Journal of Medicinal Plants Research Vol. 5(17), pp. 4279-4286, 9 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Micormorphological diversity in leaf epidermal anatomy of *Brachiaria* species using elemental dispersive spectrophotometer analysis

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Accepted 9 June, 2011

In this study the micromorphological diversity linked to different elements silica bodies, epidermal leaf anatomy and elemental dispersive spectrophotometer (EDS) analysis (LM, SEM) of five species of genus *Brachiaria* was carried out. The technique of EDS analysis for grass taxonomy is used first time in Pakistan. This examination indicates variations in leaf blade epidermal characters such as shapes of silica bodies, prickles, and microhair and macrohair form. EDS analysis of the phytoliths determines a new structure used as an aid in phytosystematic characterization. The quantitative analysis provides silicon as a new taxonomic character to distinguish different species of the genus *Brachiaria*.

Key words: Epidermal anatomy, micropalynological diversity, elemental dispersive spectrophotometer.

INTRODUCTION

Brachiaraia Griseb, is a genus of annual and perennial grasses belonging to the tribe Paniceae, sub-family, Panicoideae of the family Poaceae. This genus is represented by a total of about 50 species of which 17 are reported from India (Bor, 1960). Trinius (1826) first recognized *Brachiaria* as a section of *Panicum* on the basis of its anatomical evidence. The section was elevated to generic level by Grisebach (1853). The recent studies indicate that the anatomy can be used in delimiting species, genera, tribes and sub-tribes. Under this respect, the work of Prat (1932, 1936) and Metcalfe (1960) have contributed considerable anatomical data about this. Amarasinghe and Watson (1988) have studied stomata ontogenesis and ultrastructure of microhairs in Poaceae.

Fahmy (2007) stated that silicon (Si) accumulation in organs and cells is one of the most prominent characteristics of plants of the family Poaceae. Elemental dispersive spectrophotometer (EDS) analysis provides important anatomical characters in correlation with the morphological distribution of the silica bodies such as in many cases the cell type from which the phytolith derived can be identified by the shapes of silica bodies. EDS analysis of the silica bodies in grasses are constructed for various purposes ranging from their taxonomical description to classification and quantitative analysis of silica bodies (Terrel and Wergin, 1979).

Mainly in relation to anatomy, the present research presents a standard basis for grouping of grasses focusing on the silica bodies giving their specific organizational detail that helps in the identification of *Brachiaria* species particularly and grasses generally. The present study is a step towards preparing a systematic inventory of grass phytoliths. EDS analysis (SEM) showed the elemental composition of phytoliths and a great variation was observed among the percentage of the elements among different species of

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Table 1. Diversity of epidermal features among *Brachiaria* species.

Characteristics	B. *distachya*	*B. eruciformis*	*B. ramosa*	*B. reptans*	*B. villosa*
Short cells	In rows of 5 or more cells	In rows of 6 or more cells	In rows of 6 or more cells	In rows of 5 or more cells	In rows of 7 or more cells
Silica bodies	In between cross shaped and dumb-bell shaped	Mostly dumb-bell shaped and cross shaped and in between dumb-bell and nodular shaped	Mostly dumb-bell shaped and cross shaped	In between dumb-bell shaped and cross shaped, nodular shaped and narrowly oval shaped	Dumb-bell shaped, cross shaped and horizontally elongated
Macro-hairs	Between the veins, 60-130 µm	Between the veins, 90-165 µm	Between the veins, 55-60 µm	Between the veins, 70-270 µm	Between the veins, 40-110 µm
Micro-hairs	Between the veins, 7-12 µm	Between the veins, 25-40 µm	Between the veins, 10.1-10.9 µm	Between the veins, 4-5 µm	Between the veins, 20-40 µm
Hooks	Abundantly present, 1.5-3 µm	Rarely present, 1-2.5 µm	Abundantly present, 2-3 µm	Rarely present, 0.5-1 µm	None seen
Stomata	With 2-3 rows, dome-shaped subsidiary cells, 6-7 µm	With 2-3 rows, triangular-shaped subsidiary cells, 4-5 µm	With 1-2 rows, triangular-shaped subsidiary cells, 3-4 µm	With 2-5 rows, triangular-shaped subsidiary cells, 2-3 µm	With 3-11 rows, triangular-shaped subsidiary cells, 5-8 µm
Long cells	Two types, with lobed ends alternating the stomata and broad long cells, which are not lobed, with sinuous walls, 14-15 µm	Thick sinuous walls, 8-27 µm	Thick sinuous walls, 7-23 µm	Two types with thick sinuous walls longer in length 10-10.5 µm and with non-sinuous walls shorter in length 3-8 µm	Thick sinuous walls, 10.6-38 µm

