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Full Length Research Paper

Elemental dispersive spectrophotometer analysis and morpho-anatomical characterization of *Panicum* species from Pakistan

Shabnum Shaheen[1]*, Mushtaq Ahmad[2], Farah Khan[1], Muhammad Zafar[2], Rana Abrar Hussain[3], Sumaira Rani[1], Zaryab Khalid[1], Rabail Ayub[1] and Amina Younis[1]

[1]Department of Botany, Lahore College for Women University, Lahore Pakistan.
[2]Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan.
[3]Science and Technology University of Education Lahore, Pakistan.

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The present study was conducted to identify, evaluate and compare the anatomical data and elemental composition of the *Panicum* species present in Pakistan. The study is based on the six species of the genus *Setaria* represented in Pakistan. The elemental dispersive spectrophotometer (EDS) detector was used for the first time in Pakistan for quantitative and qualitative determinations of the elements present in the phytoliths of grasses. Leaf epidermal anatomy was found taxonomically useful, which helps at the species level and showed that in all the six species of the genus, leaf is bifacial. Epidermal cells show the difference in shape of silica bodies, prickles, micro hairs and macro hairs. A lot of variation was observed in the silica bodies of the genus *Panicum* showing it as a new taxonomical tool during EDS analysis.

Key words: Elemental dispersive spectrophotometer, morpho-anatomical characterization, *Panicum* spp.

INTRODUCTION

Panicum is one of the grass genuses, commonly recognized in the floras of the world (Bor, 1960). *Panicum* is a member of the tribe Paniceae and is easily distinguishable from all other members of the tribe on the basis of the leaf blade anatomy. Using leaf blade epidermal characteristics Prat (1936) and Metcalfe (1960) supported the inclusion of *Panicum* in the Paniceae. The present study describes the biological significance and implications of leaf anatomical characteristics and also indicates that epidermal leaf blade anatomy is taxonomically useful. On the basis of the leaf epidermal anatomy, we can distinguish among different species of the genus. It also discusses the extent to which leaf epidermal features might be utilized in the systematic consideration in view of their perceived similarities in structural and reproductive biology. Most of these characters are diagnostic and have been used for making keys. While studying leaf blade epidermis, the presence or absence of microhairs is widely used as a taxonomic character (Prat, 1936; Metcalfe, 1960; Watson and Dallwitz, 1985).

Along with the anatomical characters, the purpose of the present study is also to investigate the role of grass phytolythis in the phyto-systematics. Phytolith research has also proved to be highly valuable to argue botanists as phytoliths are siliceous and maintain their morphological integrity and become a microfossil of that plant. Microfossil phytolith have been used for the reconstruction of pale environments (Fisher et al., 1987; Lewis, 2001) as indicators of ancient industrial and agricultural practices (Rosen, 1992; Rosen, 1999). Due to the siliceous nature of grass phytoliths, commonly known as silica bodies, the grasses can be classified taxonomically by comparison with a large collection of silica particles in each species. In the present study the EDS analysis emerged as a useful taxonomic feature to delimit the species on the basis of the silicon percentage in grass phytoliths.

