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Full Length Research Paper

Systematic anatomy and elemental dispersive spectrophotometer analysis of genus *Pennisetum* from Pakistan

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Microscopic examination of five species of genus *Pennisetum* was carried out from Pakistan. The investigation of the species was based on the evidence from the epidermal leaf anatomy (LM and scanning electron microscopy (SEM) and elemental dispersive spectrophotometer analysis. Leaf epidermal anatomy of the selected species showed variation in size and shape of stomatal cells, micro and macro hairs, trichomes, silica bodies, hooks, papillae and long cells. Leaf epidermal anatomy proved a significant tool for the identification and classification in systematics. Energy dispersive spectrometer (EDS) emerged as a new taxonomic tool in the classification of the selected species and was done first time for the genus *Pennisetum*.

Key words: Systematic anatomy, energy dispersive spectrometer (EDS), *Pennisetum.*

INTRODUCTION

The genus *Pennisetum* belongs to the grasses, which are a large, diverse and successful group of family Poaceae of monocotyledonous plants. There are about 10,000 species that are categorized as grasses by taxonomists who traditionally follow embryonic, architectural and anatomical characters (Clayton and Renvoize, 2006). The *Poaceae* is generally divided into 5 (or 6) sub families: Poaideae, Panicoideae, Bambusoideae, Chloridoideae, Arundinoideae and sometimes Oryzoideae, a subtype of Bambusoideae. The genus *Pennisetum* belongs to the subfamily Panicoideae. Hubbard (1948) and Bor (1960) placed the genus *Pennisetum* in the tribe Paniceae, close to the genus *Cenchrus.* The generic name *Pennisetum* has been derived from two Latin words, Penna and Seta, meaning feather and bristles that is, feathery bristles (Brunken, 2008). The genus includes about 140 species distributed in the world (Chaudhary, 1989). *Pennisetum* is an economically important genus as it comprises many grain crops such as *Pennisetum americanum* and some species are also used as common fodder such as *Pennisetum orientale*. Some species are of medicinal imporatance such as *Pennisetum divisum* (Sujatha et al., 1989). The *Pennistum* is also considered as a great source of millets. Millets are generally considered as minor crops and they are also used as both for forage and grains and when used as a grain they are considered as a cereal (Brunken, 2008). Despite the economic importance and worldwide distribution of the genus *Pennisetum*, the members of this genus have received relatively little attention from systematics.

As the previously published account of this genus provides only the traditional scheme of systematics so has posed many taxonomic confusions and problems. These traditional taxonomic methods are based on gross morphology. Also the use of anatomical characters through light microscopy in taxonomy became a routine procedure. So the purpose of present study was to produce a classification system drawing upon evidence from not only the morpho-anatomical characters through light microscopy but also through scanning electron microscopy in conjugation with the elemental dispersive

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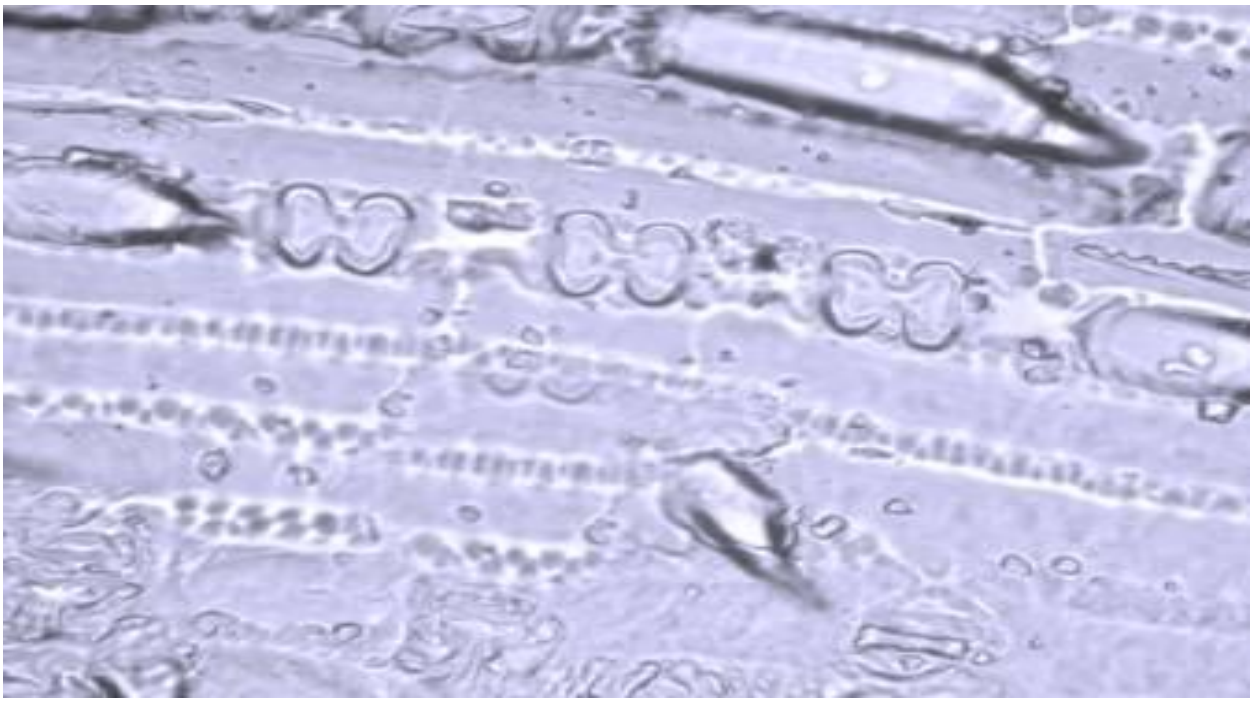

