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Full Length Research Paper

Foliar epidermal anatomy as an aid to the identification of grasses in tribe Aveneae (subfamily Pooideae, Poaceae) from salt range of Pakistan

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In the present studies foliar epidermal anatomy regarding 7 species belonging to 5 genera of tribe Aveneae (Poaceae) was carried out. The studies revealed that different leaf anatomical characters, such as presence or absence of rounded short cells, length of long cells, size of stomatal complex and shape of silica bodies, are helpful in the identification of different species and genera within the tribe. Large cells with maximum length and largest stomatal complex are found in *Avena sp.* In genus *Agrostis, Phalaris and Koeleria* variations exist in the morphology of silica bodies. The studies revealed that diversity in different anatomical characters present within the species and genera is a valuable tool for the differentiation and correct identification of species in tribe Aveneae.

Key words: Foliar, Aveneae, Poaceae, salt range, Pakistan.

INTRODUCTION

The tribe Aveneae includes more than 57 genera in the world, mainly found in temperate regions of both hemispheres and extends to mountainous regions of the tropics. In Pakistan tribe Aveneae is represented by 17 genera and 55 species. In Salt Range seven species of this tribe belonging to 5 genera are present, in which genus *Avena* and *Polypogon* has two species each, while other genera *Agrostis*, *Koeleria* and *Phalaris* are represented by one species each (Ahmad et al., 2009). These species generally grow in the early spring. *Agrostis*, *Koeleria* and *Polypogon* are found in the shady and moist places, while *Polypogon* is also present in marshy places. *Phalaris* and *Avena sp* are the serious weeds of wheat fields. Aveneae is a large heteromorphous tribe, in which different genera show variations morphologically, but the species within the genus are quite similar morphologically, e.g the genus *Polypogon* and *Avena.* The leaf epidermal characters have proved to be very important in providing information of taxonomic importance (Ogie-Odia et al., 2010). Epidermal traits, that is, epidermal cells, stomata and hairs have proved to be an important tool in delimitation of taxa in many plant families (Metcalfe and chalk, 1950 - 1989; Ditsh et al., 1995; Barthlott et al., 1998; Stenglein et al., 2003). It is confirmed that leaf epidermal features assist to elucidate taxonomic relationships at different levels (Prat, 1936; Stebbins, 1956; Metcalfe, 1960; Ellis, 1979; Palmer and Tucker, 1981; Palmer et al., 1985; Davila and Clark, 1990; Cai and Wang, 1994; Mejia-Saules and Bisbey, 2003) and these leaf epidermal characters have a significance in grass systematics and characterization of broad groups within the grasses, particularly subfamilies and tribes.

Of all the non reproductive organs, leaf is the most commonly used in plant taxonomy (Stace, 1965, 1984) and leaf epidermis is of prime importance in solving taxonomic problems parallel with cytology. In tribe Aveneae *Agrostis viridis* seems quite similar to genus *Polypogon* and there is confusion in identifying *Polypogon monospeliensis* from *Polypogon fugax* and

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Avena fatua from *Avena ludoviciana* on the bases of morphological studies, so foliar epidermal studies will help in identification and to solve the taxonomic problems in tribe Aveneae.

MATERIALS AND METHODS

Dried leaves were placed in boiling water by using water bath, to soften the leaves until they become unfolded and were used for epidermal scraping. Fresh leaves were used directly for anatomical studies. Leaf samples were prepared according to the modified method of Cotton (1974) who followed Clark (1960) technique. The fresh or dried leaves were placed in a tube filled with 88% lactic acid kept hot in boiling water bath for about 50 to 60 min. Lactic acid softens the leaf tissues so that its peeling is made possible.

The abaxial and adaxial epidermis was removed, along with the mesophyll cells by using scalpel blade, until only the abaxial epidermis of the leaf remained on the tile. The epidermis was placed on the slide and mounted in clean 88% lactic acid. The micro photographs of the mounted materials were taken by using a camera mounted on Leica light microscope. Different anatomical observations were made on the different species of grasses collected from Salt Range of Pakistan. The following anatomical characters of both abaxial and adaxial epidermis were studied.

