Role of leaf epidermis in identification and differentiation of grasses in tribe Chlorideae (Poaceae) from Pakistan

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In the present investigations, leaf epidermal studies of 5 species belonging to 3 genera of tribe Chlorideae (Poaceae) from salt range of Pakistan were carried out. The investigated species were Chloris barbata Sw, Chloris dolicostachya Lag, Cynodon dactylon Pers, Tetrapogon cenchriformis (A.Rich) Clayton and Tetrapogon villosus Desf. The size of stomatal complex, long cells, shape of subsidiary cells, silica bodies are found to be important in differentiation of different taxa. T. villosus is characterized by large cells having maximum length while T. cenchriformis is differentiated from T. villosus and other taxa by large stomatal complex, while smallest stomatal complex is observed in C. dactylon. Bicelled microhairs with hemispherical distal cell are present only in genus Chloris while found absent in C. dactylon (rarely present on adaxial surface) and Tetrapogon species. The presence of microhairs is not a constant character, as in C. dactylon microhairs may be present or absent on the abaxial side. It may be due to habitat or environmental variations. Saddle shaped silica bodies are found in all the species of the tribe that is a characteristic feature of chloridoid type grasses.

Key word: Epidermis, Chlorideae, Grasses, Pakistan.

INTRODUCTION

Tribe Chlorideae is one of the important tribes of family Poaceae. This tribe is represented by 45 genera throughout the tropics. In Pakistan, tribe Chlorideae has 7 genera with 14 species (Nasir and Ali, 1970, 2002). In the present studies from Salt Range of Pakistan 3 genera Tetrapogon, Chloris and Cynodon having 5 species are collected. Tetrapogon and Chloris have two species each, while one species of Cynodon is present. Sometimes there is confusion in identification and differentiation of species of the same genus on the basis of morphology, so anatomical studies assist in solving problem of identification. Leaf epidermis study provides valuable data regarding the identification of grasses and is recognized as a source of useful taxonomic characters, because of variations in leaf characters that are taxonomically useful (Barkworth, 1981). The arrangement of epidermal cells on the adaxial surface of the leaf blade is generally quite different from that of the abaxial surface. Most epidermal cells are classified as long cells or short cells. Long cells vary in length and width, wall thickness, and the extent to which the walls are sinuous, papillate or pitted. Significant differences in long cells exist among genera and even among species of the same genus (Gould, 1969). Leaf epidermal traits i.e. epidermal cells, stomata and microhairs have been proved valuable in identification and differentiation of different taxa (Stenglein et al., 2003). The shape of silica bodies varies among different grass species from round or oblong to linear, crescent shaped, dumbbell shaped, nodular, sinuous, saddle shaped or cross shaped. Macrohairs also vary greatly in size, shape and wall thickness (Gould, 1969) and these leaf epidermal

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characters are of great value in grass systematic and characterization of broad groups within the grasses, particularly subfamilies and tribes (Ahmad, et al., 2011). Leaf epidermal features are important to clarify taxonomic relationships in different taxa (Davila and Clark, 1990; Cai and Wang, 1994; Mejia–Saules and Bisbey, 2003; Ogie-Odia et al., 2010). So the aim of the present investigations is to carry out leaf epidermal studies to identify and differentiate grass species in tribe Chlorideae.

MATERIALS AND METHODS

Fresh leaves of five species of grasses 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 leaves were placed in a tube filled with 88% lactic acid in water bath for about 50 to 60 min. The abaxial and adaxial epidermis from middle portion of the leaf was removed, along with the mesophyll cells by using scalpel blade. 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. The following anatomical characters of both abaxial and adaxial epidermis was studied.

1. Length, width and shape of long cells, whether sinuous, slightly sinuous or non sinuous.
2. Short cells and papillae in intercostal zone are present or absent.
3. Number of rows of long cells between two costal zones.
4. Length and width of stomatal complex and shape of guard and subsidiary cells.
5. Length, width and shape of macrohairs and hooks, their presence or absence.
6. Length, width and shape of silica bodies and short cells and their distribution in costal zone.

RESULTS

**Chloris barbata (Abaxial leaf surface)**

**Long cells:** with sinuous walls, number of rows between two costal zones is 6 to 7, rectangular, conspicuously elongated;

**Short cells:** Cross shaped, present over and between the veins; solitary and sometimes paired; arranged in rows of 2 to 3 cells.