Figure 1. Prickles and silica bodies of *P. americanum.*

spectrophotometer, as the leaf epidermal anatomy is now considered as one of the most important tools in understanding the identification, classification and relationships of various species, genera and families of the angiosperms. In the family Poaceae the epidermal characters attained a high degree of differentiation and specialization. As early Prat in 1932 to 1936 used anatomical features in the classification of grasses and believed that the anatomical features were reflecting their true relationships more exactly than any other characters. So the aim of the present study was to explore the morpho-anatomical characters of the five species of the genus *Pennisetum* trough LM, SEM and EDS detector. EDS technique is used first time in systematics as a new tool for the identification and classification of the genus *Pennisetum*.

MATERIALS AND METHODS

The *Pennisetum* species listed were used in the present investigation to gain knowledge about the morpho-anatomical characterization. (1) *Pennisetum americanum* (2) *Pennisetum divisum* (3) *Pennisetum lanatum* (4) *Pennisetum orientale* (5) *Pennisetum flaccidum.* The fresh as well as the herbarium specimens of all the five species of the genus *Pennisetum* were used for the anatomical study. The peelings were made by scraping the epidermis with a sharp blade following the method described by the Cotton (1974) and Clark (1960) but with a little modification (Shaheen et al., 2010). The preparations of abaxial and adaxial epidermis of leaf samples were then observed under light microscope. For SEM the abaxial and adaxial epidermis of leaves were placed on the stubs and after gold coating put into SEM for getting digital images. The method was followed by the Terrel and Wergin (1979) and Hilu and Wright (1984) who concluded that SEM has proven to be extremely useful for examining the structure of leaf epidermis in extent. Also the SEM/EDS analysis of abaxial and adaxial epidermis of leaf samples was carried out. The quantitative analysis of phytoliths were taken, especially the mass percentage of Silicon was calculated in order to make a comparison between the different taxa of genus *Pennisetum*. Then the microphotographs of leaf samples through LM, SEM and "mapping" of the composition of the elements in the form of graphs were taken by using SEM/EDS analysis.

RESULTS AND DISCUSSION

P. americanum (L.) Leeke

The distinctive epidermal anatomical features of *P. americanum* are presented in Figure 1 showing dumb-bell shaped silica bodies and long cells are present between the veins with thick sinuous walls and beak shaped with sharp pointed end prickles are abundantly present between the veins shown in Table 1 whereas EDS analysis is presented in Figure 2 and shows that the mass percentage of Silicon is 20.63.

P. divisum (Gmel.) Henr

The distinctive epidermal anatomical features of *P. divisum* are presented in Figure 3 having cross-shaped

Table 1. Distinguishing summary of leaf epidermal anatomy among the species of genus *Pennisetum.*

Characteristics	*P. americanum*	*P. divisum*	*P. flaccidum*	*P. lanatum*	*P. orientale*
Short cells	In rows of 3 to 4 cells	In rows of 2 to 3 cells	In rows of 2 or more cells	In rows of 2 or more cells	In rows of 2 or more cells
Silica bodies	Dumb-bell shaped	Cross-shaped and nodular shaped	Cross-shaped to dumb bell shaped	Cross-shaped to dumb bell shaped	Dumb-bell shaped
Macro-hairs	None seen	Between the veins, 30 to 40 μm	None seen	None seen	None seen
Micro-hairs	Between the veins, 6 to 8 μm	Between the veins, 5 to 6 μm	None seen	Between the veins, 9 μm	Between the veins, 5 to 6 μm
Prickles	Between the veins, beak shaped, 6 to10 μm	Between the veins and also over the veins, beak shaped, knife shaped, 6 to 10 μm	Between the veins and also over the veins, beak shaped, knife shaped, 4 to 7 μm	Between the veins and also over the veins, beak shaped, knife shaped, 3 to 4 μm	Between the veins and also over the veins, beak shaped, knife shaped, 4 to 6 μm
Hooks	None seen	Present, 2 to 3 μm	Present, 3 μm	None seen	Present, 2 μm
Stomata	With 5 to 6 rows, low dome-shaped subsidiary cells, 5 to 6 μm	With 5 to 6 rows, almost circular shaped subsidiary cells, to 6 μm	With 2 to 3 rows, low dome-shaped subsidiary cells, 7 to 8 μm	With 5 to 6 rows, almost circular shaped subsidiary cells, 3 to 4 μm	With 5 to 7 rows, low dome-shaped subsidiary cells, 5 to 6 μm
Long cells	Thick sinuous walls, 1 to 30 μm	Slightly sinuous walls, 25 to 30 μm	Thick sinuous walls, 10 to 25 μm	Thick sinuous walls, 15 to 20 μm	Thick sinuous walls, 15 to 25 μm