Length, width and shape of long cells. Short cells and papillae in intercostal zone are present or absent. Number of rows of long cells between two costal zones, length and width of stomatal complex and shape of guard and subsidiary cells. Shape of subsidiary cells, low or high dome shaped or triangular. Length, width and shape of microhairs, bicellular or unicellular and shape of distal and basal cells present or absent. Length and width of macrohairs, hooks and their shape, their presence or absence. Length, width and shape of silica bodies and short cells and their distribution in costal zone.

RESULTS

The data are arranged systematically as thus explain;

Agrostis viridis Gouan.

Abaxial intercostal long cells, having thin and non sinuous walls, some cells are narrow at the ends than in the middle, 142.5 to 190 µm long and 15 to 20 µm wide. Number of rows of long cells between two costal zones is 3 to 6. Number of stomatal rows between two costal zones is1 to 2. Stomatal complex is 37.5 to 40 µm long and 17.5 to 21.25 µm wide, guard cells are somewhat dumb bell shaped, subsidiary cells parallel sided. Macrohairs none seen. Microhairs none seen Hooks are 52.5 to 60 µm long and 12.5 to 16.25 µm wide.

Costal zone

Silica bodies are slightly dumb bell shaped, cross shaped or slightly rectangular in shape and are 18.75 to 23.75 µm long and 7.5 to 10 µm wide. Short cells are 27.5 are 45 µm long and 7.5 to 8.75 µm wide. Prickles are with bulbous base, pointed at one end is 72.5 to 75 µm long and 20 µm wide (Figure 1a).

Adaxial intercostal zone

Adaxial intercostal long cells are with straight walls and not having uniform diameter is 125 to 287.5 µm long and 15 to 25 µm wide. Number of rows of long cells between two costal zones is 3 to 4. Number of stomatal rows between two costal zones is 1 to 2. Stomatal complex is 35 to 37.5 µm long and 18 to 20 µm wide, guard cells thick in the middle, subsidiary cells parallel sided and very thin walled. None of the Microhairs were seen. Hooks are broad at the base and are 47.5 to 60 µm long and 47 to 60 µm wide.

Costal zone

Silica bodies are slightly rectangular in shape or irregularly shaped and are 18 to 20 µm long and 6.0 to 8.75 µm wide. Short cells are 23.75 to 25 µm long and 8.75 to 11.25 µm wide. Angular prickles are 40 to 52.5 µm long and 20 to 23.75 µm wide (Figure 1b).

Avena fatua Linn.

Abaxial intercostal zone

Abaxial intercostal long cells are very long and straight walls and are 250 to 625 µm long and 20 to 22.5 µm wide. Number of rows of long cells between two costal zones, 6 to 12. Number of stomatal rows between two costal zones is 1 to 3. Stomatal complex is 36.25 to 65 µm long and 17.5 to 27.5 µm wide, guard cells are with uniform diameter, not narrow in the middle and subsidiary cells are parallel sided and are 10 to 12.5 µm long and 5 to 7.5 µm wide. None of the Microhairs and Hooks were seen.

Costal zone

Silica bodies are slight cubical in shape and are 5 to 12.5 µm long and 3.75 to 7.5 µm wide. Angular prickles are 50 to 75 µm long and 20 to 25 µm wide (Figure 2a).

Adaxial intercostal zone

Adaxial intercostal long cells are with straight walls, not uniform in width and are 192.5 to 475 µm long and 27.5 to 35 µm wide. Number of rows of long cells between two costal zones is 3 to13. Number of stomatal rows between two costal zones is 1 to 4. Stomatal complex is 58.25 to 61.25 µm long and 22.5 to 27.5 µm wide, guard cells are with uniform diameter and are 10 to 11.25 µm wide and subsidiary cells are parallel sided and are 6.25 to 8.25 µm wide. None of the Microhairs and Hooks were seen.

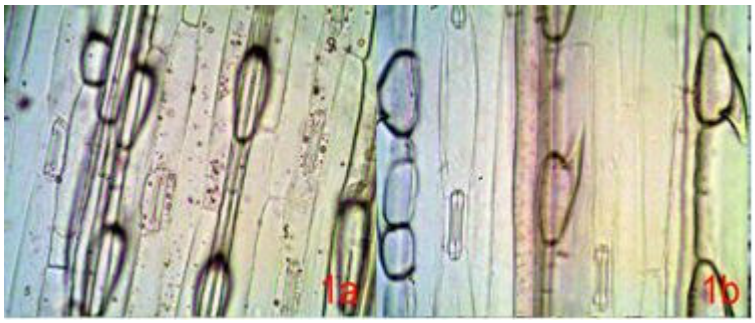

Figure1. (a) *Agrostis viridis*, Abaxial leaf surface; (b) Adaxial leaf surface.