the genus *Brachiaria*.

MATERIALS AND METHODS

Leaves from fresh as well as dried specimens were used for anatomical studies. The dried material was taxonomically confirmed by comparing with herbarium specimens of the Quaid-e-Azam University Islamabad (ISL). The LM of abaxial and adaxial epidermis of leaf samples was carried out following the method of Cotton (1974) and Clark's (1960) but with a little modification (Ahmad et al., 2010). For EDS analysis the abaxial and adaxial epidermal leaf samples were placed on stubs and after the gold coating put into SEM and then quantitative analysis of phytoliths were taken, especially the mass percentage of silicon was calculated in order to make a comparison between the different taxa. SEM in conjugation with EDS (SEM/EDS) makes possible the quick resolution of tough analytical problems effectively, timely and economically (Terrel and Wergin, 1979). The microphotographs of epidermal features, silica deposition and distribution were taken using light microscope and scanning electron microscope equipped with an energy dispersive X-ray spectrometer (SEM-EDXS).

RESULTS AND DISCUSSION

Leaf epidermal anatomy

Anatomical studies revealed clear-cut differences in size and shapes of short cells, silica bodies, microhairs with basal and distal cells, macrohairs, hooks, stomatas and long cells of the genus *Brachiaria*. The long cells on both surfaces possess rectangular shape and thick sinuous walls in majority of the taxa except in *Brachiaria eruciformis* where the long cells are only slightly sinuous. In *Brachiaria distachya* and *B. reptans* more than five short cells and cork cells are fused together in a row whereas in the *B. eruciformis* and *Brachiaria ramosa* more than six cells are fused together. In the *Brachiaria villosa* more than seven cells are fused together in a row (Table 1). According to Sharma and Kaur (1983) 5 short cells including silica cells and cork cells are fused together in a row in *B. ramosa*.