*Corresponding author. E-mail: shabnumflora@hotmail.com

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

Characteristics	*P. antidotale*	*P. atrosanguineum*	*P. capillare*	*P. miliaceum*	*P. psilopodium*	*P. sumatrense*	*P. turgidum*
Short cells	In rows of 2-3 cells	In rows of 3 or more cells	In rows of 2-3 cells	In rows of 5 or cells	In rows of 2-3 cells	In rows of 3 or more cells	In rows of 5 or more cells
Silica bodies	In between the cross shaped and dumb-bell shaped	Cross-shaped, dumb-bell shaped and nodular shaped	Cross-shaped and dumb-bell shaped	In between the cross shaped and dumb-bell shaped	Dumb-bell shaped and nodular shaped	In between the cross shaped and dumb-bell shaped	Cross-shaped to nodular shaped
Macro-hairs	Between the veins, 40-80 µm	Between the veins, 30-50 µm	Between the veins, 60-80 µm	Between the veins, 60-70 µm	None seen	None seen	Between the veins and also over the veins, 20-40 µm
Micro-hairs	Between the veins and also over the veins, 10-20 µm	Between the veins, 5-7 µm	Between the veins, 5-6 µm	Between the veins, 4-5 µm	Between the veins, 3-6 µm	Between the veins, 3-4 µm	Between the veins, 5-10 µm
Prickles	Between the veins and also over the veins, two types, beak shaped and knife shaped 5-10 µm	Between the veins and also over the veins, beak shaped, 6-8 µm	Between and also over the veins, two types, narrow beak and elongated distil portion and broad beak and short and blunt distil portion, 5-12 µm	Over the veins, beak shaped with sharp pointed end, basal portion broad, 10-12 µm	Between and also over the veins, two types, beak shaped and knife shaped 3-7 µm	Between and also over the veins, two types, narrow beak and elongated distil portion and broad beak and short and blunt distil portion, 3-7 µm	Between and also over the veins, two types, narrow beak and short and blunt distil portion and broad beak and elongated distil portion, 5-8 µm
Hooks	None seen	None seen	None seen	Rarely present, 2 µm	1-2 µm	1-2 µm	1-2 µm
Stomata	With 4-5 rows, rectangular shaped subsidiary cells, 3-5 µm	With 5-6 rows, rectangular shaped subsidiary cells, 4-5 µm	With 2-3 rows, rectangular shaped subsidiary cells, 4-6 µm	With 6-8 rows, rectangular shaped subsidiary cells, 5-6 µm	With 2-3 rows, low dome-shaped subsidiary cells, 3 µm	With 2-3 rows, low dome-shaped subsidiary cells, 2-3 µm	With 2-3 rows, low dome-shaped subsidiary cells, 3-4 µm
Long cells	Thick sinuous walls, 10-12 µm	Thick sinuous walls, 10-25 µm	Thick sinuous walls, 10-20 µm	Thick sinuous walls, 20-40 µm	Smooth walled or thin sinuous walls, 5-10 µm	Thick sinuous walls, 5-20 µm	Slightly sinuous walls, 10-15 µm

MATERIALS AND METHODS

Leaves from living and dried specimens of *Panicum antidotale, Panicum atrosanguineum, Panicum capillare, Panicum miliaceum, Panicum psilopodium*, *Panicum sumatrense* and *Panicum turgidum* were used for anatomical studies. Fresh leaves were collected from different localities in Pakistan and the dried leaves from the herbarium of Quaid-i-Azam University, Islamabad. Leaves samples for light microscopy (LM) were prepared according to the method of Cotton (1974) who followed Clark's (1960) technique, but with a little modification

