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Full Length Research Paper

Identification and differentiation of Leaf epidermal obtained from Species of Setaria weeds in Pakistan

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Leaf epidermal anatomy of 5 species of Setaria weeds collected from salt range was carried out to identify and differentiate these species, as there is confusion in identification of these species on the basis of morphology. Variations in different leaf epidermal characters are observed in different *Setaria* species. *Setaria glauca* is identified by the absence of prickles on the abaxial side, while prickles are present on both abaxial and adaxial side of *Setaria viridis*, *Setaria intermedia*, *Setaria italica* and *Setaria verticillata*. Two types of silica bodies are observed in *Setaria* species. Cross shaped silica bodies are observed in *S. verticillata*, while in other *Setaria* species dumb bell shaped silica bodies are found. Microhairs were present only on the abaxial side of *S. glauca*, while absent in other species, so this character also distinguishes it from other species. Largest stomatal complex is found in *S. glauca*, while in *S. intermedia,* stomatal complex is smaller as compared to other species. It is observed that different leaf anatomical characters assist in identification and differentiation of different *Setaria* species.

Key words: Leaf epidermal, Setaria, poaceae, salt range.

INTRODUCTION

The genus *Setaria* belongs to the grass family Poaceae, sub family Panicoideae, which is a very large cosmopolitan family consisting of about 50 to 60 tribes, 660 genera and 9000 species throughout the world (Olorode, 1984; Hutchinson and Dalziel, 1972). Genus *Setaria* is represented by 125 species in the tropical regions of the world and 6 species in Pakistan (Cope, 1982). Out of these, five species are present in the Salt Range of Pakistan (Ahmad et al., 2009). The only world wide cultivated cereal of this genus is *Setaria italica* (L.) P. Beauv, that is used to cure chicken pox in some areas of Pakistan (Ahmad et al., 2010). Weedy species of *Setaria* are one of the worst weed groups interfering with world agriculture and other disturbed and managed habitats (Kashavarzi and Seifali, 2007).

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The grass family Poaceae is noted for its wide diversity and complexity and so has posed many problems to the taxonomists using the traditional methods based on gross morphology (Srivastava, 1978) and morphological characters are some how overlapped at specific and intraspecific levels, however anatomical characters in *Setaria* species are of diagnostic value (Kashavarzi and Seifali, 2005a). Before the end of 19th century, taxonomists were confined to the features of reproductive organs, as floral characters were considered to provide the most valuable characters to taxonomic affinities (Nwokeocha, 1996). Of all the non reproductive organs, the leaf is the most widely used in plant taxonomy (Stace, 1965, 1984). Srivastava (1978) described the leaf epidermis as the second most important character after cytology for solving taxonomic problems.

The variations in leaf epidermal anatomical characters that is, stomata, long cells, microhairs, silica bodies shape and size are helpful in identifying the species and

these epidermal traits have proved to be an important tool in delimitation of taxa in many plant families (Barthlott et al., 1998; Stenglein et al., 2003).

The greatest complexity and diversity of silica bodies is found in most advanced panicoideae (Prat, 1932; Clayton, 1981). As there is difficulty in identifying different *Setaria* species and distinguish them from each other on the bases of their morphology, so leaf epidermal anatomical studies were carried out to determine the pattern of variations in epidermal studies, leaf epidermal features helpful in identification and to assess their value in species identification. Such studies are of particular value for the scientists, who need to identify small scraps of plant material for example, Pharmacognosists (in drugs), forensic experts (as clues) and in gut and faeces for animal dieticians (Stace, 1980).

MATERIALS AND METHODS

Leaves of the five *Setaria* species were used for anatomical studies. Dried leaves were placed in boiling water for few minutes to soften the leaves until they became unfolded and were ready for epidermal scrapping. 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 tissues of leaves due to which its peeling off is made easy.

To prepare the abaxial surface, the leaf was placed keeping its adaxial surface upward and then it was flooded with 88% cold lactic acid. The adaxial epidermis was cut across the leaf using a sharp scalpel blade and scrapped away together with the mesophyll cells until only the abaxial epidermis of the leaf remained on the tile. The epidermis was placed outside and mounted in clean 88% acetic acid.

Same procedure was followed to prepare the adaxial epidermis but the leaf was placed in the uppermost part of the abaxial surface. The photographs of these mounted materials were taken using a camera (35 mm) mounted on the microscope.