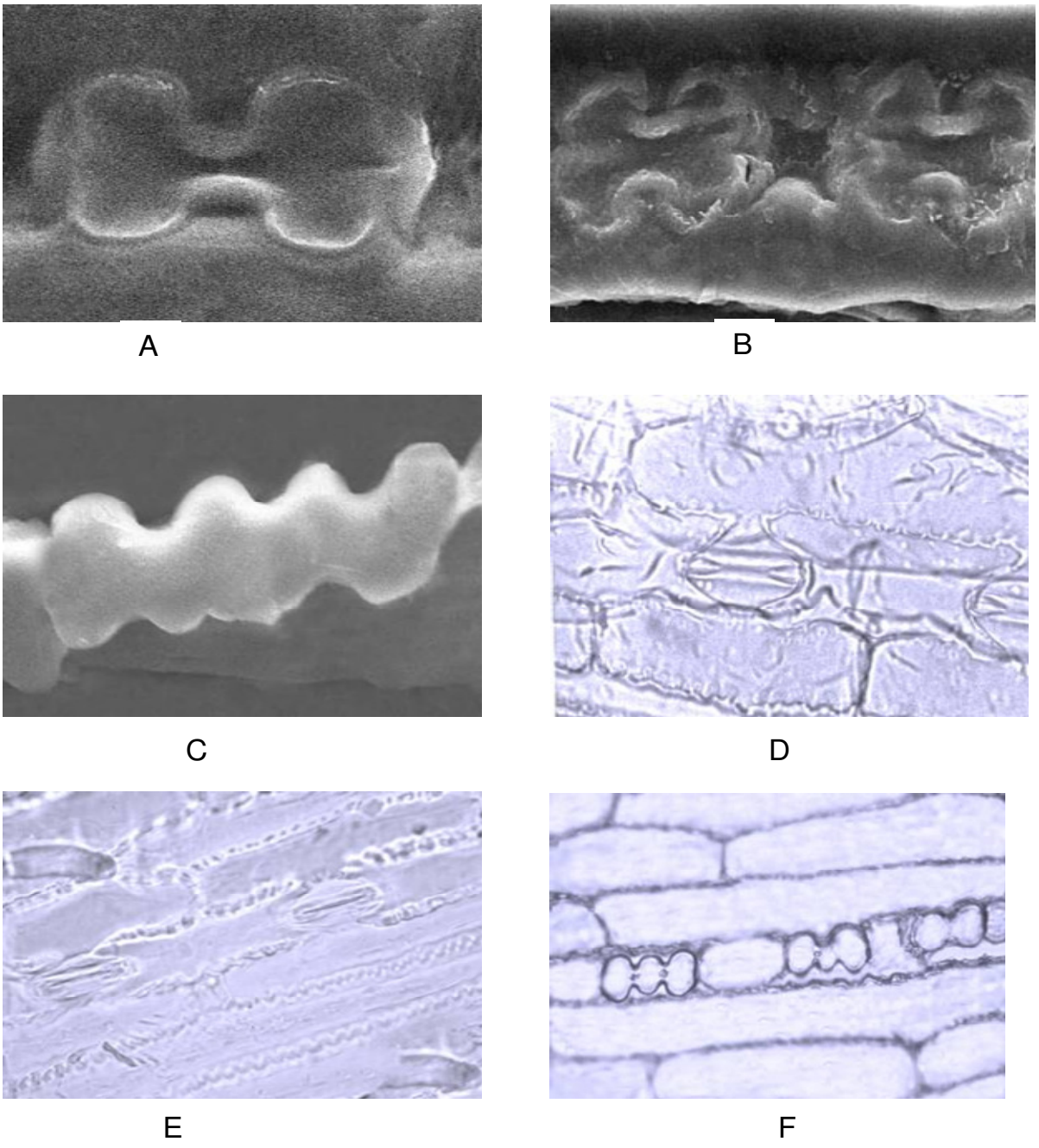

Plate 1. (a) SEM of dumb-bell shaped Silica body (*B. villosa*), (b) SEM of cross shaped Silica Body (*B. ramosa)*, (c) SEM of nodular shaped Silica body (*B. reptans*), d) Single celled micro-hair (*B. eruciformis*), (e) Thick sinuous walled long cells *(B. villosa)*, (f) In between dumb-bell shaped silica bodies (*B. reptans)*.

The shape of silica bodies plays an important role in the identification of the species of the genus *Brachiaria.* There is a great variation in the shape of silica bodies of the genus *Brachiaria.* Dumb-bell shaped and cross-shaped silica bodies were observed in in *B. ramosa, B. villosa* and *B. eruciformis* (Plate 1a and b). Horizontally elongated shaped silica bodies were present in the abaxial surface in *B. villosa*. Cross-shaped, nodular-shaped, oval shaped and intermediate between cross-shaped and dumb-bell shaped silica bodies were observed in *B. reptans* in the abaxial surface (Plate 1c) whereas adaxial surface possess dumb-bell shaped and cross-shaped silica bodies only. These results in this respect corroborate with the findings of Sharma and Kaur (1983).

Microhairs were observed in all the species.