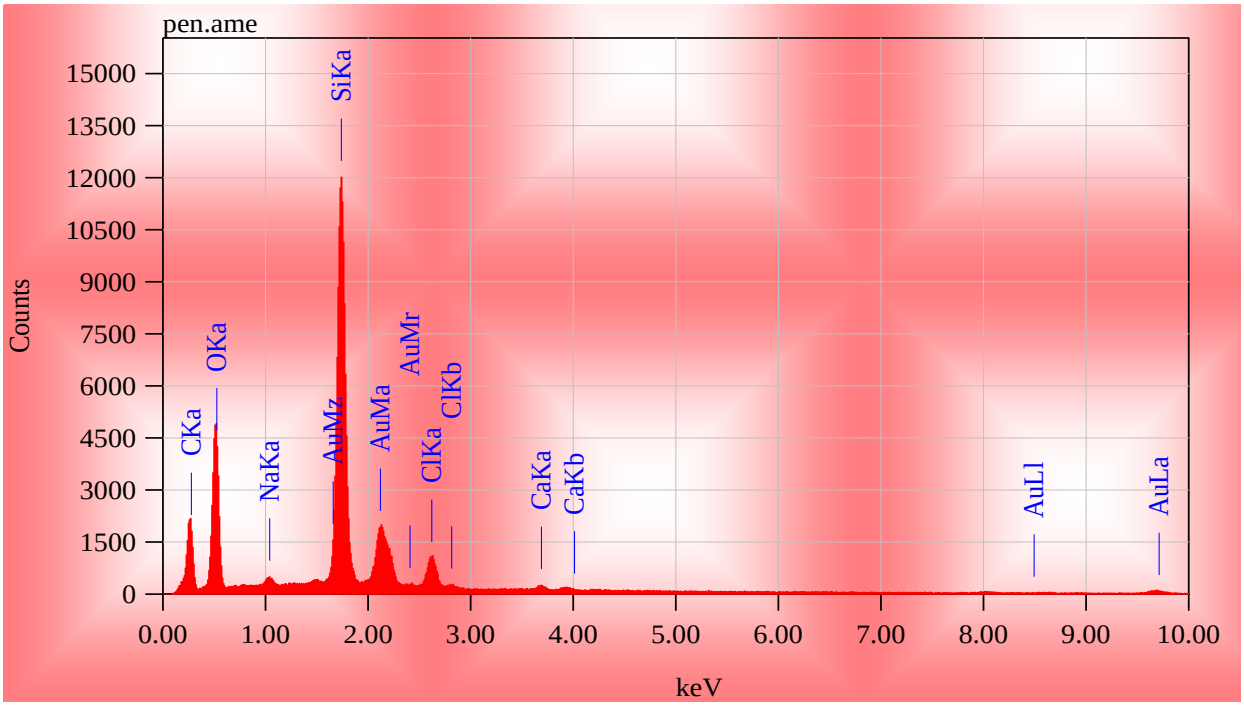

Figure 2. EDS analysis of *P. americanum.*

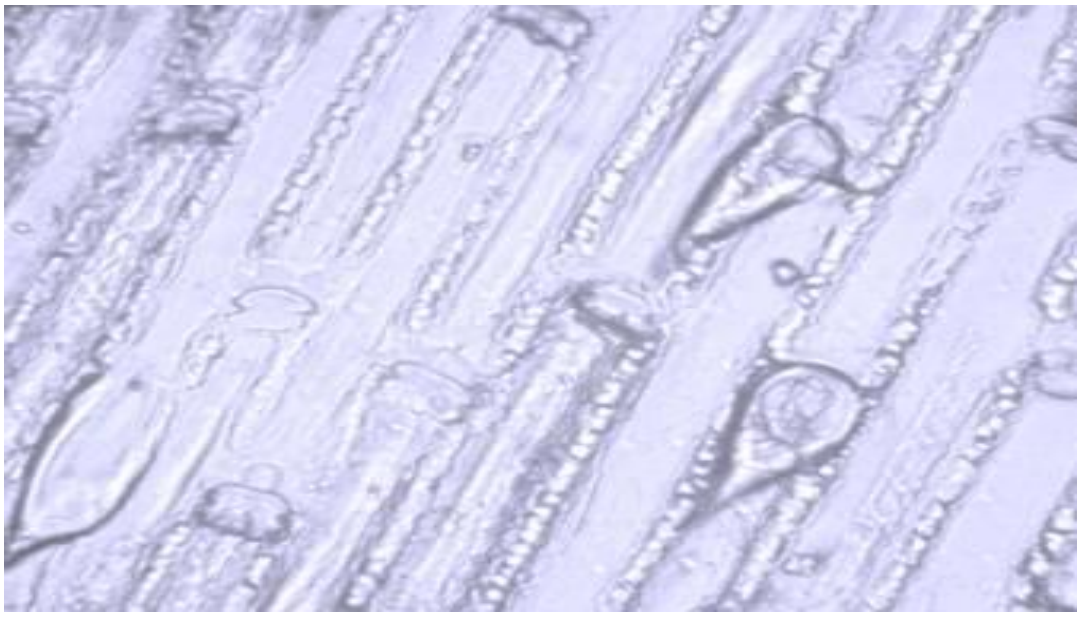

Figure 3. Long cells, prickles and silica bodies of *P. divisum*.

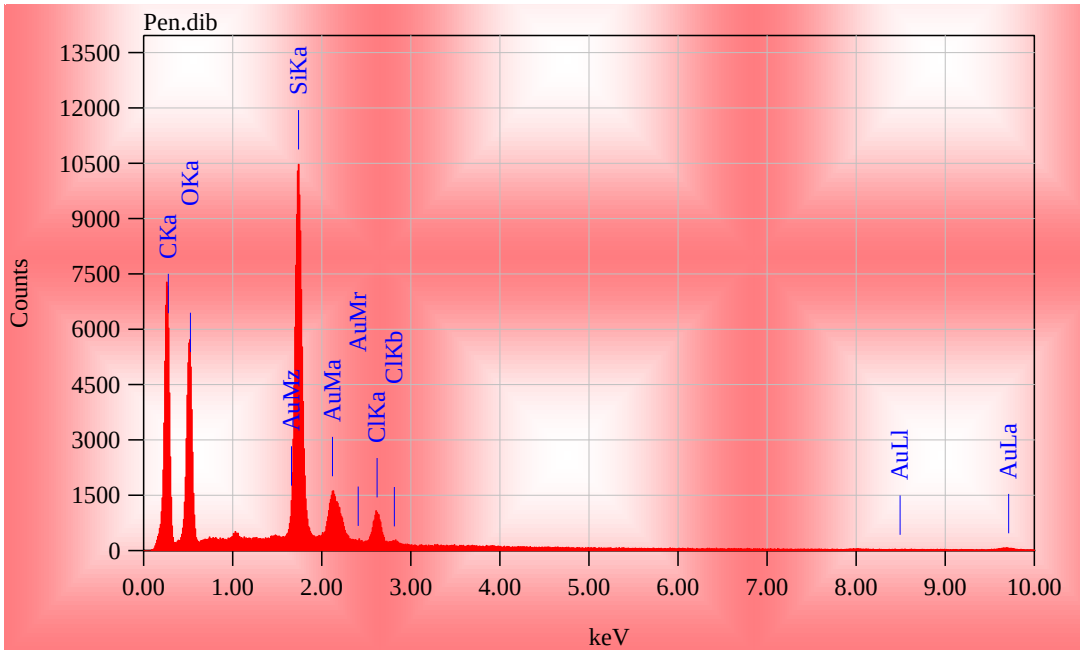

Figure 4. EDS analysis of *P. divisum.*

and nodular shaped silica bodies. Micro-hairs are rarely present between the veins and prickles are abundantly present between the veins and also over the veins which are beak shaped with much broader base and knife shaped (Table 1). EDS analysis is presented in Figure 4 and it shows that the mass percentage of Silicon is 12.45 and shows the lowest value in genus *Pennisetum.*

***P. flaccidum* Griseb**

The distinctive epidermal anatomical features of *P. flaccidum* are presented in Figure 5, short-cells are over the veins, in a rows of 2 or more cells, each containing a closely paired silica body and cross-shaped to dumb-bell shaped silica bodies are present. Stomata are present