**Prickle hair:** Absent. Unequal angular prickles also present on leaf margins.

**Micro hair:** Bicelled, distal cell is shorter than basal cell and is hemispherical.

**Macro hair:** none seen.

**Hooks:** Present

**Stomatal complex:** Guard cells are dumb bell shaped and subsidiary cells are triangular.

**Silica bodies:** saddle shaped, with 2 to 3 layers (Figure 1).

**Chloris barbata (Adaxial leaf surface)**

**Long cells:** Adaxial intercostal long cells are with sinuous walls, 42.5 to 53.25 μm long and 8.75 – 8.80 μm wide. Numbers of rows of long cells between two costal zones are 5 to 8 with sinuous walls.

**Short cells:** Short cells are with sinuous walls, 10 to 17.5 μm long and 6.25 to 10 μm wide.

**Prickle hair:** Present.

**Micro hair:** none seen.

**Macro hair:** none seen.

**Hooks:** Present

**Stomatal complex:** Guard cells are dumb bell shaped and subsidiary cells are triangular.

**Silica bodies:** Saddle shaped (Figure 2).

**Chloris dolicostachya (Abaxial leaf surface)**

**Long cells:** Abaxial intercostal long cells are with thin sinuous walls, 60 to 65 μm long and 10 to 12.5 μm wide.
**Chloris dolicostachya**, Leaf abaxial surface.

**Figure 3. Chloris dolicostachya**, Leaf abaxial surface.

**Chloris dolicostachya**, Leaf adaxial surface.

**Figure 4. Chloris dolicostachya**, Leaf adaxial surface.

**Cynodon dactylon**, leaf abaxial surface, showing saddle shaped silica bodies, and long cells with deep sinuous walls

**Figure 5. Cynodon dactylon**, leaf abaxial surface.

**Micro hair:** Microhairs are bi-celled; distal cell is shorter than basal cell. Distal cell is hemispherical in shape and with rounded apices while basal cell is with rounded base, 21.25 to 23.75 μm long and 7.5 to 8.75 μm wide.

**Macro hair:** none seen.

**Hooks:** none seen

**Stomatal complex:** Stomatal complex is 15.25 to 20 μm long and 15.25 to 17.5 μm wide, guard cells are dumb bell shaped and subsidiary cells are triangular to high dome shaped.

**Silica bodies:** Silica bodies are saddle shaped, 12.5 to 20 μm long and 11.25 to 15 μm wide (Figure 3).

**Chloris dolicostachya (Adaxial leaf surface)**

**Long cells:** Adaxial intercostal long cells are with sinuous walls, 32.5 to 55 μm long and 6.25 to 10 μm wide. Numbers of rows of long cells between two costal zones are 5 to 7. Number of stomatal rows between two costal zones are 1 to 2.

**Short cells:** Short cells are 12.5 to 16.25 μm long and 8.75 to 10 μm wide.

**Prickle hair:** Prickles are 25 to 30 μm long and 8.75 to 10 μm wide.

**Micro hair:** Microhairs are bicelled and both cells are almost equal or distal cell is shorter than basal cell and distal cell is hemispherical.

**Macro hair:** Absent

**Hooks:** Absent

**Stomatal complex:** Stomatal complex is 15 to 17.5 μm long and 12.5 to 13.75 μm wide.

**Silica bodies:** Silica bodies are saddle shaped, 7.5 – 8.0 μm wide horizontally and 11 – 12.25 μm wide vertically (Figure 4).

**Cynodon dactylon (Abaxial leaf surface)**

**Long cells:** Abaxial intercostal long cells are with thin sinuous walls, 39.5 to 52.5 μm long and 10 to 15 μm wide while interstomatal cells are with, deeply sinuous walls and with concave ends. Numbers of rows of long cells between two costal zones are 6 to 10. Numbers of stomatal rows between two costal zones are 1 to 3.

**Short cells:** Short cells are with sinuous walls, 15 to 17.5 μm long and 6.25 to 10 μm wide. Rounded bodies (cork cells) are present between the short cells.

**Prickle hair:** Prickles are pointed at the tip, 40 to 42.5 μm long and 10 to 13 μm wide.

**Micro hair:** Absent.

**Macro hair:** Absent

**Hooks:** Absent

**Stomatal complex:** Stomatal complex is 15 to 16.25 μm long, 11.25 to 12.5 μm wide, guard cells are dumb bell shaped, and subsidiary cells are low to high dome shaped.
Silica bodies: Silica bodies are saddle shaped, 7.0 to 7.5 μm horizontally and vertical diameter is 10 to 11.25 μm and silica bodies are 7.5 to 10 μm long and 6.5 to 7.5 μm wide (Figure 5).