Anatomical observations were made on available representative specimens for the taxa. The specimen of 5 species of the genus *Setaria* are preserved in the herbarium of Quaid-i-Azam University Islamabad. A high variability was found in the length and width of different characters, therefore the typical size is given. To obtain the typical size, numerous measurements were made on each of the representative specimens.

RESULTS

The results of this study show the key to the species of *Setaria* on the basis of leaf epidermal anatomical characters:

1. *S. glauca*: The silica bodies that are dumb bell shaped have an average length of more than 25 ∝m, while the silica bodies that are cross shaped, have an average length of 17.5 to 12.5 ∝m.
2. *S. verticillata*: The long macrohairs present adaxially are 32.5 to 36 µm long, tapering towards the apex. Also, macro-hairs are present adaxially with 33 to 40.5 µm length
3. *S. viridis:* Hooks are present on the abaxial surface, with an average length of more than 17 ∝m. Also, they are present on the abaxial surface, with an average length of 15 ∝m or less than 15 ∝m.
4. *S. italic:* The stomata are found with low dome shaped subsidiary cells on the abaxial side and high dome shaped subsidiary cells on the adaxial side.
5. *S. intermedia:* The stomata are found with low to high dome shaped subsidiary cells.

Setaria glauca L. Beauv.

Adaxial side

Costal zone: The prickles are 45 to 85 ∝m · 25 mm and they have a beak like projection at one end. The number of cells between two prickles is 4 to 6. Short cells are 15 to 20 mm · 7.5 mm. The silica bodies and short cells are alternatively present to each other in this zone. However, the silica bodies are dumb bell shaped, with 27.5 to 30 mm · 10 to 12.5 mm.

Intercostal zone: In the intercoastal zone, the microhairs are 22.5 to 30 ∝m · 7.5 ∝m, and they are bicelled and blunt at the end. The hooks, which are 60 to 55 ∝m · 25 to 30 ∝m, are beaked at one end, while the stomata is 47.5 to 52.5 ∝m · 22.5 to 25 ∝m. The guard cells are dumb bell shaped, while the subsidiary cells are dome shaped. The number of rows of cells between two costal zones is 6 to 7, while the number of stomatal rows between two costal zones is 2. The long cells are 120 to 620 ∝m · 35 to 40 ∝m, although its walls are slightly sinuous. However, long cells having stomata are longer than other cells (Figure 1a).

Abaxial side

Costal zone: In the coastal zone, the average number of rows of silica bodies is 30 to 32.5 ∝m · 12.5 ∝m, although 1 silica body is dumb bell shaped. The prickles are absent and the short cells are 15 · 10 ∝m. However, silica bodies and short cells are present alternatively to each other.

Intercostal zone: In the intercostal zone, microhairs are 20 to 22.5 ∝m · 7.5 to 10 ∝m. Also, they are bicelled and blunt at the end with 255 × 7.5 µm. The hooks are absent and the stomatal are complex from 45 to 60 ∝m · 30 to 27.5 ∝m. The guard cells are dumb bell shaped, while the subsidiary cells are low dome shaped. The number of rows of cells between two costal zones is 9 to 19, while the number of stomatal rows between two costal zones is 2 to 6. The long cells are 70 to 450 ∝m · 3 0 to 35 ∝m, and

