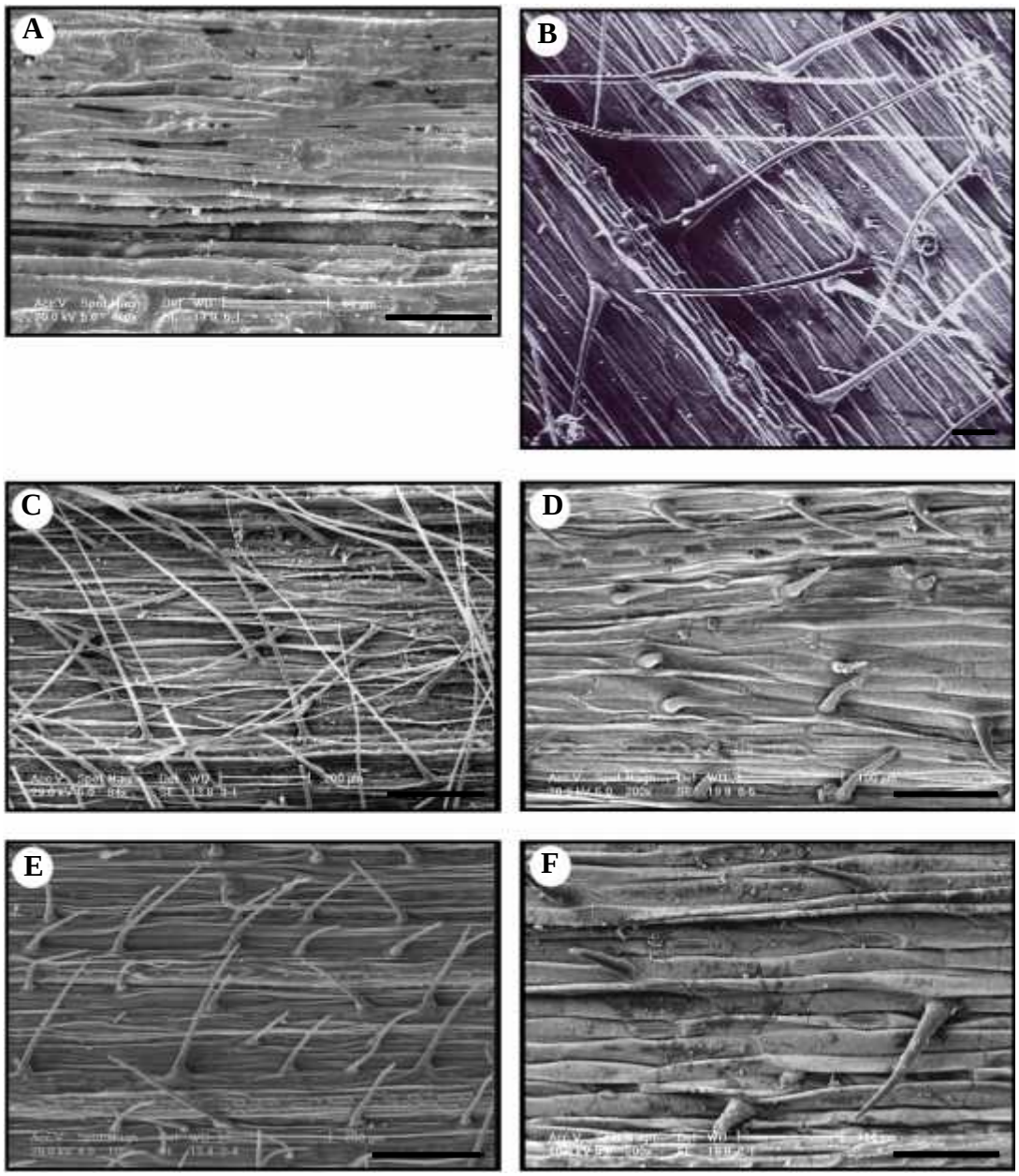

Fig. 1. A-F, abaxial epidermis of the leaf in *Bromus* species; A, *B. arvensis*; B, *B. brizifomis*; C, *B. gedrosianus*; D, *B. intermedius*; E, *B. japonicus*; F, *B. oxyodon*. Scale bar: A, B=50 µm; C, E=200 µm; D, F=100 µm.