**Cynodon dactylon:** (Adaxial leaf surface)

- **Long cells**: Adaxial intercostal long cells are with thin sinusuous walls, 22.5 to 37.5 μm long and 7.5 to 10 μm wide. Numbers of rows of long cells between two costal zones are 2 to 3.
- **Short cells**: Short cells are with sinusuous walls, 16 to 18.75 μm long and 8 to 10 μm wide. Rounded bodies or cork cells are present between silica bodies and short cells, 6.25 to 8.75 μm long and 6.25 to 8.75 wide.
- **Prickle hair**: Prickles are 12.5 to 20 μm long and 6.25 to 7.5 μm wide.
- **Micro hair**: Absent.
- **Macro hair**: Absent.
- **Hooks**: Absent.

**Stomatal complex**: Guard cells are dumb bell shaped, subsidiary cells are triangular or high dome shaped 23.75 to 25 μm long and 12.5 to 17.5 μm wide.

**Silica bodies**: Silica bodies are saddle shaped, with 1 to 3 layers, 6.25 to 10 μm long and 6.25 to 7.5 μm wide (Figure 6).

**Tetrapogon cenchriformis** (Abaxial leaf surface)

- **Long cells**: Abaxial intercostal long cells are with irregular sinusuous walls, 31.5 to 55 μm long and 10 to 12.5 μm wide. Number of rows of long cells between two costal zones are 4 to 8 and number of stomatal rows between two costal zones are 2. Long cells in costal zone are also with sinusuous walls, present over the veins with silica bodies, 36.25 to 37.5 μm long and 8.75 to 9.37 μm wide.
- **Short cells**: Short cells are with sinusuous walls, 16 to 18.75 μm long and 8 to 10 μm wide. Rounded bodies or cork cells are present between silica bodies and short cells, 6.25 to 8.75 μm long and 6.25 to 8.75 wide.
- **Prickle hair**: Angular prickles are present at the margins, 32 to 35 μm long and 8 to 10 μm wide.
- **Micro hair**: Absent.
- **Macro hair**: Absent.
- **Hooks**: Absent.

**Stomatal complex**: Stomatal complex is 20 to 27.5 μm long and 20 to 22.5 μm wide, guard cells are dumb bell shaped, 5 μm wide and subsidiary cells are high dome shaped, 6.25 μm wide.

**Silica bodies**: Silica bodies are saddle shaped and dumb bell shaped. Saddle shaped silica bodies are 9 to 10 μm wide horizontally and vertical diameter 10 to 11.25 μm wide while dumb bell shaped silica bodies are 15 to 17.5 μm long and 7.5 to 8.75 μm wide (Figure 7).

**Tetrapogon cenchriformis** (Adaxial leaf surface)

- **Long cells**: Adaxial intercostal long cells are with sinusuous walls, 37.5 – 72.5 μm long and 10 – 11.25 μm wide. Number of rows of long cells between two costal zones are 6 – 7. Number of stomatal rows between two costal zones are 2 – 3.
- **Short cells**: Short cells are with sinusuous walls, 16 – 18.75 μm long and 8 – 10 μm wide. Rounded bodies or cork cells are present between silica bodies and short cells, 6.25 – 8.75 μm long and 6.25 – 8.75 wide.
- **Prickle hair**: Angular prickles are present at the margins, 32 – 35 μm long and 8 – 10 μm wide.
- **Micro hair**: Microhairs are bicelled, basal and distal cells are almost equal, distal cell is rounded at the tip, basal cell having a rounded structure, 22.5 – 26.75 μm long and 12.5 μm wide.
- **Macro hair**: Macrohairs are rounded at the base, 8.0 – 15 μm long and 7.5 – 12.5 μm wide.
Hooks: Absent

Stomatal complex: Stomatal complex is 18.75 - 19.37 μm long and 16.25 – 17.5 μm wide, guard cells are dumb bell shaped, 5 μm wide and subsidiary cells are high dome shaped, 6.25 μm wide.

Silica bodies: Silica bodies saddle shaped, 8.5 - 10 μm wide horizontally and 13.75 - 15 μm wide vertically. Short cells with sinuous walls, 10 – 11.25 μm long and 8.75 – 10 μm wide. Prickles are abundant in the costal zone, 41 – 42.5 μm long and 12 – 16.25 μm wide (Figure 8).

*Tetrapogon villosus* (Abaxial leaf surface)

Long cells: Abaxial intercostal long cells are with sinuous walls, 42.5 to 92.5 μm long and 10 to 13.75 μm wide. Numbers of rows of long cells between two costal zones are 2 to 3. Number of stomatal rows between two costal zones is 1.