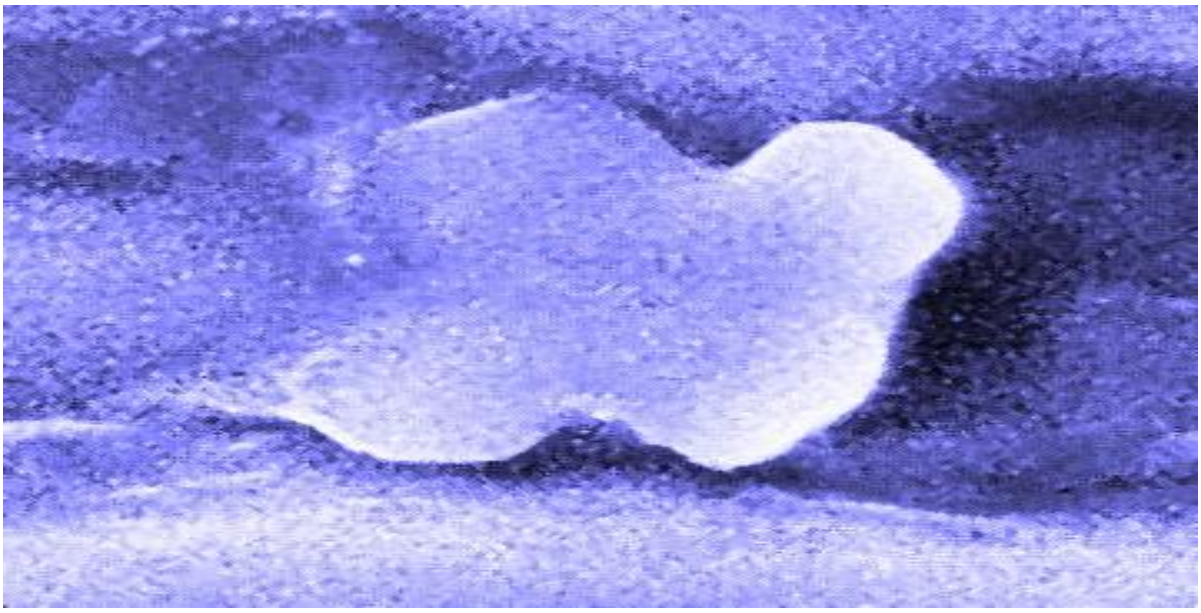

Figure 5. SEM Silica body of *P. flaccidum.*

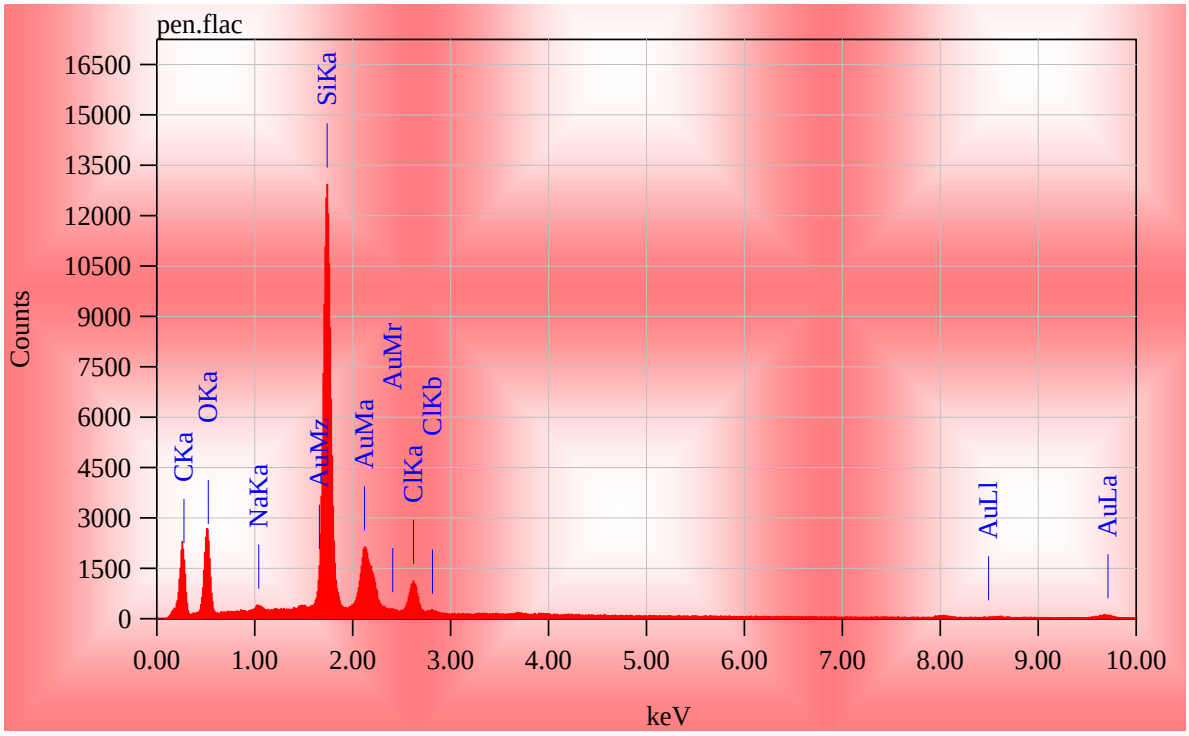

Figure 6. EDS Analysis of *P. flaccidum.*

with 2 to 3 rows in intercostal zones, often tending to be with low dome-shaped subsidiary cells (Table 1). EDS analysis is presented in Figure 6 and it shows that the mass percentage of Silicon is 22.86 and shows the highest value in genus *Pennisetum.*

P. lanatum Klotzsch

The distinctive epidermal anatomical features of *P. lnatum* are presented in Figure 7. Silica bodies are cross-shaped to dumb-bell shaped and long cells are present