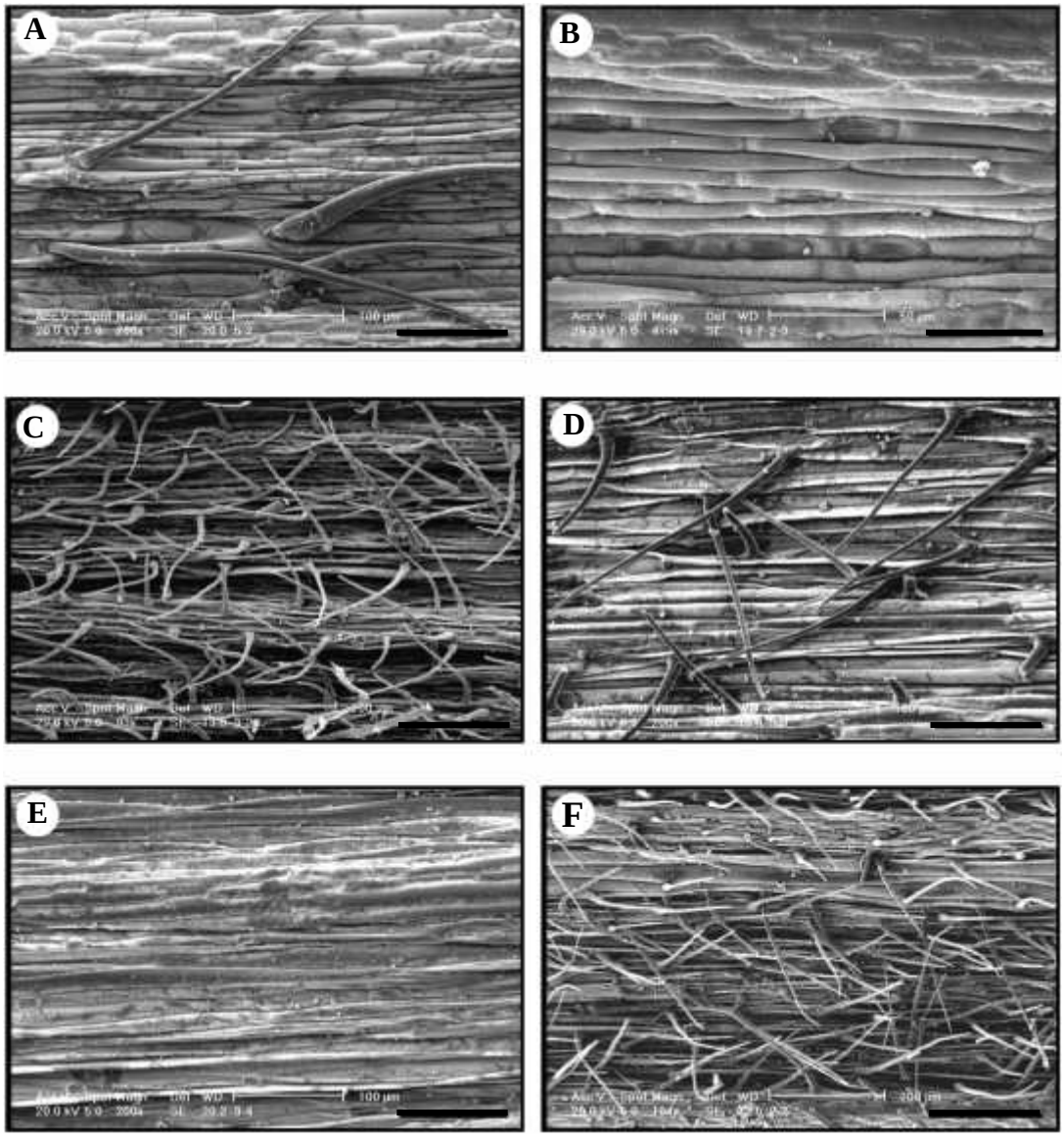

Fig. 2. A-F, abaxial epidermis of the leaf in *Bromus* species; A, *B. racemosus*; B, *B. scoparius*; C, *B. fasciculatus*; D, *B. madritensis*; E, *B. rubens*; F, *B. sericeus*. Scale bar: A, D-E=100 µm; B=50 µm; C, F=200 µm.

