Journal of Medicinal Plants Research Vol. 5(24), pp. 5803-5809, 30 October, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Systematic application of palyno-anatomical characterization of *Setaria* species based in scanning electron microscopy (SEM) and light microscope (LM) analysis

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Accepted 25 August, 2011

This study based on multiple parameters including morphology, epidermal leaf anatomy and palynological (light microscopy and scanning electron microscopy) systematics of 5 species of genus *Setaria* (Poaceae). The study is confined to five species of the genus *Setaria* that is, *Setaria glauca, Setaria italica*, *Setaria tomentosa, Setaria verticillata* and *Setaria viridis*. In this study, the main emphasis was on palyno-anatomical characterization used as an aid in systematics. The scanning electron microscopic analysis of grasses, particularly *Setaria* species, has been reported for the first time in Pakistan, and it shows a lot of variation within the species. From this study, *S. tomentosa* is recorded as a new report in the Flora of Pakistan. It is stated that the study based on classical and modern approaches is very useful for systematic delimitation of problematic taxa like *Setaria*.

Key words: Palyno-anatomical, elemental dispersive spectrophotometer, scanning electron microscopy, *Setaria* (Poaceae).

INTRODUCTION

Grasses exhibit a great diversity in inflorescence forms. The overall pattern is sufficiently complex that it is difficult to analyze inflorescence evolution. The complexity of the problem is reduced by examining one group of grasses, the panicoid "bristle clade," which exhibits a less complex pattern of variation. (Andrew and Elizabeth, 2002). *Setaria* is one of the grass genus, commonly recognized in the floras of the world. Bor (1960) reported that *Setaria* P. Beauv, is a genus of annual and perennial grasses belonging to the tribe Paniceae, sub-family Panicoideae of the family Gramineae. It is represented by nearly 125 species distributed throughout the temperate and tropical regions of the world. In the Indian sub-continent, the genus is represented by a total of 17 species.

Palyno-anatomical characterization has assumed great significance in plant taxonomy and the advancements in the microscopy have led to the effective use of parameters for taxonomic purposes. The purpose this of study was to assess the significance of pollen and leaf epidermal characters for systematic applications. Several of the pollen features are of diagnostic importance thus one can usually distinguish different grass species based on palynological characterization. The main aim of this study is to correlate morphological features of *Setaria* and its species with palyno-anatomical characters using light microscopic and scanning electron microscopic tools.

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MATERIALS AND METHODS

Morphology

For morphological study preserved specimens of *Setaria* species from the herbarium of Quaid-I-Azam University (ISL) and fresh specimens from the field were used. 1 to 5 specimens per species were used for assessment of morphological characters, 3 to 5 values were for each character of a representative species. Morphological characteristics were reconfirmed by using various Floras (Townsend and Guest, 1968; Tutin and Heywood, 1972; Hooker, 1875; Nasir and Ali, 1982; Saldanha and Nicolson (1976) and Hooker and K.C.S.I. 1885a, 1894).

Leaf epidermal anatomy

Leaves from fresh and dried specimens were used for anatomical studies. Leaves samples were prepared according to the method of Cotton (1974) who followed Clark's (1960) technique, but with a little modification (Ahmad et al., 2010). Leaves were soaked in lactic acid for a few minutes to make them soft unfolded and than remove epidermis. Different epidermal characteristics including qualitative and quantitative were determined using light microscope (Mejii- MX 5200H, Japan). The microphotographs of epidermal features are taken using Leica light microscope (LM).

Scanning electron microscopic study of pollen

Polleniferous material from dried specimens was used to prepare slides by adopting the method of Zafar et al. (2011). Various characterization of pollen that is, shape, type, structure, diameter, exine thickness, P/E ratio and sculpturing were determined using light microscope (Mejii – MX 5200H, Japan). Scanning Electron Microscopy (SEM) was used for imaging of pollen with distinct sculpturing.

RESULTS AND DISCUSSION

Morphology

Morphologically the varied length, width and color of spikes are different in different species. Skerman and Riveros (1990) and Rosen (2009) stated that in *Setaria italica*, the inflorescence had main stalk with shortened branching bearing spikes and bristles. A cluster of hairs is present in the most of the species, but in case of *Setaria glauca* cluster of hairs is absent at rachilla. Spikelets seen in each species are enclosed by involucres, composed of whorls of bristles. The size and nature of the bristles varies in different species. In some cases, the bristles are hairy for example, *S. glauca* (Table 1).