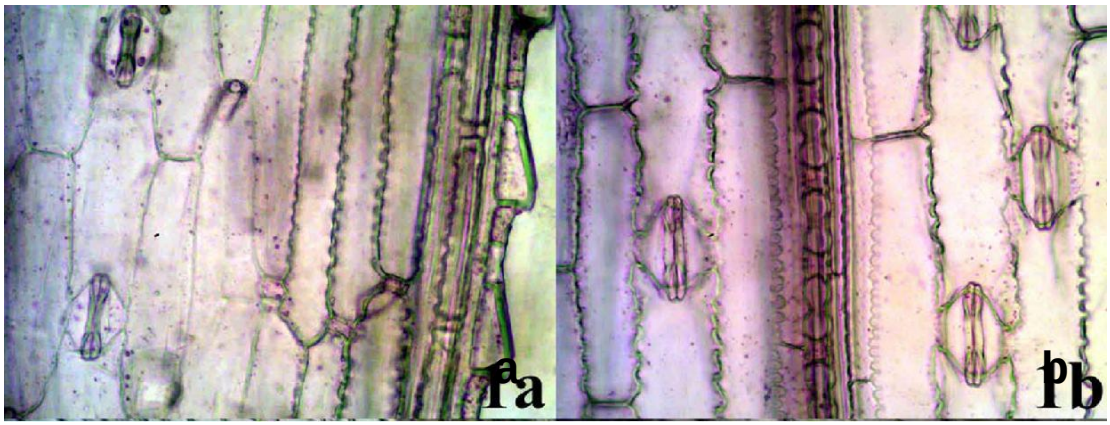

Figure 1. (a) *Setaria glauca*, Leaf adaxial epidermis, (b) Leaf abaxial epidermis.

and are slightly sinuous or straight, with a rectangular shape. Also, they are present, adjacent to the costal zones and are deeply sinuous. However, some long cells are wide in the middle and are narrow towards the sides (Figure 1b).

***Setaria viridis* (Linn.) P. Beauv.**

Adaxial side

Costal zone: In the coastal zone, the average number of rows of the silica bodies, which are dumb bell shaped, is 1 to 3, while the prickles are 27.5 to 35 ∝m · 10 to 15 ∝m. The short cells are 7.5 to 17.5 ∝m · 5 ∝m, although the silica bodies and short cells are present alternatively to each other in this zone. However, the silica bodies are dumb bell shaped with 15 to 17.5 ∝m · 5 ∝m.

Intercostal zone: In the intercoastal zone, the microhairs are 15 to 17.5 ∝m · 5 ∝m, and they are bicellular and blunt at the tip. The hooks are absent and the macrohairs are absent as well, but the stomatal complex is 22.5 to 25 ∝m · 17.5 to 20 ∝m. The guard cells are dumb bell shaped, while the subsidiary cells are dome shaped. The number of rows of long cells between two costal zones is 2 to 3, while the long cells themselves are 85 to 200 ∝m · 17.5 to 25 ∝m. However, the walls are wavy (Figure 5a).

Abaxial side

Costal zone: In the coastal zone, the average number of rows of silica bodies is 1. The prickles are 25 to 35 ∝m · 12.5 ∝m, while 2 to 6 cells are found between two prickles. The silica bodies are dumb bell shaped with 12.5 to 20 ∝m · 5 ∝m, while the short cells are 10 to 15 ∝m · 5 to 7∝m. However, the silica bodies and short cells are present in this zone alternatively to each other.

Intercostal zone: In the intercoastal zone, microhairs are 25 to 27.5 ∝m · 5 ∝m. The macrohairs are bicelled and absent, while the hooks are rounded at the base and are pointed at one end with 17.5 · 15 ∝m. The stomatal complex is 20 to 35 ∝m · 15 to 30 ∝m; moreover, the guard cells are dumb bell shaped, while the subsidiary cells are dome shaped. The number of rows of cells between two costal zones is 6 to 11, while the number of stomatal rows between two costal zones is 1 to 2. However, the long cells are 62.5 to 430 ∝m · 12.5 to 15 ∝m, although the walls are slightly wavy (Figure 5b).

***Setaria italica* (Linn.) P. Beauv.**

Adaxial side

Costal zone: In the coastal zone, the average number of rows of silica bodies is 1 to 3. The prickles are 5 to 15 ∝m · 7.5 ∝m and are frequently present in this zone. The silica bodies are 15 ∝m · 5.0 to 7.5 ∝m, and are dumb bell in shape.

Although, the short cells are 5 to 15 ∝m · 7.5 ∝m, the silica bodies and short cells are present in this zone alternatively to each other.

Intercostal zone: In the intercoastal zone, microhairs are 25 · 5 ∝m, and are bicelled and blunt at the end. Both the macrohairs and hooks are absent in this zone. The stomatal complex is 22.5 to 25 ∝m · 20 to 22.5 ∝m. The guard cells are dumb bell shaped, while the subsidiary cells are tall dome shaped. The number of rows of cells between two costal zones is 6 to 10, while the number of stomatal rows between two costal zones is 2. However, the long cells are 80 to 115 ∝m · 10 ∝m, although long cells having stomata are much longer, as compared to

other cells (Figure 3a).