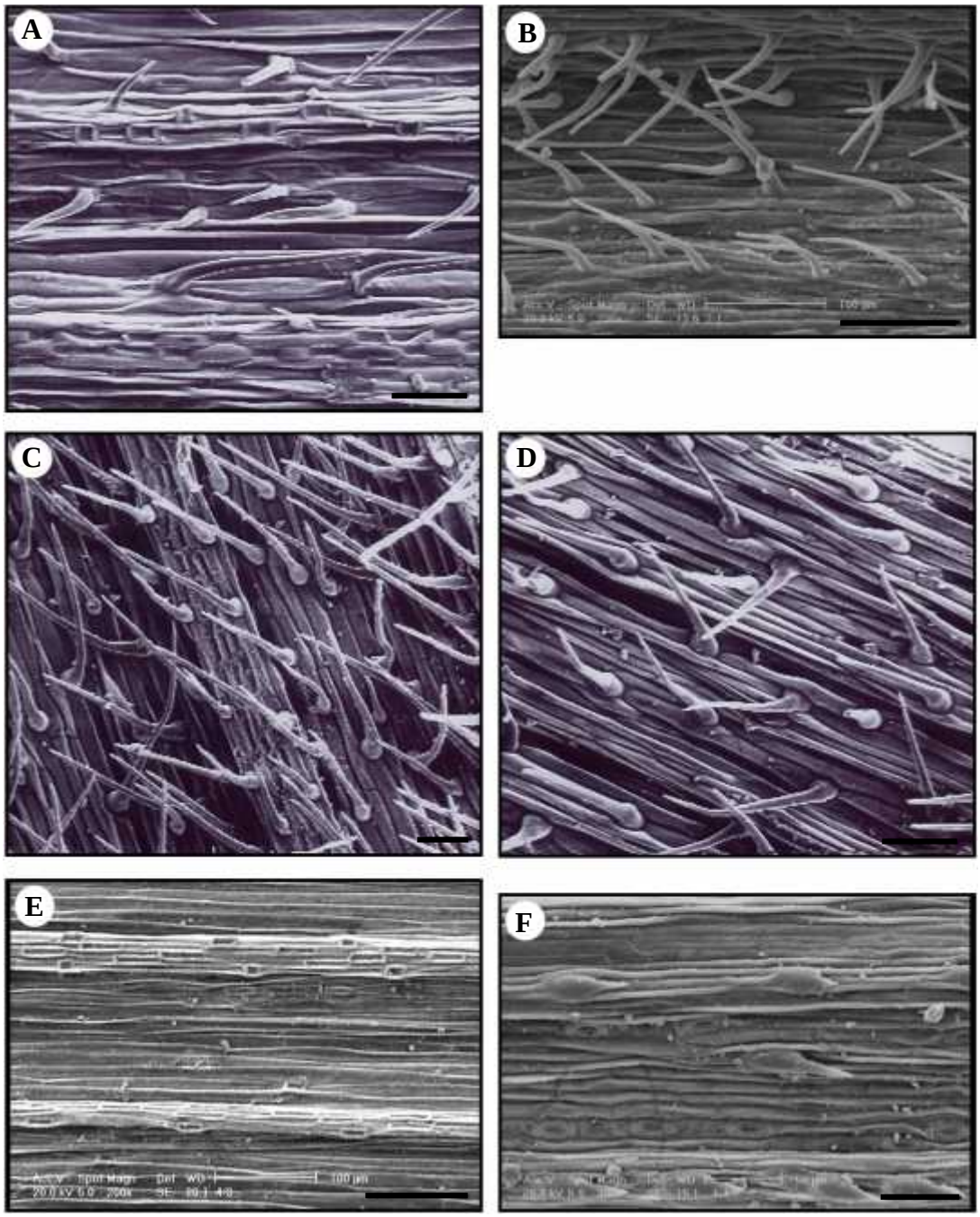

Fig. 3. A-F, abaxial epidermis of the leaf in *Bromus* species; A, *B. sterilis*; B, *B. tectorum*; C, *B. danthoniae*; D, *B. gracillimus*; E, *B. benekenii*; F, *B. biebersteinii*. Scale bar: A,C-D, F=50 µm; B,E=100 µm.