The length, width and nerves on the glumes are also diagnostic, for example, *Setaria tomentosa* nerves are present on the upper and lower glume, glumes are 5-nerved. *B. villosa*, the lower glume is 3-nerved and the upper glume is 5-nerved. Townsend and Guest (1968) and Renvoize (2005) reported that in *S. glauca* upper glume observed is shorter *S. italica* upper glume and in *Setaria verticillata*, upper glume is as long as the spikelet.

Similarly, the length of lemma range and palea is also a characteristic feature. In *S. tomentosa*, the lemma length is 0.3 to 0.4 mm whereas in *S. verticillata*, the lemma is 0.2 mm long. Many species, the length of the lower lemma is analogous to spikelet length. *S. verticillata* has very minute palea that is, 0.1 mm long whereas in *S. glauca* the palea is as long as the lemma (Table 1).

Leaf epidermal anatomy

The epidermal leaf blade anatomy is taxonomically useful. On the basis of leaf epidermal anatomy, we can distinguish among different species of the genus *Setaria*. Ishtiaq et al. (2001) studied abaxial and adaxial leaf epidermal anatomy of four species having morphological resemblances. Our findings showed variation in short and long cells, silica bodies, macro and micro-hairs, presence and absence of stomata and shape of subsidiary cells. Most of these characters are diagnostic and have been used for making keys.

In *S. glauca*, *S. italica* and *S. viridis*, more than five cells are fused together in a row in the costal region whereas more than four cells are arranged in a row in the costal region in the *S. verticillata* and more than six cells are arranged in a row in the *S. tomentosa*. In the genus *Setaria*, both the abaxial and adaxial surfaces possess almost the same silica bodies. Dumb-bell and nodular shaped silica bodies are found in *S. glauca* and *S. tomentosa* (Figure 1) while dumb-bell to cross-shaped silica bodies in *S. verticillata* are present (Figure 3). Sharma and Kaur (1983) mentioned that silica bodies alternating with cork cells are present in the costal regions only in all the species of *Setaria* except *S. plicata* where they are observed in both the costal and intercostal regions. In the genus *Setaria* most diagnostic feature is the shape of silica bodies, which is helpful at specific level.

Prat (1936), Dahlgren and Clifford (1982) and Shouling (2006) reported that congruence between grass phylogeny and morphological patterns in silica bodies has been customarily implied for a long time by grass taxonomists. A great variation in mass percentage of silicon is observed in the genus *Setaria*. *S. italica* shows the lowest value as 9.54% (Figure 2) whereas *S. viridis* shows the highest value as 19.60% (Figure 5). It is found that the mass percentage of silicon is an important feature for taxonomic debate in the genus *Setaria*.

Macro- and micro-hairs are found in all the species of the genus *Setaria*. Watson and Dallwitz (1988) stated that the presence or absence of micro-hairs is widely used as a taxonomic character. Johnston and Watson (1976) and Fahmy (2007) reported that morphologically micro-hairs are generally classified into three major types, the chloridoid type, with relatively short, broad and thick walled cap cells, the panicoid type with relatively long, narrow and thin walled and elongated cap cells with very long basal cells and ovoid cap cells. These

Table 1. Morphological distinctions among the species of genus *Setaria* L.

Characteristics	*S. glauca*	*S. Italica*	*S. tomentosa*	*S. verticillata*	*S. viridis*
Habit	Bunched annual	Summer annual	Annual or herbaceous perennial	Loose lytufted annual	Summer annual
Culms	Spreading to erect	Erect or somewhat geniculate at the base	Decumbent	Erect	Erect or geniculate
Leaf sheaths	Glabrous or sparsely hairy	Glabrous, puberulent or pubescent	Glabrous or pubescent	Glabrous or papillose	Glabrous to papillosepilose
Leaf sheath margins	Scabrid	Ciliate	Scabrous	Scabrous	Densely ciliate
Spikelets	Ovate	Elliptic to obovate	Ovoid to ellipsoid	Ellipsoid	Ellipsoid
Lower glume	Ovate	Ovate	Obovate	Ovate	Oblate
Lower glume apex	Acute	Acute	Obtuse or acute	Acute	Obtuse
Upper glume	Ovate	Elliptic	Boat shaped	Ovate	Elliptic
Upper glume apex	Acute	Acute or obtuse	Obtuse or acute	Acute	Obtuse
Glumes nerves	5-nerved	3-7 nerved	3-7 nerved	3-5 nerved	3-5 nerved
Lemma	Ovate	Elliptic or oblong	Ovate	Ovate	Elliptic
Lemma apex	Obtuse	Acute or obtuse	Acuminate	Acute	Obtuse
Lemma surface	Strongly rugose	Finally rugose	Finally rugose	Finally rugose	Finally rugose
Lemma nerves	5-nerved	5-7 nerved	5-nerved	5-nerved	5-nerved