Abaxial side

Costal zone: In the coastal zone, the prickles are 32.5 to 40 ∝m · 20 to 22.5 ∝m, and they are slightly beaked at one end. The silica bodies are dumb bell shaped with 7.5 to 15 ∝m, while the short cells are 7.5 · 5 ∝m.

Inter costal zone: In the intercoastal zone, the microhairs are 20 to 27.5 ∝m · 5 ∝m, in that both the hooks and macrohairs are absent. The stomatal complex is 20 to 25 ∝m · 10 to 15 ∝m. The guard cells are dumb bell shaped, while the subsidiary cells are low dome shaped. The number of rows of long cells between two costal zones is 6 to 10, while the number of stomatal rows between two costal zones is 2. The long cells are 62.5 to 150 ∝m · 12.5 to 15 ∝m, and its walls are slightly sinuous. However, the long cells having stomata are much longer as compared to other cells (Figure 3b).

***Setaria verticillata* (Linn.) P. Beauv.**

Adaxial side

Costal zone: Here, the average number of rows of silica bodies is 1 to 3. The prickles are 42.5 to 45 ∝m · 22.5 to 25 ∝m and are beaked at one end. A total of 2 to 9 cells are found between the prickles. The short cells are 7.5 to 17.5 ∝m · 7.5 to 10 ∝m, while the silica bodies are cross shaped with 12.5 to 17.5 ∝m · 7.5 to 10 ∝m. However, the silica bodies and short cells are alternatively present to each other in this zone.

Intercostal zone

Here, the microhairs and hooks are absent, but the stomatal complex is 25 to 30 ∝m · 25 to 27 ∝m. The guard cells are dumb bell shaped, while the subsidiary cells are dome shaped. The number of rows of long cells between two costal zones is 7 to 9, while the number of stomatal rows between two costal zones is 2. The long cells are 45 to 137.5 ∝m · 20 to 30 ∝m and its walls are sinuous. However, the cells having stomata are much longer than other cells (Figure 4a).

Abaxial side

Costal zone: In this zone, the average number of rows of silica bodies is 1. The prickles are 42.5 to 45 ∝m · 15 to 20 ∝m, and are beaked at one end. It is observed that 3 to 9 cells are present between two prickles. The short cells are 12.5 to 15 ∝m · 12.5 to 15 ∝m. Although, the silica bodies are 12.5 to 20 ∝m · 7.5 to 10 ∝m, they are x shaped or dumb bell shaped.

Inter costal zone: Here, the microhairs are 17.5 to 22.5 ∝m · 5 to 7.5 ∝m, but they are blunt at one end. Both the macrohairs and hooks are absent in this zone. The stomatal complex is 25 to 27.5 ∝m · 17.5 to 25 ∝m. The guard cells are dumb bell shaped, while the subsidiary cells are dome shaped. The long cells are 45 to 225 ∝m · 17.5 to 25 ∝m, but its walls are sinuous.

However, the cells having stomata are much longer than other cells (Figure 4b).

***Setaria intermedia* Roem. and Schult**

Adaxial side

Costal zone: In this zone, prickles are 50 to 55 ∝m · 20 to 22.5 ∝m, and they are pointed at one end. The silica bodies are dumb bell shaped, with 15 to 20 ∝m · 5 to 7.5 ∝m. The short cells are 17.5 ∝m · 7.5 ∝m. However, the silica bodies and short cells are present alternatively to each other in this zone.

Intercostal zone: Here, microhairs are 20 · 5 ∝m, and are bicelled and blunt at the end. The microhairs, as well as the hooks, which are 25 to 30 ∝m · 17.5 ∝m are absent. The stomatal complex is 20 to 22.5 ∝m · 17.5 to 20 ∝m. The guard cells are dumb bell shaped, while the subsidiary cells are dome shaped. The number of rows of cells between two costal zones is 8 to 10, while the number of stomatal rows between two costal zones is 2 to 3. The long cells are 62.5 to 150 ∝m, and they have sinuous walls. The cells having stomata are much longer than other cells (Figure 2a).

Abaxial side

Costal zone: In this zone, prickles are 27.5 to 40 ∝m · 15 to 17.5 ∝m. The silica bodies are dumb bell shaped, with 15 · 5 ∝m. The short cells are 7.5 to 10 ∝m · 7.5 ∝m. However, the silica bodies and short cells are present alternatively to each other in this zone.