Short cells: Short cells are with sinuous walls, 20 to 27.5 μm long and 7.5 to 11.25 μm wide. Silica bodies and short cells are arranged in a row

Prickle hair: Angular prickles are at the margins of costal zone, obliquely projected into pointed tip, 87.5 to 95 μm long and 17.5 to 20 μm wide.

Micro hair: Absent

Macro hair: Absent

Hooks: Absent

Stomatal complex: Stomatal complex is 17.5 to 18.75 μm long and 15 to 17.5 μm wide, guard cells are dumb bell shaped and subsidiary cells are high dome shaped.

Guard cells are 3.75 to 7.5 μm wide and subsidiary cells are 6.25 to 7.5 μm wide. Microhairs, macrohairs and hooks are absent.

Silica bodies: Silica bodies are saddle shaped, 12.5 to 13.75 μm long and 7 to 8.75 μm wide. Number of rows of silica bodies are 1 to 4 (Figure 9).

*Tetrapogon villosus* (Adaxial leaf surface)

Long cells: Adaxial intercostal long cells are with sinuous walls, 62.5 to 65 μm long and 12.5 to 16.25 μm wide. Numbers of rows of long cells between two costal zones are 4 to 5. Number of stomatal rows between two costal zones is one.

Short cells: Short cells are with sinuous walls, 25 to 27.5 μm long and 7.0 to 8.75 μm wide.

Prickle hair: Angular prickles are at the margins of costal zone, obliquely projected into pointed tip, 87.5 to 95 μm long and 17.5 to 20 μm wide.

Micro hair: Absent

Macro hair: Absent

Hooks: Absent

Stomatal complex: Stomatal complex is 20 to 22 μm long and 15 to 16.25 μm wide, guard cells are dumb bell shaped, and subsidiary cells are high dome shaped.

Silica bodies: Silica bodies are saddle shaped, 12.5 to 13.75 μm long horizontally and 10 to 16.25 μm wide vertically (Figure 10).
DISCUSSION

The studies of leaf epidermis in all species of tribe revealed that all intercostal long cells on abaxial and adaxial surface are with sinuous walls, however the walls are thin sinuous in Clorhls dolicostachya and Cynodon dactylon (Figures 5 and 6). In Tetrapogon cenchriformis long cells with irregular sinuous walls are observed. Long cells with maximum length (92.5 µm) are present in Tetrapogon villosus. It is found that size of stomatal complex and shape of subsidiary cells are important characters in identification and differentiation of different taxa. The largest stomatal complex is observed in T. cenchriformis that is 20 to 27.5 µm long and 20 to 22.5 µm wide and small in C. dactylon which ranges in length from 15 to 16.25 µm long and 11.25 to 12.5 µm wide. Peterson et al. (2011) observed low dome shaped or triangular stomata in new genus Elloschioa of subfamily Chloridoideae.

In the present studies, subsidiary cells are triangular to high dome shaped in genus Chloris, low to high dome shaped in C. dactylon and high dome shaped in Tetrapogon species, indicating that stomatal complex proved to be useful taxonomic feature in this tribe. Chaudhary et al. (2001) found that in C. dactylon stomata are with triangular subsidiary cells, silica bodies are saddle shaped, and microhairs with hemispherical distal cells are present, while macrohairs are absent. In the present investigations, C. dactylon showed triangular and low to high dome shaped subsidiary cells and micro hairs are not observed abaxially (Figure 5) but a few microhairs are present adaxially (Figure 6). Freire et al. (2005) also observed the presence of microhairs in C. dactylon. Metcalfe (1960) studied 3 species of C. dactylon collected from different localities and found that silica bodies are saddle shaped, stomata are with triangular or low dome shaped subsidiary cells, and microhairs were present in all the species examined. The present findings are similar to that of Metcalfe except the microhairs which are absent on abaxial side in Cynodon, in the present studies. It may be due to environmental variations as C. dactylon is a wide spreading grass, which varies considerably in habit. Bi-celled microhairs (21.25 to 23.75 µm) with hemispherical distal cell shorter than basal cell are found only in genus Chloris (Figures 1 and 3), while found absent in C. dactylon (rarely present on adaxial surface) and Tetrapogon species. All the species in the tribe have saddle shaped silica bodies as according to Prat (1934, 1961) and Watson and Johnston (1986), the chloridoïd type is characterized by globose or club shaped bi-cellular microhairs and saddle shaped silica bodies.

REFERENCES