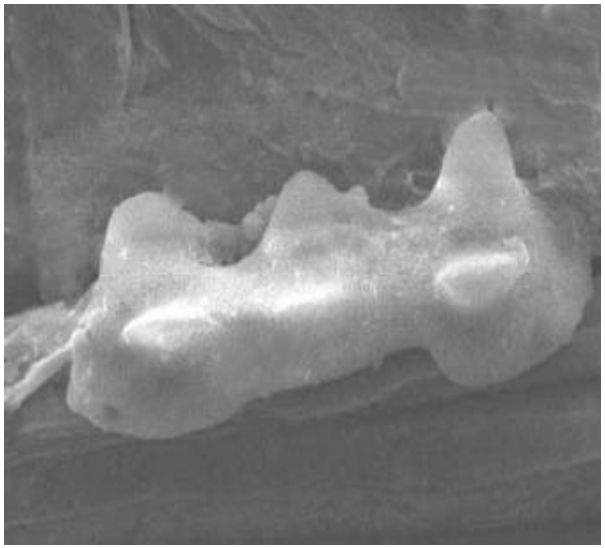

Figure 1. Setaria glauca SEM of leaf epiderma silica body (abaxial epidermis).

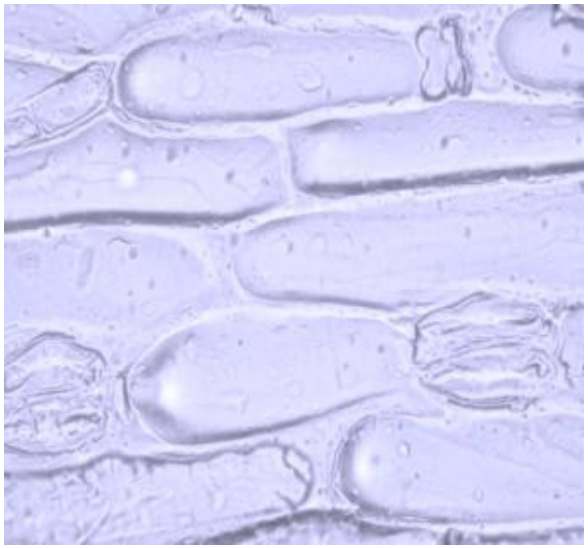

Figure 2. *Setaria tomentosa* Stomata and non-sinuous walled long cells (abaxial epidermis).

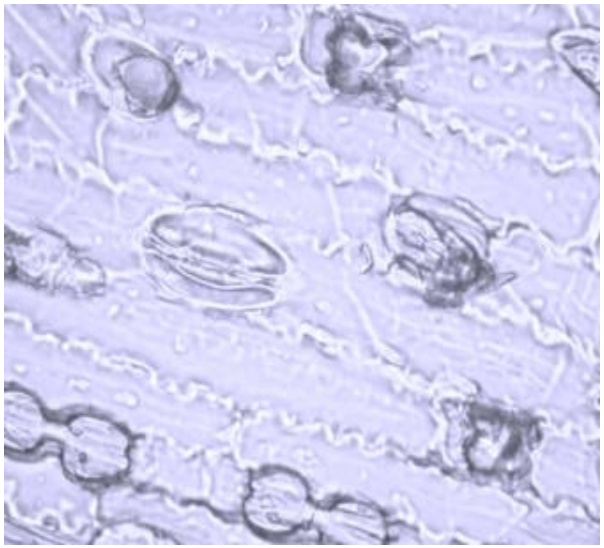

Figure 3. *Setaria verticillata* Dumb-bell shaped and cross shaped silica bodies (abaxial epidermis).