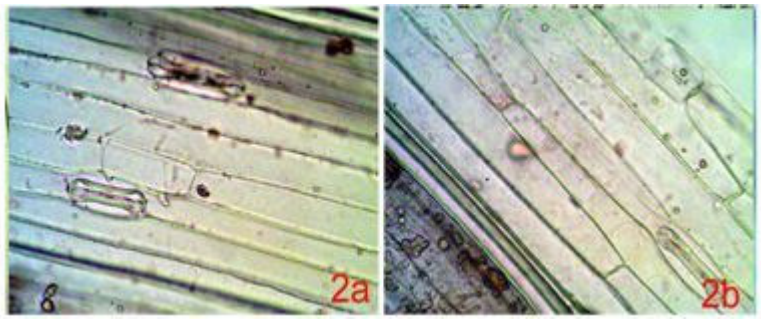

Figure 2. (a) *Avena fatua,* Abaxial leaf surface; (b) Adaxial leaf surface.

Costal zone

Silica bodies are 6.0 to 12.25 µm long and 5.0 to 6.5 µm wide. Prickles are 45 to 50 µm long and 20 to 25 µm wide (Figure 2b).

***Avena ludoviciana* Dur**.

Abaxial intercostal zone

Abaxial intercostal long cells are with straight walls, not uniform in width and are 192.5 to 475 µm long and 27.5 to 35 µm wide. Number of rows of long cells between two costal zones is 7 to 14. Number of stomatal rows between two costal zones is 1 to 3. Stomatal complex is 82.5 to 90 µm long and 25 to 26.5 µm wide, subsidiary cells are parallel sided and they are 5 to 7.5 µm wide, guard cells are with uniform width in the middle and they are 10 to 11.5 µm wide; none of the Microhairs and Hooks seen.

Costal zone

Silica bodies are with elongated, thick, non sinuous walls, some cells having transverse striations; they are 68.5 to 550 µm long and 8.75 to 25 µm wide. Prickles are with elongated bulbous bases, pointed at one end and they are 62.5 to 87.5 µm long and 20 to 22.5 µm wide (Figure 3a).

Adaxial intercostal zone

Number of rows of long cells between two costal zones is 6 to11. Number of stomatal rows between two costal zones is 2 to 4. Stomatal complex is 61.5 to 62.5 µm long and 22 to 25 µm wide. None of the Microhairs and Hooks seen.

Costal zone

Silica bodies are with elongated, thick, non sinuous wall and they are 32.5 to 75 µm long and 6.25 to 7.5 µm wide. Angular prickles present at the margins and in the mid of costal zone are 28.25 to 52.5 µm long and 13.75 to 15 µm wide (Figure 3b).

***Koeleria argentea* Griseb.**

Abaxial intercostal zone

Abaxial intercostal long cells are with non sinuous walls,

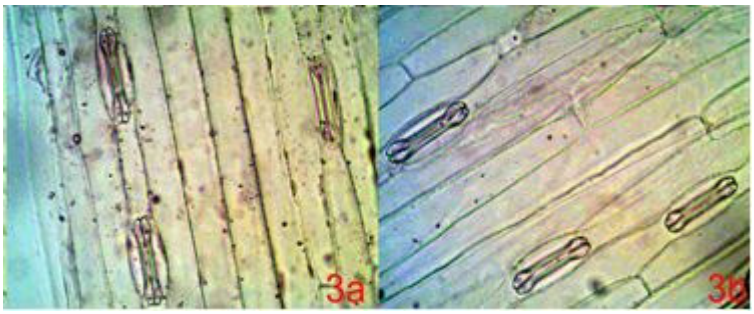

Figure 3. (a). *Avena ludoviciana*, Abaxial leaf surface. (b). Adaxial leaf surface.