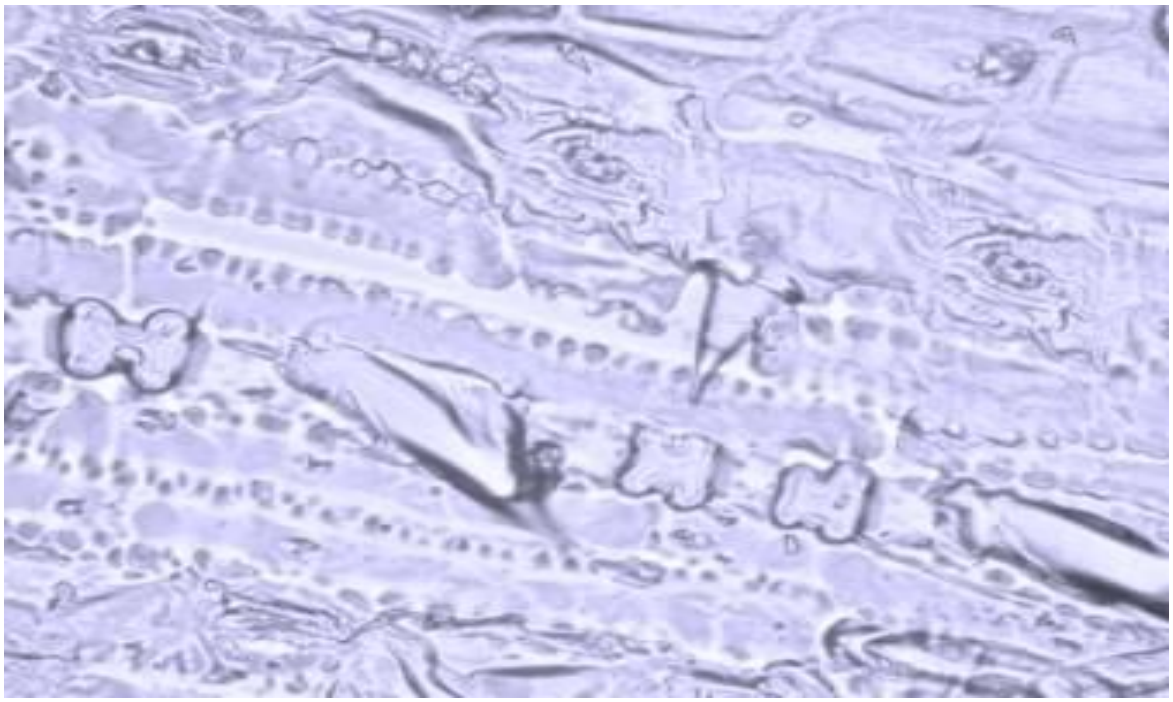

Figure 7. Silica bodies and prickles of *P. lanatum.*

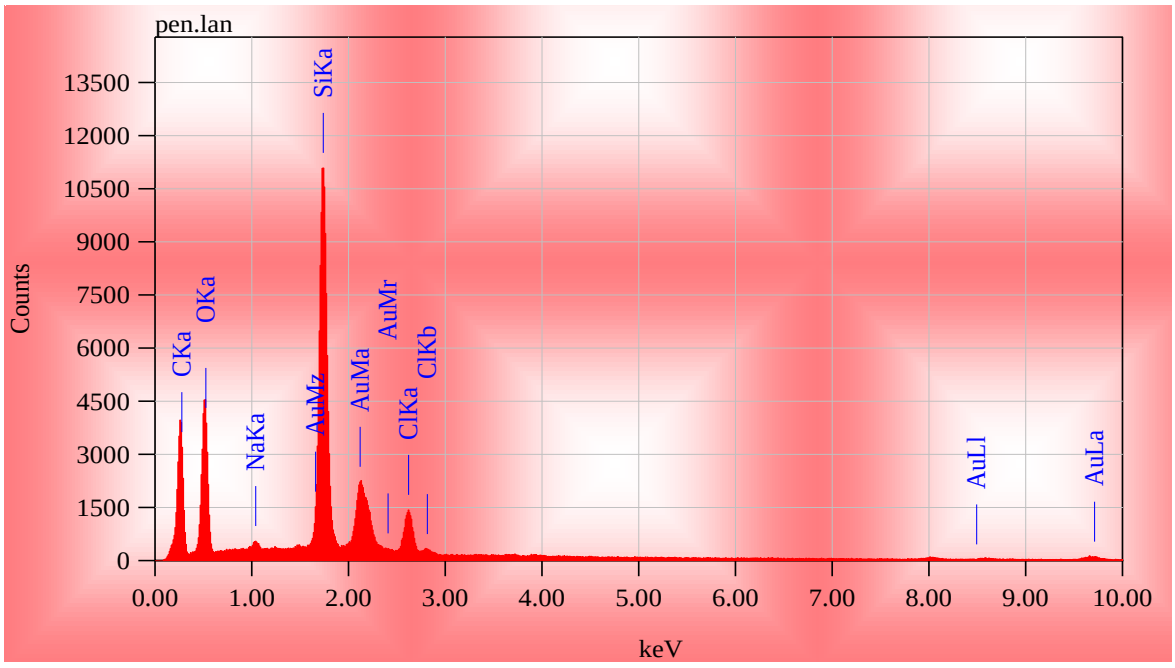

Figure 8. EDS Analysis of *P. lanatum.*

between the veins with thick sinuous walls (Table 1) and EDS analysis is presented in Figure 8 and it shows the elemental composition of phytoliths. The mass percentage of Silicon is 16.21 and confirms the siliceous nature of phytoliths.