Inter costal zone: In this zone, microhairs are 20 · 5 ∝m and are blunt at the end. The macrohairs are absent, while the hooks, which are 15 · 10 ∝m are beaked at one end. The stomatal complex is 17.5 to 25 ∝m · 12.5 to 17.5 ∝m. The guard cells are dumb bell shaped, while the subsidiary cells are dome shaped. The number of rows of long cells between two costal zones is 4 to 9, while the number of stomatal rows between two costal zones is 1 to 2. Long cells are 100 to 165 ∝m · 10 to 15 ∝m, and are slightly sinuous. However, the long cells having stomata

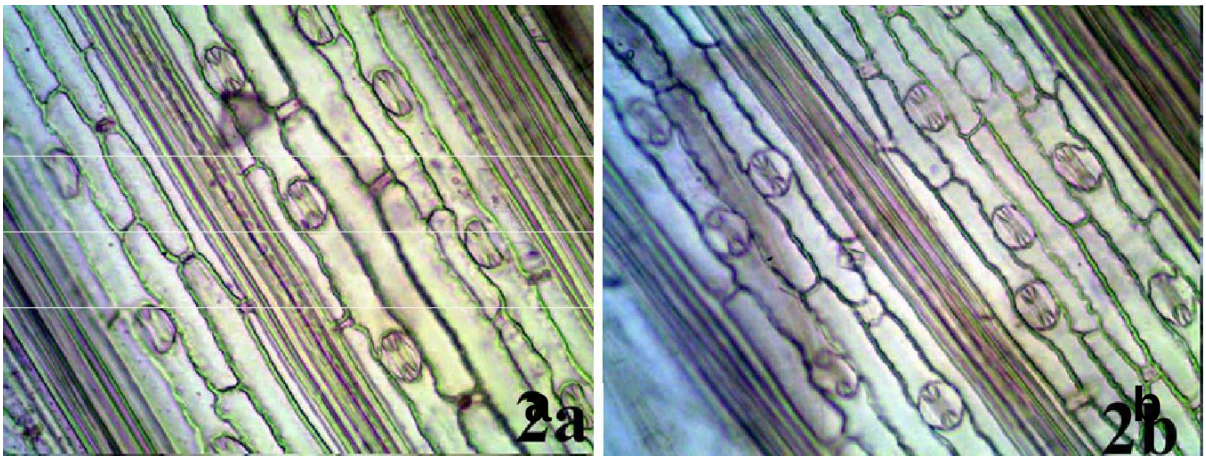

Figure 2. (a) *Setaria intermedia*, Leaf adaxial epidermis, (b) Leaf abaxial epidermis.

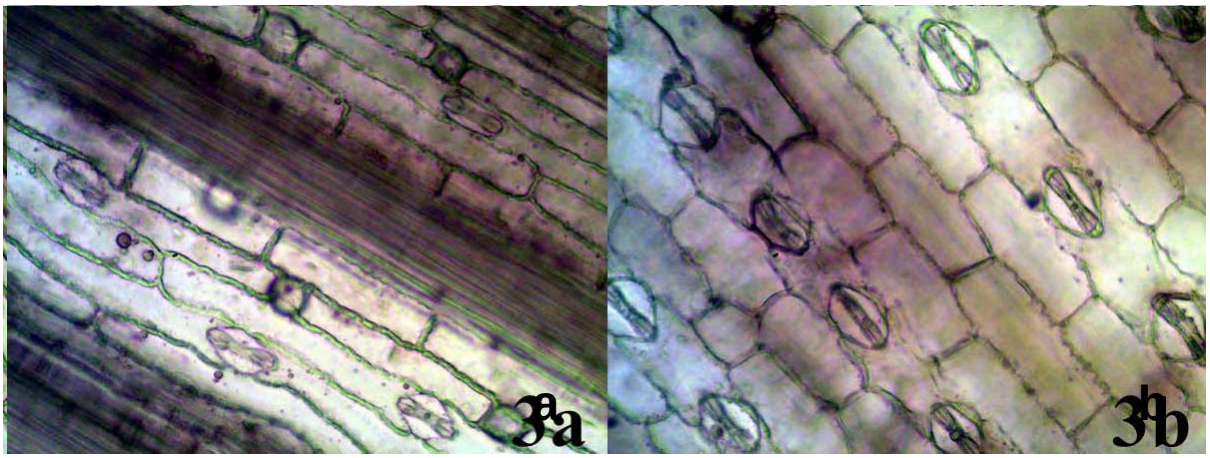

Figure 3. (a) *Setaria italica*, Leaf adaxial epidermis, (b) Leaf abaxial epidermis.

are much longer as compared to other cells (Figure 2b).