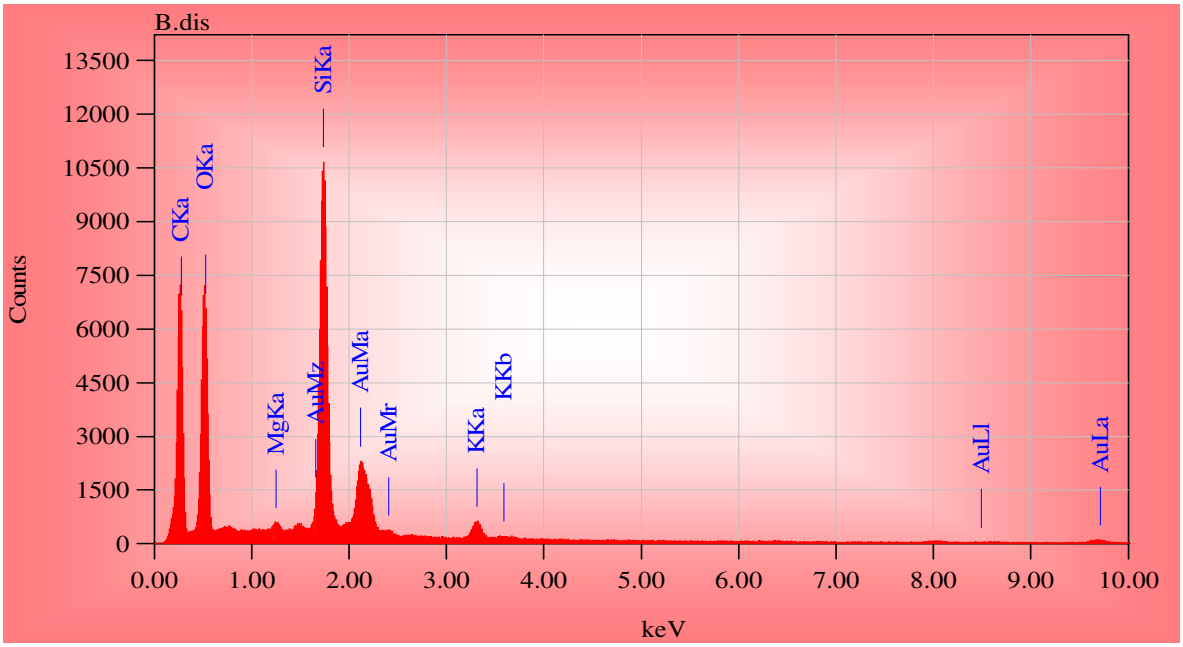

Figure 1. EDS analysis of *B. distachya.*

In *B. eruciformis, B. ramosa* and *B. villosa* microhairs were absent in abaxial surface, but present in adaxial surface whereas in *B. distachya* microhairs are present only in the abaxial surface. *Brachiaria reptans* possess microhairs on both abaxial and adaxial surfaces. The microhairs in the genus *Brachiaria* are of panicoid type as classified by Metcalfe (1960).

Metcalfe (1960) did not observe any macrohairs in *B. reptans,* but in this study macrohairs were observed in the intercostal zone of both the abaxial and adaxial epidermis in *B. reptans*. Macrohairs were found on the abaxial surface only in *B. distachya* and *B. ramosa* whereas *B. eruciformis* possess macrohairs on the adaxial surface only. The results of this investigation are quite different from the findings of Sharma and Kaur (1983).

The prickles were absent on both surfaces of leaf epidermis of all the species of genus *Brachiaria.* Sharma and Kaur (1983) in their studies concluded that prickles in the form of hooks were present in *B. distachya*. Stomata were observed in the intercostal regions of both surfaces in all the species of the genus. Shoulaing et al. (1996) considered, the tall dome shaped subsidiary cells as primitive while parallel and triangular subsidiary cells as advanced characters.

The variations in the leaf epidermis features in the different species of genus *Brachiaria* are taxonomically useful both at specific and sub-specific level. The presence or absence of macrohairs, microhairs and prickles, the distribution and morphology of long and short cells, silica bodies and the stomatal features are important diagnostic characters, which can be utilized for the identification and separation of various species of *Brachiaria.*

Elemental dispersive spectrophotometer (EDS) analysis

The phytoliths were observed through SEM and their siliceous nature was confirmed by EDS technique and was classified taxonomically by comparison with a large collection of silica particles in each species. The EDS data from the silica bodies showed the presence of different elements including carbon, oxygen, silicon, magnesium, sodium, potassium, calcium and chlorine. All the EDS data results have carbon and oxygen. As the phytoliths are of siliceous nature, so in reviewing the percentage of silicon, a distinct comparison of the species within the genera can be seen.