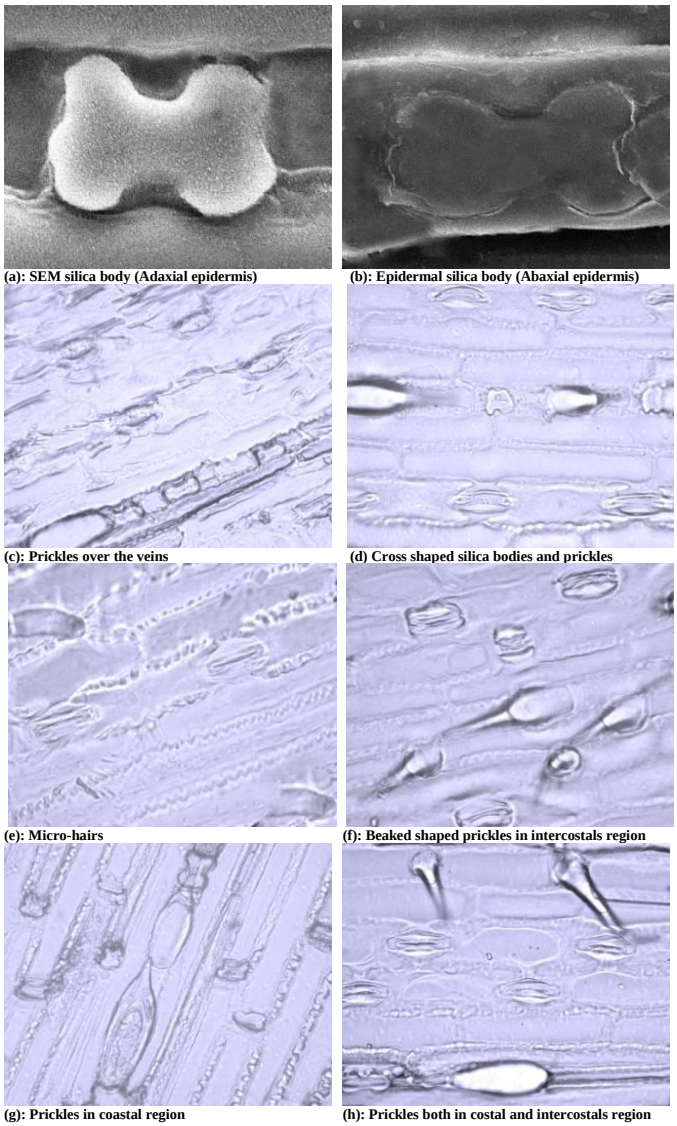


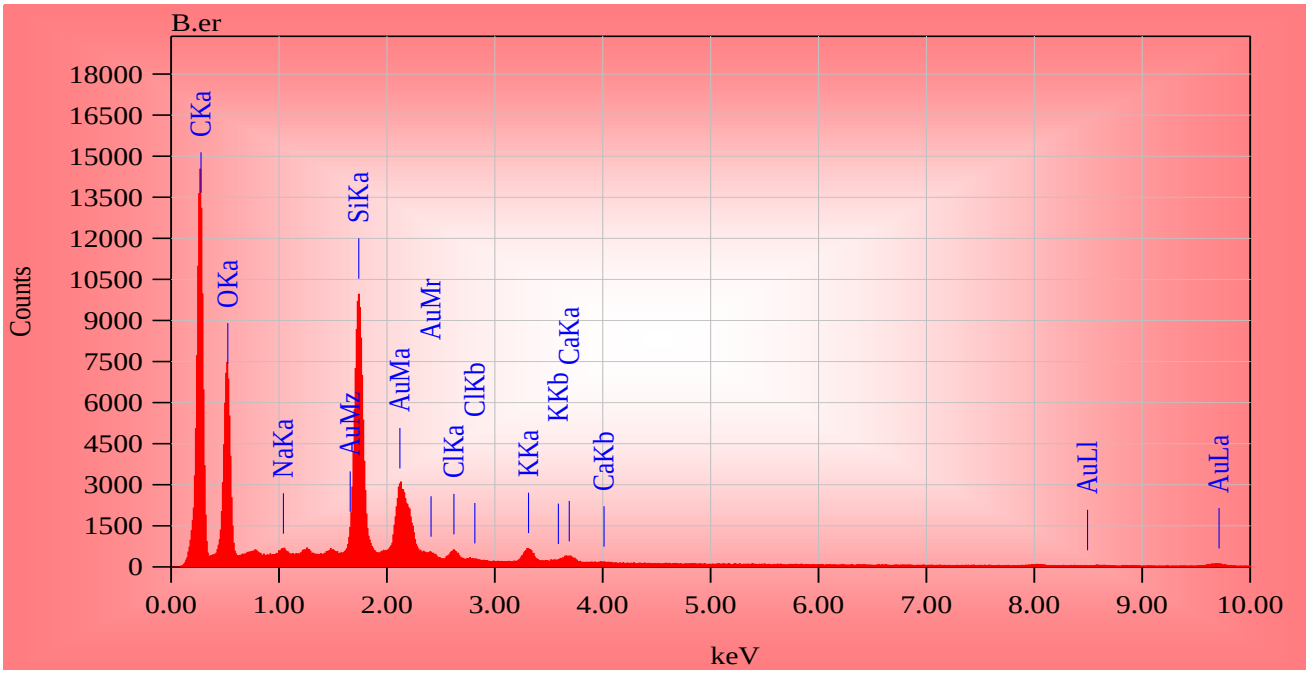
Figure 1. SEM and light microscopic leaf epidermis anatomy.