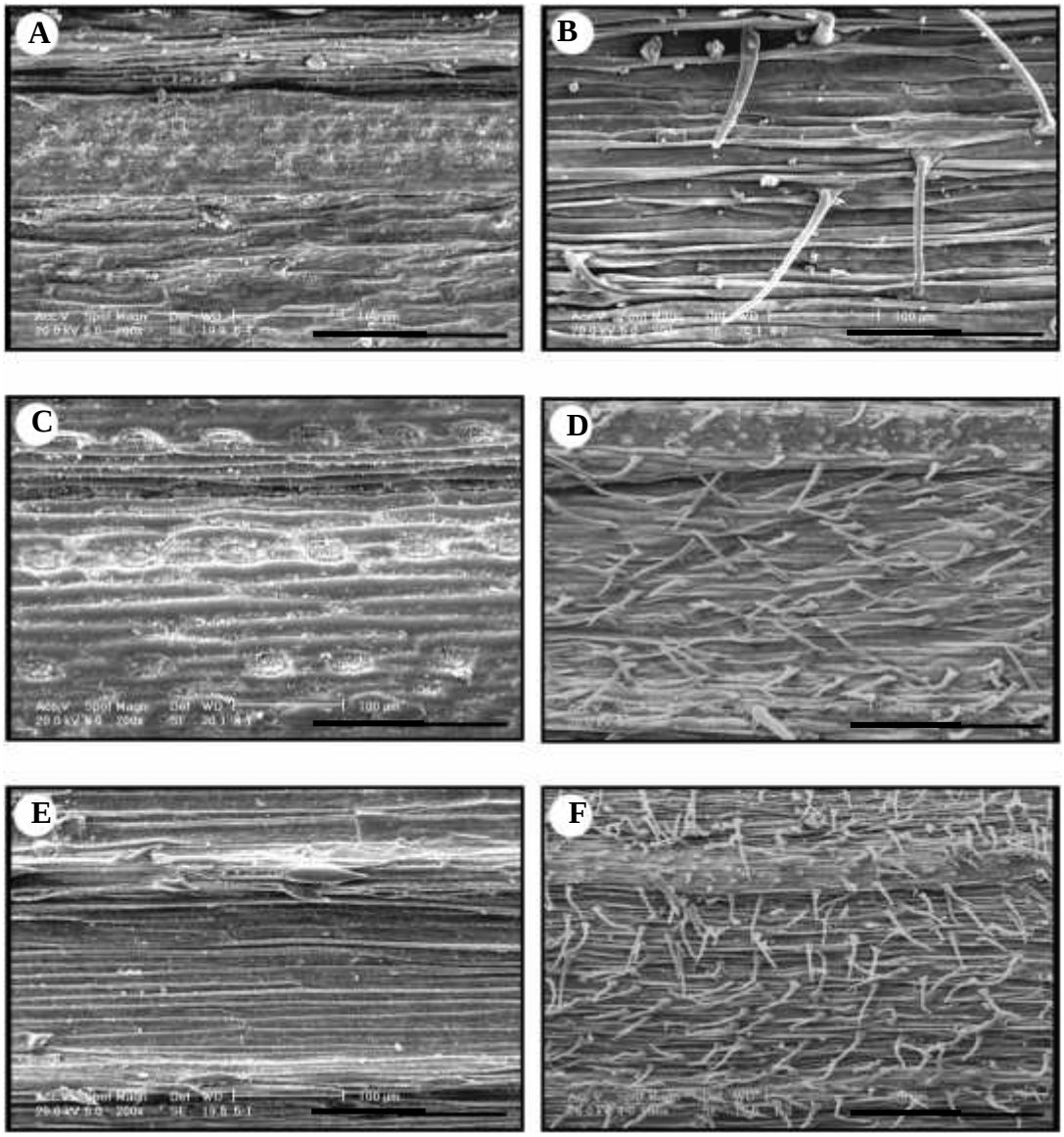

Fig. 4. A-F, abaxial epidermis of the leaf in *Bromus* species; A, *B. cappadocicus*; B, *B. frigidus*; C, *B. inermis*; D, *B. kopetdaghensis*; E, *B. ramosus*; F, *B. riparius*. Scale bar: A-C,E=100 µm; D, F=200 µm.