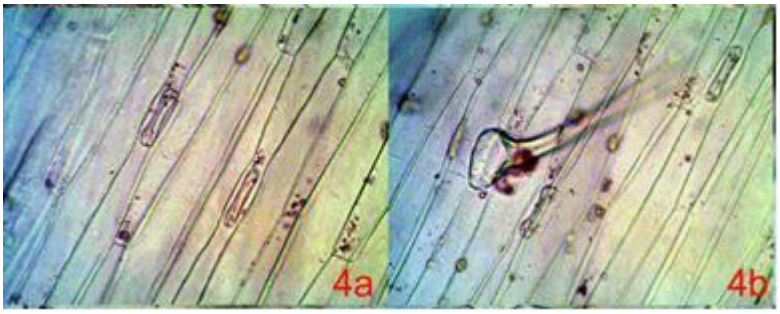

Figure 4. (a) *Koeleria argentia*, Abaxial leaf surface. (b). Adaxial leaf surface.

not uniform in diameter, most cells are cubical, broad in the middle and 205 to 310 µm long and 17.5 to 32.5 µm wide. Number of rows of long cells between two costal zones is 5 to 8. Number of stomatal rows between two costal zones is 1 to 2. Stomatal complex is 35 to 37.5 µm long and 15 to 17.5 µm wide, guard cells are not narrow in the middle and subsidiary cells are very thin walled, parallel sided or low dome shaped. None of the Microhairs were seen. Macrohairs are swollen at the base and pointed towards the tip and they are 112.5 to 185 µm long and 20 to 22.5 µm wide. None of the Hooks were seen.

Costal zone

Silica bodies are horizontally elongated and with smooth or sinuous outline. Angular prickles are 42.5 to 47.5 µm long and 13.75 to 15 µm wide (Figure 4a).

Adaxial intercostal zone

Adaxial intercostal long cells are with non sinuous walls, slightly broad in the middle and they are 142.5 to 332 µm long, 12 to 28.75 µm wide. Number of rows of long cells between two costal zones is 5 to 10. Number of stomatal rows between two costal zones is 1 to 3. Stomatal complex is 35 to 45 µm long and 16.25 to 17.5 µm wide, guard cells are not narrow in the middle and subsidiary cells are very thin walled. Microhairs none seen, macrohairs are with bulbous base and they are 165 to 512.5 µm long and 20 to 32.5 µm wide. Hooks were not seen.

Costal zone

Silica bodies are elongated and with sinuous or smooth outline and with many layers of silica bodies. Prickles are 47.5 to 58.75 µm long and 8.75 to16.25 µm wide (Figure 4b).

Phalaris minor Retz.

Abaxial intercostal zone

Abaxial intercostal long cells are with thin, non sinuous walls and they are 90 to 270 µm long, 18 to 22 µm wide. Number of rows of long cells between two costal zones is 5 to 15. Number of stomatal rows between two costal zones is 2 to 3. Stomatal complex is 44 to 46 µm long and 22 to 25 µm wide, guard cells are dumb bell shaped and subsidiary cells are parallel sided or low dome shaped. None of the Microhairs and Hooks were seen.

Costal zone

Silica bodies are with rounded ends and smooth. Short

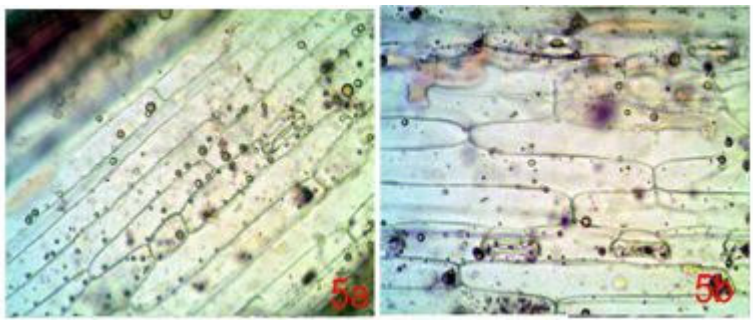

Figure 5. (a) *Phalaris minor*, Abaxial leaf surface; (b) Adaxial leaf surface.

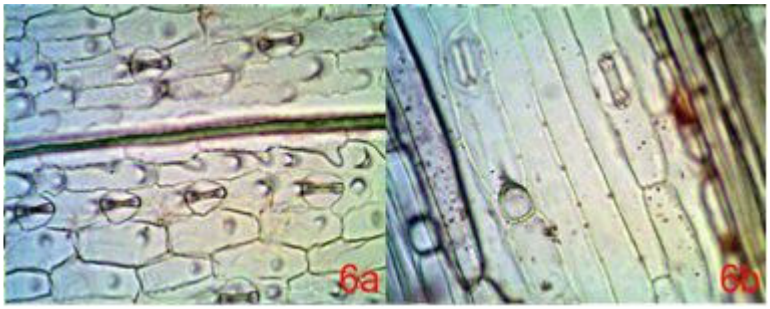

Figure 6. (a)*Polypogon fugax,* Abaxial leaf surface; (b) Adaxial leaf surface.

cells are with sinuous walls (Figure 5a).