(Shaheen et al., 2010). For EDS analysis the leaf samples were put into scanning electron microscope (SEM) after gold coating on stubs. The method of Terrel and Wergin (1979) was followed with a little modification for SEM/EDS analysis. The abaxial and adaxial leaf epidermis were prepared for quantitative analysis of different elements including carbon, oxygen, silicon, magnesium, sodium, potassium, calcium and chlorine. The phytoliths were observed by SEM and their siliceous nature was confirmed by EDS. As the phytoliths are of siliceous nature, so the percentage of silicon was reviewed in order to make a distinct comparison of the species within the genera. The microphotographs of these mounted materials were obtained by using light microscope and SEM.

RESULTS AND DISCUSSION

Leaf epidermal anatomy

Renvoize (1995) mentioned that although the occurrence of siliceous cells in Poaceae is considered usual, in some species the short cells may be represented exclusively by the siliceous cells. In this paper *P. antidotale*, *P. capillare* and *P. psilopodium*, short cells were arranged in rows of 2 to 3 cells whereas *P. turgidum* had short cells arranged in rows of five or more cells (Table 1). The shapes of silica bodies showed variation among different species. *P. antidotale*, *P. miliaceum* and *P. sumatrense* had silica bodies in between the cross-shaped and dumb-bell shaped (Figure 1a and b) whereas only dumb-bell shaped and nodular shaped silica bodies were observed in *P. psilopodium* (Figure 1c). *P. turgidum* comprises of cross-shaped to nodular shaped silica bodies (Figure 1d). These findings incorporate with the results of Ishtiaq et al. (2001). Majority of the *Panicum* species possessed macro-hairs in the intercostal regions except *P.*



ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.3037

Element	(keV)	mass%	Error%	At%	Compound	mass%	Cation	K
C K	0.277	36.76	0.10	50.50				13.2827
O K	0.525	38.41	0.24	39.61				32.0595
Si K	1.739	14.83	0.11	8.71				17.3794
K K	3.312	1.00	0.18	0.42				1.2405
Au M	2.121	9.01	0.49	0.75				7.6515
Total		100.00		100.00				

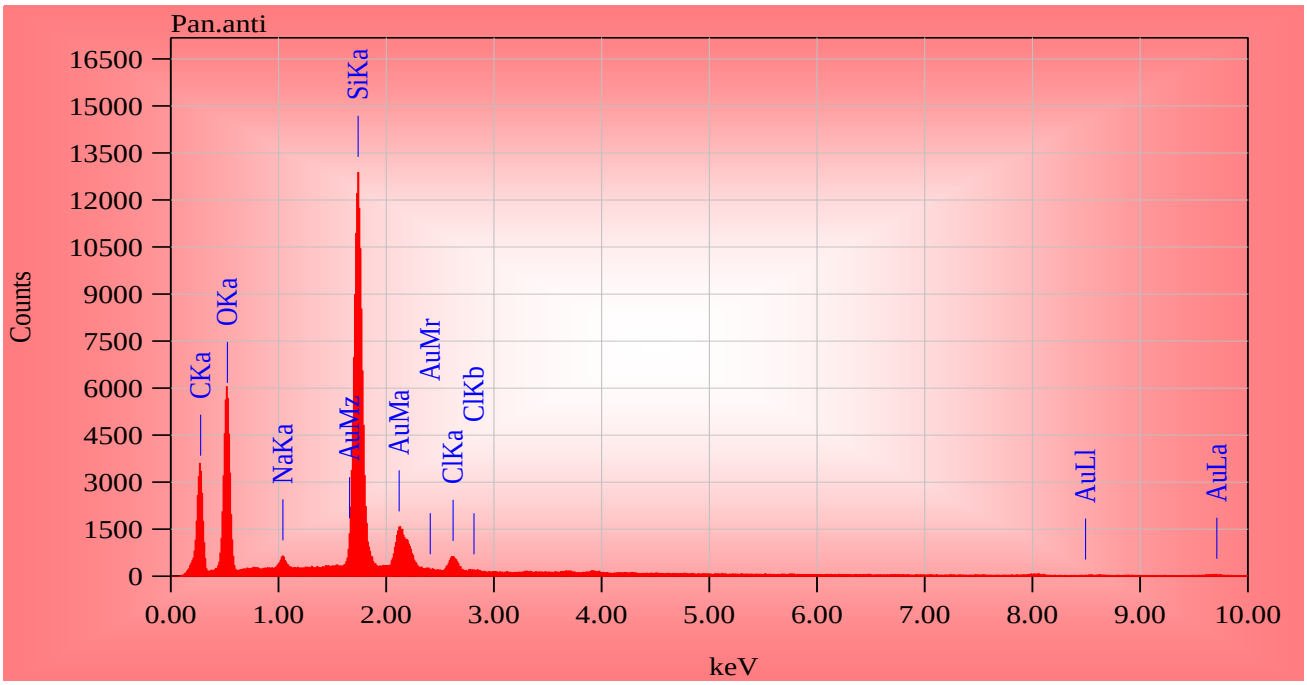
Figure 2. EDS Analysis of *P. atrosanguineum.*