Fahmy (2007) stated that the phytoliths of grasses are of particular interest, as they possess important morphological features, which have encouraged many investigators to identify these plants. In the genus *Brachiaria*, the *B. eruciformis* shows the lowest value of silicon as 8.35% (Figure 2 and Table 3) whereas *B. distachya* shows the highest value as 12.43% which constitutes a major portion of silica bodies and plays a vital role in their composition indicating that this feature is very useful at the species level (Figures 1 to 5 and Tables 2 to 6). The EDS analysis of phytoliths of grasses has been carried out first time in Pakistan and showed a lot of variation within the species and a distinct

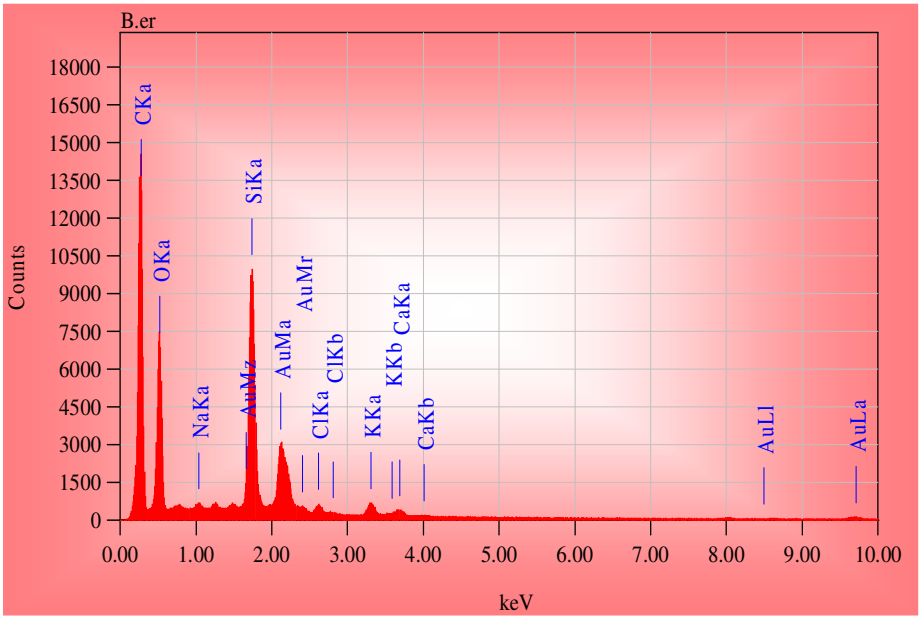

Figure 2. EDS analysis of *B. eruciformis.*

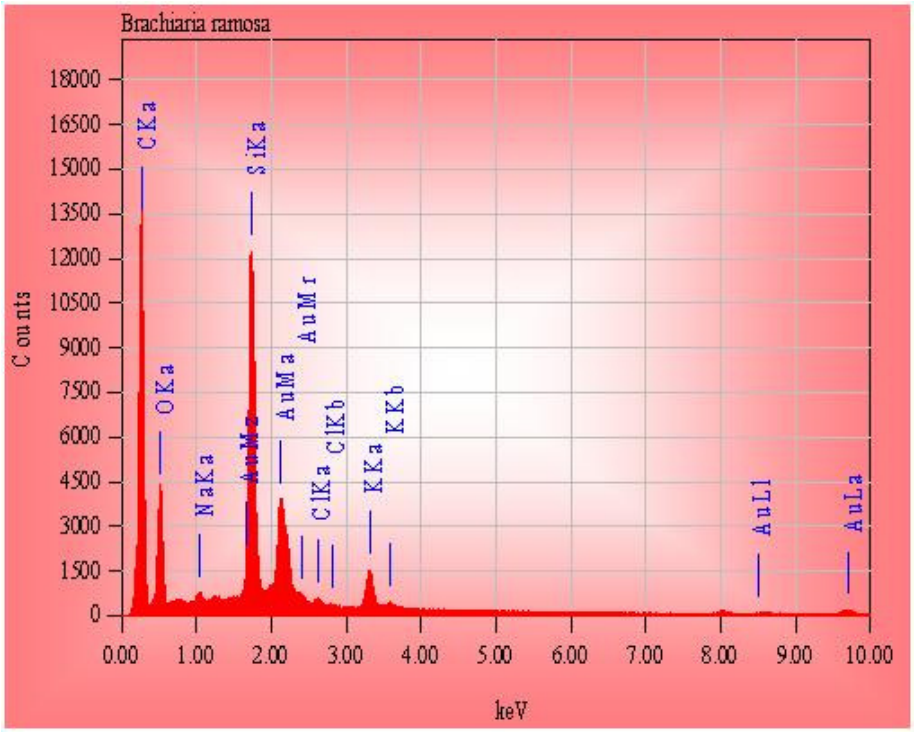

Figure 3. EDS analysis of *Brachiaria ramosa*

comparison of the species within the genera can be seen. The EDS analysis of grasses can be used as a taxonomic tool for the classification of grasses into different groups.