DISCUSSION

Anatomical studies have been used successfully to clarify taxonomic status and also help in the identification of species. Anatomical studies showed variations in size of microhairs, short cells, silica bodies, long cells, hooks, stomata, as well as in the shape of different organelles of different *Setaria* species. The size and shape of organelles were different on both the abaxial and adaxial surfaces of leaf epidermis because of the bifacial leaf which also confirmed the findings of Shouliang et al. (1996).

In the present study, epidermal anatomy of five different species of *Setaria* was carried out, because morphological studies of grasses are not sufficient for their correct identification (Sherma, 1979). Grass leaf has two distinct costal and inter costal zones, which alternate with each other. There are minor differences in the structure of adaxial and abaxial surfaces (Chaudhary et al., 2001). The prickles were found to be absent on the abaxial surface in *S. glauca*, and present on both abaxial and adaxial side of *S. viridis*, *S. intermedia*, *S. italica* and *S. verticillata*, thus the presence of prickles only on adaxial surface of *S. glauca* distinguishes it from the rest of *Setaria* species (Figure 1a). Two types of silica bodies were observed in *Setaria* species. Cross shaped silica bodies were observed in *S. verticillata*, while in other *Setaria* species dumb bell shaped silica bodies were found (Figures 1b and 2a). The evolutionary trend in silica bodies is from dumb bell shaped to cross shaped silica bodies (Shouliang et al., 1996).

Microhairs were present on both the abaxial and adaxial side of the leaf epidermis of all the species. The

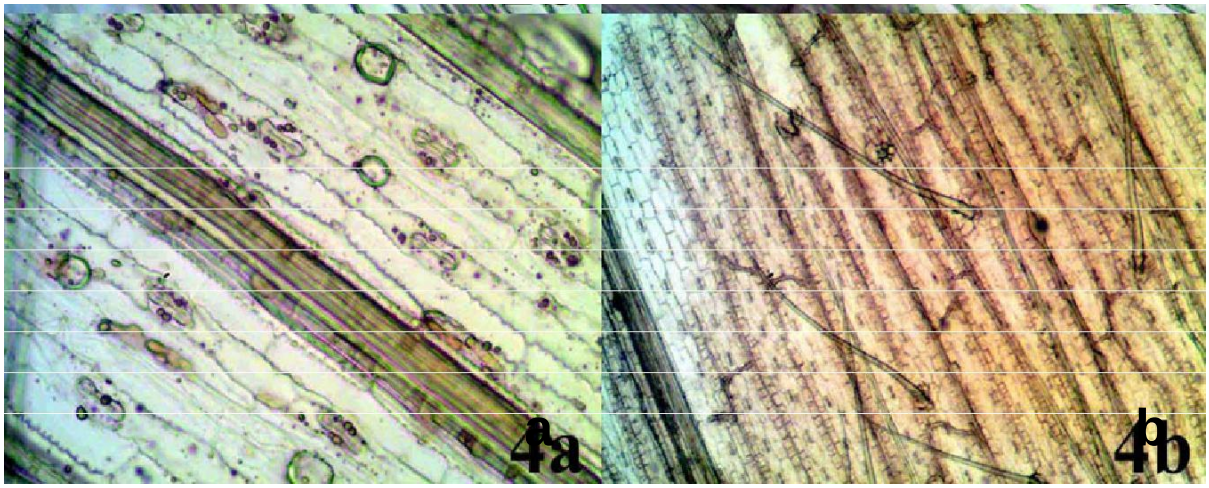

Figure 4. (a) *Setaria verticillata*, Leaf adaxial epidermis showing macrohairs, **(b)** Leaf abaxial epidermis.