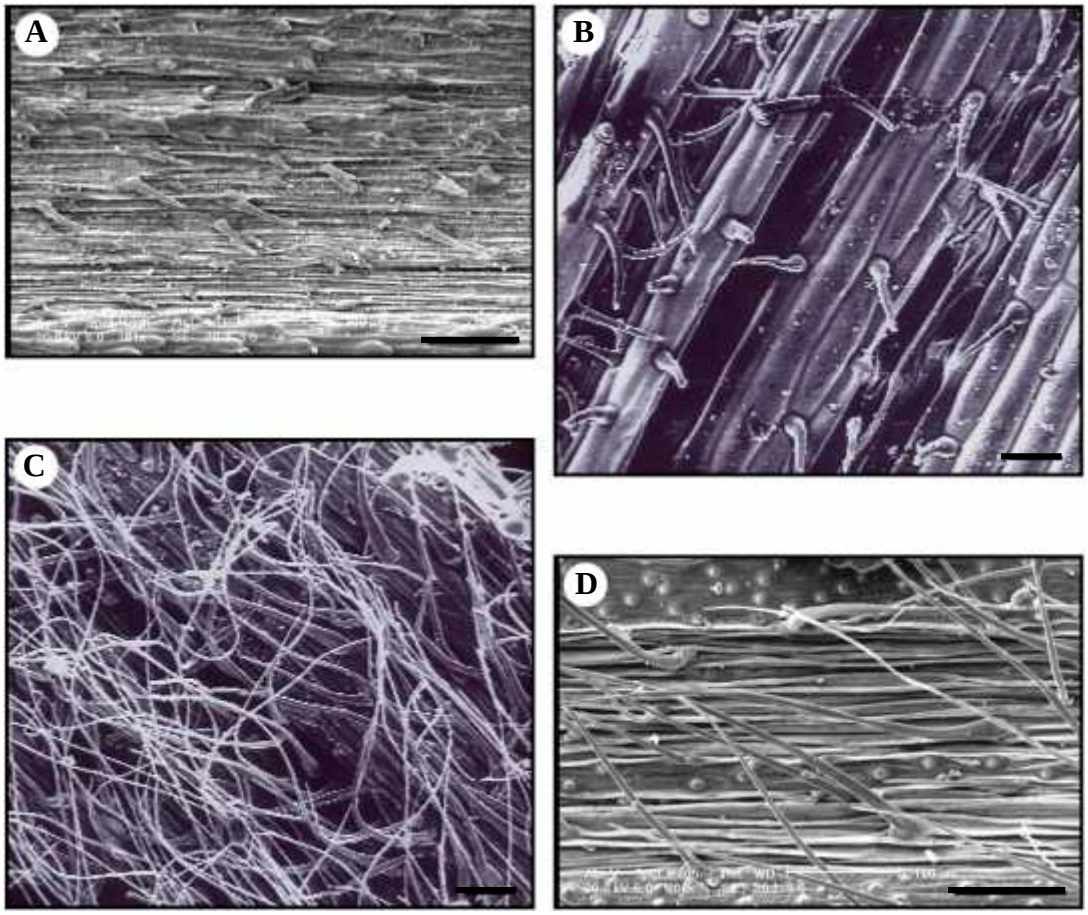

Fig. 5. A-D, abaxial epidermis of the leaf in *Bromus* species; A, *B. stenostachyus*; B, *B. tomentellus*; C, *B. tomentosus*; D, *B. variegatus*. Scale bar: A, D=100 µm; B-C=50 µm.