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

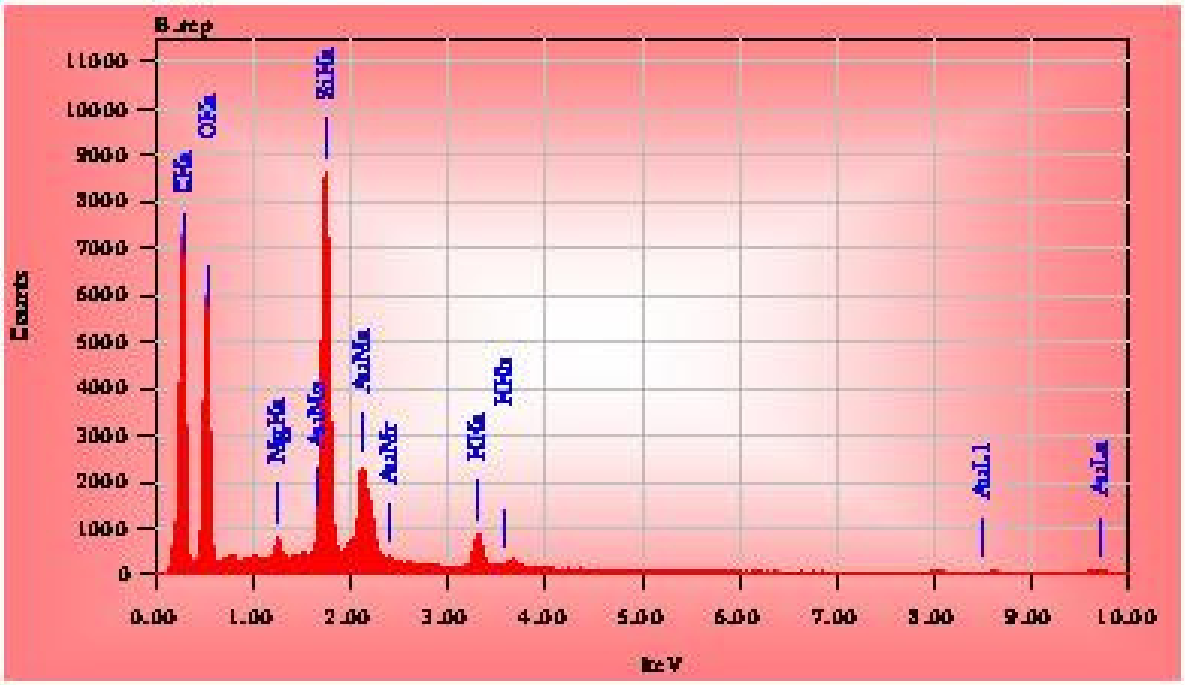

Figure 4. EDS analysis of *Brachiaria reptans.*

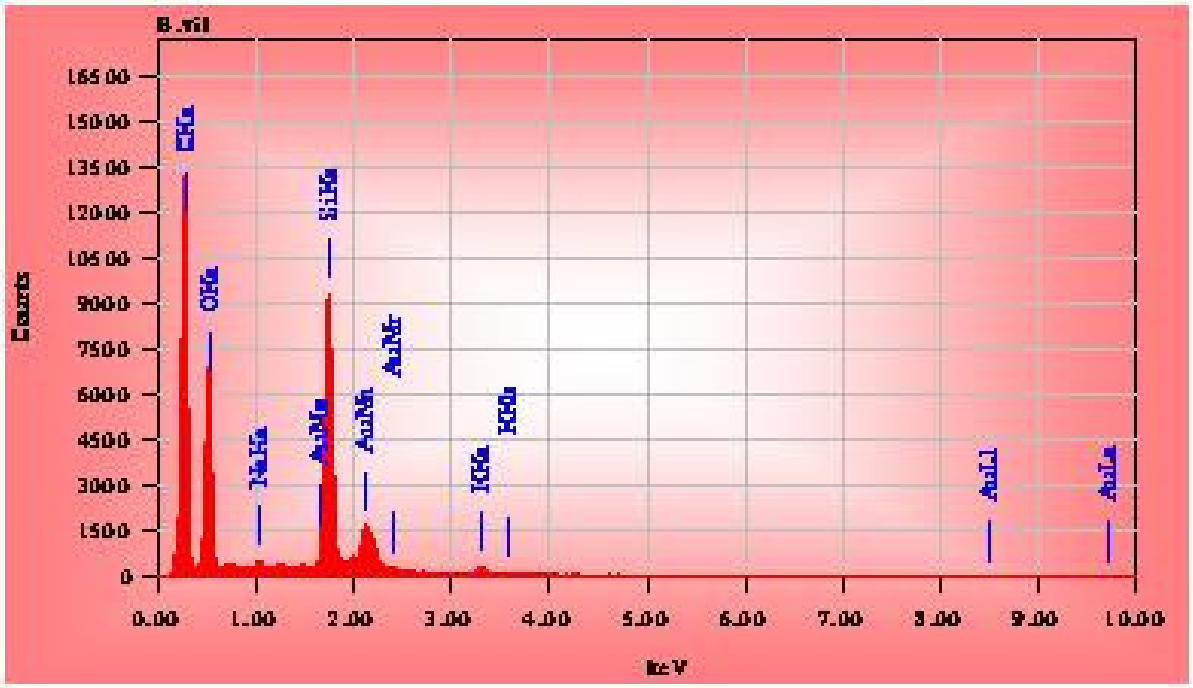

Figure 5. EDS analysis of *Brachiaria villosa*

identification of *Brachiaria* 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

Table 2. ZAF method standardless quantitative analysis (Figure 1).

Fitting coefficient: 0.3040					
Element	**(keV)**	**Mass (%)**	**Error (%)**	**Compound mass (%)**	**Cation K**
C K	0.277	39.52	0.09	53.98	15.8346
O K	0.525	36.29	0.25	37.21	29.8752
Mg K	1.253	0.36	0.11	0.24	0.4318
Si K	1.739	12.43	0.11	7.26	14.9441
K K	3.312	1.09	0.18	0.46	1.3970
Au M	2.121	10.31	0.48	0.86	9.1645
Total		100.00		100.00	

Table 3. ZAF method standardless quantitative analysis (Figure 2).