***P. orientale* L. C. Rich**

The distinctive epidermal anatomical features of *P. orientale* are presented in Figure 9. Prickles are present between the veins and also over the veins, beak shaped

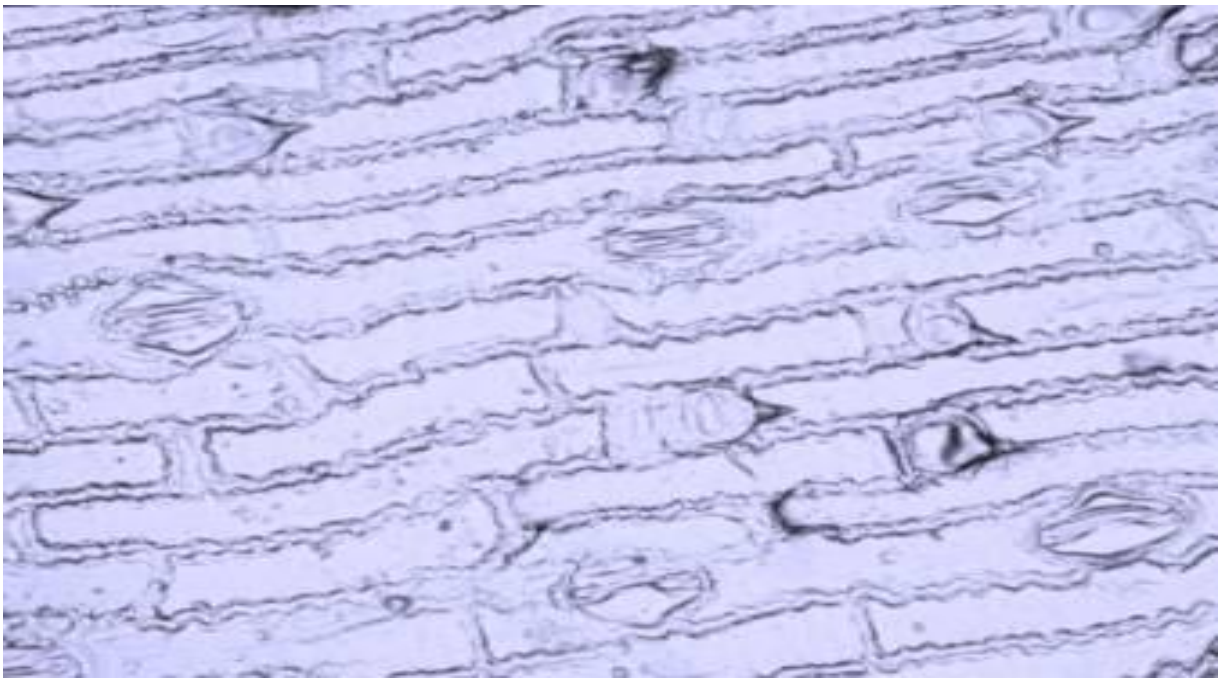

Figure 9. Long cells, stomata and prickles of *P. orientale.*

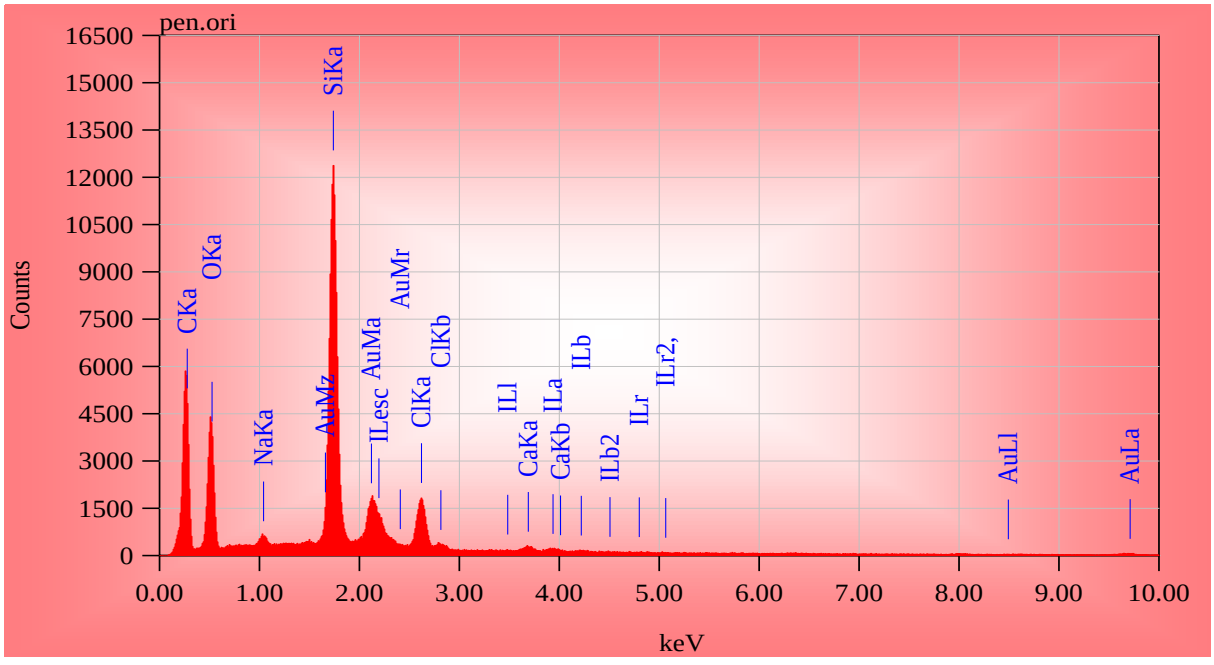

Figure 10. EDS Analysis of *P. orientale.*

with much broader base and knife shaped (Table 1). EDS analysis is presented in Figure 10 and it shows that the mass percentage of Silicon is 15.04, which play a vital role in the composition of silica bodies. A lot of variation was observed in the morpho-anatomical characters of different species of genus *Pennisetum*. Leaf epidermal anatomy was found taxonomically useful, which helps at the species level and showed that in all the five species of the genus leaf is bifacial. In *P. americanum*, dumb-bell shaped silica bodies are found (Figure 1) whereas

P. divisum comprises of cross-shaped and nodular shaped silica bodies (Figure 3). As indicated in the present research, features of the leaf epidermal anatomy are useful at specific level. *P. americanum* and *P. divisum* can be distinguished on the basis of the shape of silica bodies and number of rows of short cells. In *P. americanum*, short-cells are arranged predominantly in rows of 3 to 4 cells in the costal regions whereas in majority of the other taxa, the short cells are arranged in rows of two or more cells. Tateoka et al. (2009) said that epidermal characters are very useful for the differentiation of the different taxa at higher level.

Macro-hairs are absent in majority of the species except *P. divisum*, which comprises of macro-hairs in the intercostal regions, on the adaxial surface only. Micro-hairs are present in majority of the species except *P. flaccidum*. In all the species, micro-hairs are found in the intercostal regions. *P. divisum* and *P.* orientale comprise micro-hairs in both abaxial and adaxial surfaces whereas in *P. lanatum* only abaxial surface and in *P. americanum* only adaxial surface possess micro-hairs (Table 1). Terrel and Wergin (1979) and Tateoka (2008) reported that the micro-hairs of grasses are characteristic bicellular trichomes, commonly found on the leaves, but also occurring elsewhere on the plant (for example, lemmas, paleas and lodicules). Watson et al. (2005) observed that micro-hairs are lacking in the subfamily Pooideae, but almost universally present in the other subfamilies.