Adaxial intercostal zone

Adaxial intercostal long cell are with thin non sinuous walls they are 124 to 275 µm long and 20 to 22.5 µm wide. Number of rows of long cell, between two costal zones is 6 to 15. Number of stomatal rows between two costal zones is 1 to 2. Stomatal complex is 45 to 47.5 µm long and 25 to 27.5 µm wide, guard cells are dumb bell shaped, they are 8 to 10 µm wide and subsidiary cells are parallel side or low dome shaped, they are 7 to 7.5 µm wide. None of the Microhairs and Hooks were seen.

Costal zone

Silica bodies are with rounded ends and smooth, they are 50 to 52.5 µm long and 13 .75 to 16.25 µm wide. Short cells are with sinuous walls. Prickles are 50 to 55 µm long and 14 to 17.5 µm wide (Figure 5b).

***Polypogon fugax* Nees ex Steud**

Abaxial intercostal zone

Abaxial intercostal long cells are with slightly sinuous walls and they are 102.5 to 212.5 µm long and 15 to 22.5 µm wide, rounded short cells are present between long cells, they are 12.5 to 15 µm long horizontally and 12.5 to 15.25 µm wide. Number of rows of long cell, between two costal zones is 6 to 11. Number of stomatal rows between two costal zones is 1 to 4. Stomatal complex is 35 to 36.25 µm long and 16.25 to 22.5 µm wide, guard cells are dumb bell shaped and subsidiary cells are dome shaped. None of the Microhairs and Hooks were seen.

Costal zone

Silica bodies are dumb bell shaped, they are 21.25 to 22.5 µm long and 4.0 to 5.0 µm wide. Short cells are with sinuous outline, they are 20 to 31.5 µm long and 7.5 to 8.75 µm wide. Prickles none seen (Figure 6a).

Adaxial intercostal zone

Adaxial intercostal long cells are with slightly sinuous or straight walls, they are 37.5 to 72.5 µm long and 11.25 to 15 µm wide. Number of rows of long cells between two costal zones is 5 to 7. Number of stomatal rows between two costal zones is 1 to 2. Stomatal complex is 2.5 to 25 µm long and 17.5 to 18.75 µm wide, guard cells are dumb bell shaped, subsidiary cells are high dome shaped.

None of the Microhairs were seen. Hooks were present, 15.5 to 17.25 µm long and 12 to 15.5 µm wide.

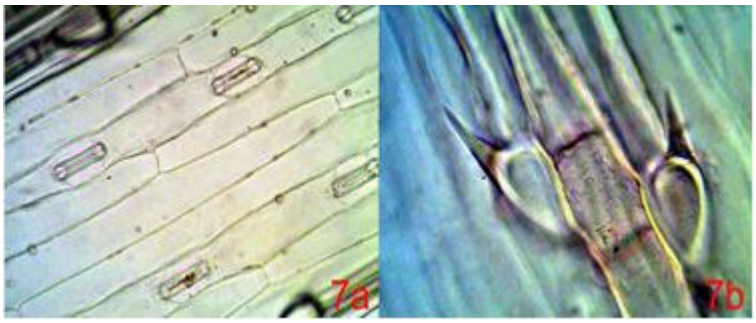

Figure 7. (a) *Polypogon monspeliensis*, Abaxial leaf surface; (b) Adaxial leaf.

Costal zone

Silica bodies are dumb bell shaped and present in continuous row, they are 17.5 to 21.25 µm long and 6.25 and 7.5 µm wide. Rounded short cells are present between long cells, prickles not seen (Figure 6b).