Table 2. Anatomical distinction of leaf epidermal features among the species of genus *Setaria.*

Characteristics	*S. glauca*	*S. italica*	*S. tomentosa*	*S. verticillata*	*S. viridis*
Short cells	In rows of 5 or more cells	In rows of 5 or more cells	In rows of 6 or more cells	In rows of 4 or more cells	In rows of 5 or more cells
Silica bodies	Dumb-bell shaped and nodular shaped	Cross-shaped, intermediate between cross-shaped and dumb-bell shaped and few dumb-bell shaped	Cross-shaped, dumb bell shaped and rarely nodular shaped	Cross-shaped and dumb bell shaped	Dumb-bell shaped, cross shaped and intermediate between dumb-bell shaped and cross shaped
Macro-hairs	Between the veins, 90-110 µm	Between the veins, 90-100 µm	Over the veins, 60-120 µm	Between the veins, 85-100 µm	Between the veins, 60-90 µm
Micro-hairs	Between the veins, 40-60 µm	Between the veins, 36-52 µm	Between the veins, 48-60 µm	Between the veins, 25-30 µm	Between the veins, 46-54 µm
Prickles	None seen	Over the veins, beak shaped, prism shaped, 5-7 µm	Over the veins, angular shaped, 3-6 µm	Between the veins, narrowly oblong, 7-10 µm	Between the veins, angular shaped, 7-9 µm
Hooks	Present, 1-1.5 µm	Present, 1-3 µm	Present, 2-6 µm	Present, 1-2 µm	Present, 2-5 µm
Stomata	With 3-4 rows, triangular shaped subsidiary cells but also with low dome shaped subsidiary cells, 5-7 µm	With 1-2 rows, triangular shaped subsidiary cells, 4-5 µm	With 1-3 rows, triangular shaped subsidiary cells, 4-5 µm	With 2-3 rows, triangular shaped subsidiary cells, 4-5 µm	With 2-3 rows, triangular shaped subsidiary cells but also with low dome shaped subsidiary cells, 4-6 µm
Long cells	Deeply undulate and sinuous walls, 20-32 µm	Moderately thin non-sinuous to markedly sinuous walls, 8-20 µm	Thick non-sinuous walls, 10-29 µm	Thin sinuous walls, 22 µm	Thin non-sinuous to markedly sinuous walls, 10.5-20 µm

morphological types also have taxonomic significance.

In this genus, prickles are found on both the abaxial and adaxial epidermis of all species except *S. glauca*, in which prickles are not observed. There is also some variation in the shape of prickles. Angular shaped prickles are found in *S. viridis* and *S. tomentosa*. Prickles are found with narrow beak and broader distil portion in *S. italica* whereas in *S. verticillata* prickles are narrowly oblong. Sharma and Kalia (1983) in their studies concluded that prickles are found in all the species except *S. sphacelata*. Both hooks and angular prickles are observed in *S. glauca*, *S. tomentosa* and *S. viridis* whereas in the remaining species, only hooks are observed.

Stomata arranged in well-defined rows in the intercostal regions, are characteristic of the majority of the species (Table 2). The subsidiary cells found, two in number, are mostly low-dome shaped, dome shaped or triangularshaped. Stomata are found in the intercostal regions of both surfaces in all the species of this genus. According to Ellis (1979) and Lewis (2001) in the Poaceae, stomata generally occur in well-defined bands in intercostal zones, and they may be classified according to the shape of subsidiary cells.

There is a great diversity in the long cells of the species of the genus *Setaria*. *S. italica* and *S. viridis* possess almost rectangular shaped, non-sinuous to markedly sinuous walls (Figure 4) whereas *S. tomentosa* possesses thick non-sinuous walls (Figure 2) and *S. verticillata* possesses thin sinuous walls. In *S. glauca*, long cells are with thin deeply undulate sinuous walls, which may be silicified. Sharma and Kalia (1983) in their studies showed that the long cells in the genus *Setaria* are distributed in the costal as well as intercostal regions on both surfaces and possess thin, non-sinuous to slightly or markedly sinuous walls in the different species.