psilopodium and *P. sumatrense* in which no macro-hairs were found (Table 1). *Panicum* species comprised micro-hairs in the intercostal regions (Figure 1e) except *P. antidotale* in which micro-hairs were found both in costal and intercostal regions.

Bi-celled micro-hairs were observed in this genus. Gilani et al. (2002) stated that the basal cell was longer than the distil cell of micro-hairs in *Digitaria abludens*, it was equal to the distil cell in *D. nodosa* and shorter than the distil cell in *D. ciliaris.* The prickles were easily observed in the majority of the species and they had been already mentioned by Renvoize (1995) and Soderstrom and Ellis (1988) for family Poaceae. Beak shaped and knife-shaped prickles were observed in majority of the taxa in both the costal and intercostals regions (Figure 1f, g and h) except *P. miliaceum*, in which prickles were found only in the costal region. No hook cells were observed in *P. antidotale*, *P. atrosanguineum* and *P. capillare* whereas *P. miliaceum*, *P. psilopodium*, *P. sumatrense* and *P. turgidum* comprised the hook cells (Table 1). It is well known that the leaf, compared with the other vegetative organs, is the best and fastest to react to changes in the environment. The influence of different ecological factors on plant organisms is best reflected on leaf morphological and anatomical structure. Variations in minute microscopic characters of leaf epidermis are used to clear up the taxonomic position of the taxa in the genus *Panicum* and different anatomical features also provides the significant anatomical data with the aim of determining their structural adaptations.

EDS analysis

EDS analysis SEM showed the elemental composition of phytoliths and a great variation was observed among the percentage of the elements between different species of the genus *Panicum.* Microfossil phytolith have been used for the reconstruction of pale environments (Fisher et al., 1997; Lewis, 2001) as indicators of ancient industrial and agricultural practices (Rosen, 1992 and Rosen, 1999). There were minor differences in mass percentage of silicon in genus *Panicum*. *P. atrosanguineum* shows the lowest value as 14.83% (Figure 2) where as *P. antidotale* showed the highest value as 19.14% (Figure 3). Phytolith research has also proved to be highly valuable to argue



ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.2769

Element	(keV)	mass%	Error%	At%	Compound	mass%	Cation	K
C K	0.277	31.55	0.12	45.50				8.8344
O K	0.525	37.49	0.22	40.59				31.9440
Na K	1.041	0.86	0.13	0.65				0.8071
Si K	1.739	19.14	0.10	11.80				22.0016
Cl K	2.621	1.23	0.13	0.60				1.4322
Au M	2.121	9.72	0.49	0.86				7.8683
Total		100.00		100.00				

Figure 3. EDS Analysis of *P. antidotale.*

botanists as phytoliths were siliceous maintain their morphological integrity and become a microfossil of that plant. The present study is a step towards preparing a systematic inventory of grass phytoliths. In the present work, it is suggested that the elemental characterization of biogenic silica through EDS analysis paired with the phytoliths morphology can be implied as a new tool to resolve taxonomic deadlock in the identification of *Panicum* species. Investigations through EDS of phytoliths evidenced that phytoliths are of siliceous nature and silica is the major component in their formation. A great range of variation as evident in the present investigation gives much hope for the taxonomists dealing with the taxonomic problems in the Poaceae.

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