Prickles are found in all the species in genus *Pennisetum*. Beak-shaped and knife-shaped prickles are present in both the costal and intercostal regions in majority of the taxa (Figures 3, 4 and 9) except *P. americanum*, which possesses only beak-shaped prickles in the intercostal regions (Table 1). No hook cells are found in the *P. americanum* and *P. lanatum* whereas the other species comprise the hook cells.

In general, the costal and intercostal zones showed the presence of long and short cells. According to Metcalfe (1960), the shape and outline of the long cells is variable, presenting from shorter elements with non-sinuous walls to long elements with sinuous thickened walls. Besides, the above-mentioned, author stated that the different kinds of long cells have been used in the solution of taxonomic problems, although he also pointed out that this character should not be used isolatedly, since intermediate forms can be used. The long cells in majority of the taxa comprise thick sinuous walls (Figure 1) except *P. divisum*, which possesses slightly sinuous walls (Figure 3). No papillae are seen in all the species of genus *Pennisetum.* Esau (2007) discussed that the silicon is found in the epidermis of mature grass leaves in the form of discrete particles (silica bodies) produced in the specialized silica cells. A great variation is observed in the mass percentage of silicon in this genus. *P. divisum* shows the lowest value as 12.45% (Figure 4) whereas the *P. flaccidum* shows the highest value as 23.58% (Figure 6). Dahlgren and Clifford (2002) concluded that the surface sculpture, shape, size and distribution pattern of silica bodies on grass epidermis are enormously variable from one species to another, and this variation has always been considered of great taxonomic value.

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