Fitting coefficient: 0.3221					
Element	**(keV)**	**Mass (%)**	**Error (%)**	**Compound mass (%)**	**Cation K**
C K	0.277	48.90	0.09	63.63	24.6131
O K	0.525	30.73	0.35	30.02	23.8418
Na K	1.041	0.31	0.17	0.21	0.3191
Si K	1.739	8.35	0.13	4.64	10.7676
Cl K	2.621	0.48	0.16	0.21	0.6509
K K	3.312	0.83	0.22	0.33	1.1470
Ca K	3.690	0.41	0.25	0.16	0.5966
Au M	2.121	9.99	0.57	0.79	9.7800
Total		100.00		100.00	

Table 4. ZAF Method standardless quantitative analysis

Fitting coefficient: 0.3141					
Element	**(keV)**	**Mass (%)**	**Error (%)**	**Compound mass (%)**	**Cation K**
C K	0.277	52.82	0.11	70.85	26.4712
O K*	0.525	20.56	0.41	20.71	15.3378
Na K	1.041	0.39	0.17	0.27	0.4585
Si K	1.739	10.46	0.13	6.0	14.8729
Cl K	2.621	0.38	0.17	0.17	0.5567
K K	3.312	2.26	0.23	0.93	3.3373
Au M	2.121	13.12	0.59	1.07	13.8760
Total		100.00		100.00	

Table 5. ZAF Method standardless quantitative analysis.

Fitting coefficient: 0.3188					
Element	**(keV)**	**Mass (%)**	**Error (%)**	**Compound mass (%)**	**Cation K**
C K	0.277	41.44	0.09	56.23	17.8062
O K*	0.525	34.68	0.27	35.32	27.9606
Mg K	1.253	0.71	0.12	0.47	0.8703
Si K	1.739	10.99	0.11	6.37	13.4065
K K	3.312	1.78	0.19	0.74	2.3238
Au M	2.121	10.40	0.50	0.86	9.4886
Total		100.00		100.00	

Table 6. ZAF method standardless quantitative analysis.

Fitting coefficient : 0.3129					
Element	**(keV)**	**Mass (%)**	**Error (%)**	**Compound mass (%)**	**Cation K**
C K	0.277	51.63	0.09	63.83	27.0390
O K	0.525	32.84	0.34	30.48	25.5482
Na K	1.041	0.37	0.16	0.24	0.3837
Si K	1.739	9.19	0.13	4.86	11.6471
K K	3.312	0.47	0.21	0.18	0.6366
Au M	2.121	5.50	0.57	0.41	5.2278
Total		100.0		100.00	

Poaceae.

REFERENCES

Ahmad MMA, Khan M, Zafar M, Arshad S, Sultana B, Abbasi H, Din SU (2010). Use of chemotaxonomic markers for misidentified medicinal plants used in traditional medicines. J. Med. Plants Res., 4(13): 1244-1252.

Amarasinghe V, Watson L (1988). Variation in Salt Secretory Activity of Microhairs in Grasses. Aust. J. Plant Physiol., 16(2): 219-229.

Bor NL (1960). Grasses of Burma, Ceylon, India and Pakistan, London (Permamon Press), p. 767.

Brown W (1977). The Kranz syndrome and its subtypes in grass systematics. Memoirs of the Torrey Botanical Club, 23: 1-97.

Clark J (1960). Preparation of leaf epidermis for topographic study. Stain. Technol., 35: 35-39.

Cotton R (1974). Cytotaxonomy of the genus Vulpia. Ph.D. Thesis, Univ. Manchester, USA.

Fahmy AG (2007). Diversity of lobate phytoliths in grass leaves from the Sahel region, West Tropical Africa: Tribe Paniceae. Plant System. Evol., 270(1-2): 1-23.

Grisebach A (1853). Brachiaria. In CF Ledebour [ed.], Flora rossica, 4: 469.

Hattersley PLW (1992). Diversification of photosynthesis. In G. Chapman [ed.], Grass evolution and domestication, pp. 38-116. Cambridge University Press, London, UK.

Hattersley PLW (1992). Diversification of photosynthesis. In G. Chapman [ed.], Grass evolution and domestication, pp. 38-116. Cambridge University Press, London, UK.

Metcalfe CR (1960). Anatomy of monocotykedons 1: Gramineae Jodrell L. B. Royal Botanic Gardens Kew. Oxford University Press.