***Polypogon monspeliensis* (Linn.) Desf.**

Abaxial intercostal zone

Abaxial intercostal long cells are with non sinuous walls, short cells are present between long cells, they are 127.5 to 255 µm long and 17.5 to 25 µm wide. Number of rows of long cell between two costal zones is 3 to 7. Number of stomatal rows between two costal zones is 1 to 2. Stomatal complex is 47.5 to 52.5 µm long and 21.25 to 22.5 µm wide, guard cells are thick in the middle, they are 7.5 µm wide, subsidiary cells are low dome shaped, 7.5 µm wide. None of the Microhairs were seen. Macrohairs are with swollen base and tapering towards the apex, they are 180 to 252.5 µm long and 10 to 13.75 µm wide. Hooks are present between long cells, base somewhat rounded, pointed at one side, they are 40 to 61.25 µm long and 5 to 12.5 µm wide.

Costal zone

Silica bodies are with straight and non sinuous walls, they are 13.25 -18.5 µm long and 12.5t to 14.5 µm wide. Prickles, 47.5 to 50 µm long and 13.75 to 15 µm wide (Figure 7a).

Adaxial intercostal zone

Adaxial intercostal long cells are with none sinuous walls. Number of rows of long cells between two costal zones is 3 to 5. Number of stomatal rows between two costal zones is 1 to 2. Stomatal complex is 40 to 41.25 µm long and 20 to 21.25 µm wide, guard cells are thick in the middle and subsidiary cells are low dome shaped. Microhairs were not seen. Macrohairs are swollen at the base and tapering to the apex, they are 150 to 287 µm long and 8.75 to 12 µm wide. Hooks were not seen.

Costal zone

Silica bodies are with none sinuous walls, they are 12 to 15 µm long and 8.5 to 13 µm wide, 3 to 4 rows of silica bodies. Prickles are 50 to 55 µm long, and 11.25 to15 µm wide (Figure7b).

DISCUSSION

The present study revealed that micro morphological characters of the leaf epidermis have a considerable value in identification. Both species of genus *Polypogon* are different from others in having rounded short cells found frequently at regular intervals between long cells. Long cells with maximum length (250 to 625 µm) are found in *A. fatua* followed by *A. sterilis*. It is observed that long cells are comparatively larger in the species of this tribe, than other species and all these species are mostly present in moist and shady habitat as Stace (1965) found that epidermal cells are large on leaves of plants from more humid and shady areas and smaller in dry areas and soil and plants grown in higher altitude. All the species of this tribe have parallel sided subsidiary cells, however low dome shaped subsidiary cells are also observed in *Koeleria argentia*, *Phalaris minor* and *P. monspeliensis* while in *P. fugax* only dome shaped subsidiary cells are observed. Largest stomata are found in genus *Avena* (Figures 2a and b) while smaller in genus *Polypogon* (Figures 6a and 7a). Absence of microhairs and macrohairs in all the species of this tribe is observed. *Koeleria argentia* is the only species in which long marcrohairs are present (Figure 4b). In genus *Polypogon*, subsidiary cells are dumb bell shaped or horizontally elongated with sinuous outline. Metcalfe (1960) studied the abaxial leaf epidermis of *P. monspeliensis* and found the same results.

Anatomical characters of leaves in grasses are used as taxonomic markers at generic and specific levels (Ahmad et al., 2010). In *Agrostis viridis* silica bodies are slightly dumb bell shaped, cross shaped or rectangular. In *P. minor* silica bodies are smooth and with rounded ends, while *K. argentia* has horizontally elongated silica bodies with smooth or sinuous outline. In genus *Avena* silica bodies are cubical in shape as in *A. fatua* and in *A. sterilis* elongated, thick silica bodies with non sinuous walls are recorded and it is observed that *A. sterilis* has silica bodies with maximum length in the whole tribe and can be distinguished from *A. fatua* by having long silica bodies. The present studies showed that morphological variations of silica bodies are present in different species of the tribe. According to Prat (1932) and Clayton (1981) complexity and diversity of silica bodies is much less elaborate in less advanced sub family Pooideae. It is observed in the present studies of silica bodies in this tribe that in most species silica bodies with straight walls are present and do not show any complexity in structure. Ying et al. (2006) conducted his studies on different species of *Calamagrostis* of tribe Aveneae and noted the leaf epidermis in *Calamagrostis* are of festucoid type and, intercostal long cells were elongated in all species and silica bodies were usually elongated with a sinuous outline. In the present studies in *K. argentia* and *A. fatua* elongated silica bodies with smooth or sinuous outline are recorded. It is observed that leaf epidermal features help in identification of different genera and species and to elucidate taxonomic relationship at species, generic and tribe level in tribe Aveneae.

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