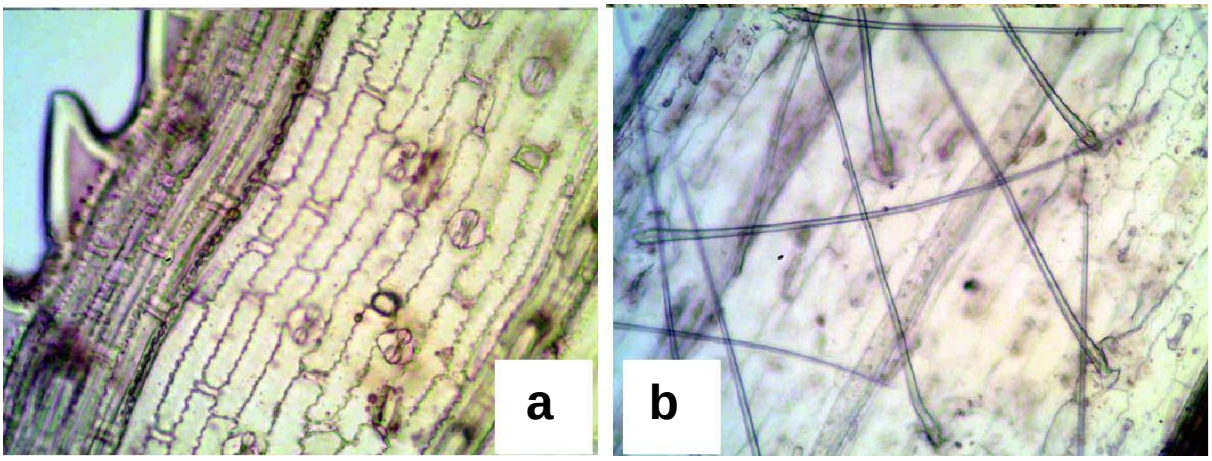

Figure 5. (a) *Setaria viridis*, Leaf adaxial epidermis showing angular prickles, (b) Leaf abaxial epidermis showing macrohairs.

microhairs were bicelled, blunt at the tip, having basal cell that is smaller than the distal cell, and the presence of bicelled microhairs is the characteristic of panicoid grasses. Macrohairs with bulbous base were present only on the abaxial side of *S. verticillata* and of *S. viridis* (Figures 4b and 5b), while absent in other species, so this character distinguishes these two species, from the other species.

Low dome shaped subsidiary cells were found on the abaxial surface of leaf epidermis and tall dome shaped subsidiary cells were observed on the adaxial side of leaf epidermis of *S. italica* (Figures 3a and b). The tall dome shaped subsidiary cells is a primitive character (Shouliang et al., 1996) and the linear dumb bell shaped stomata of grasses are generally believed to represent a more evolutionary advanced form than kidney shaped stomata (Palevitz, 1981). According to Metcalfe (1960) the shape and outline of the long cells is variable, presenting from shorter elements with non sinuous thin walls to long elements with sinuous thickened walls. The different types of long cells have been used in the solution of the taxonomic problems. Lightly sinuous long cells were observed in all the *Setaria* species except *S. glauca,* in which long cells have thin, non sinuous to slightly sinuous or straight walls, and deep sinuous long cells were found on the margins of the costal zones of the adaxial and abaxial surface (Figures 1a and b). Sharma and Kalia (1983) also observed that the long cells in the genus *Setaria* have thin, non sinuous to slightly sinuous walls in different species. *S. glauca* is recognized by its large stomatal complex, having length from 40 to 60 µm (Figure 1a and b), while in *S. intermedia,* stomatal complex is smaller (17.5 to 25 µm long) as compared to other species (Figures 2a and 2b). It is observed that different leaf anatomical characters are helpful in identification and differentiation of different *Setaria* species. *S. verticillata*

may be the advanced species on the basis of shape of silica bodies but the presence of dome shaped subsidiary cells is a primitive character and tall dome shaped subsidiary cells, as found in the adaxial side of *S. italica* is also a primitive character. The presence of both the primitive and advanced characters may lead to the conclusion that these species occupy an intermediate position in evolutionary hierarchy.

The studies revealed that foliar epidermal studies assist in identification and differentiation of different *Setaria* species. Different anatomical characters such as absence or presence of prickles on the abaxial and adaxial side, shape of silica bodies and size of stomatal complex are of prime importance in delimiting different taxa at the species and generic level.

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