Palynology

In polar view, the size of pollen grains ranged from 25 (20 to 30) to 37.5 (20 to 55) µm. *S. verticillata* appears to be

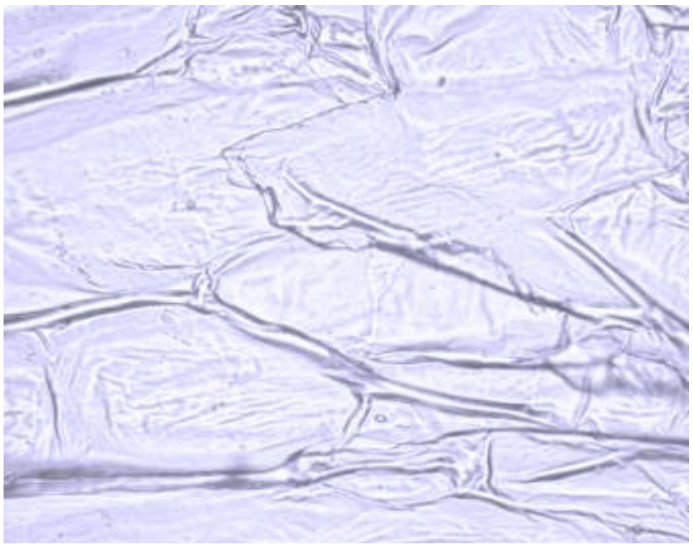

Figure 4. *Setaria italica* non-sinuous to markedly sinuous walled long cells (abaxial epidermis).

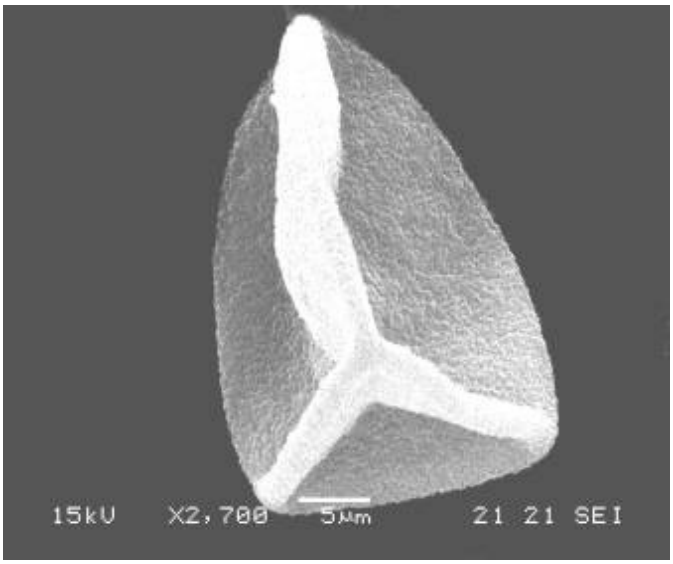

Figure 5. *Setaria viridis* angular-shaped pollen.

the smallest whereas *S. glauca* appears to be the larger in size. In equatorial diameter, the size ranged from 23 (16 to 30) to 45 (40 to 50) µm. *S. tomentosa* appears to be the smaller in size whereas *S. glauca* is the larger (Figure 6). Erdtman (1952) found uniporate pollen grains of *Hordeum vulgare* with 3 µm in diameter. Anozie (2002) studied some medicinal plants palynologically. P/E ratio ranged from 0.38 to 1.36. *S. glauca* shows the lower value whereas *S. tomentosa* shows the higher value (Table 3). Exine thickness ranged from 0.65 (0.2 to 1.1) to 0.95 (0.8 to 1.1) µm. *S. viridis* and *S. italica* show the lower value whereas *S. glauca* shows the highest value in exine thickness. Pollen grains in most of the species seen are circular in shape in polar view whereas in equatorial view, *S. glauca* possesses the spheroidal-subspheroidal, *S. italica* semi-angular and *S. viridis* angular pollen grains (Figure 8). In the genus *Setaria*, most taxa have the range of pollen fertility 75 to 96%. The highest value of pollen fertility is found in *S. tomentosa* as 96.49% and the lowest value is in *S.glauca* as 75.38% (Figure 7). The reason for the low pollen fertility could be environmental or physiological such as production of late season pollen grains. Scientists believed that the palynological studies can provide more accurate basis as additional feature for identification of plant species.

Table 3. Palynological diversity among the species of genus *Setaria*.

Characteristics	*S. glauca*	*S. Italica*	*S. tomentosa*	*S. verticillata*	*S. viridis*
Polar diameter (µm)	37.5 (20-55)	27.5 (25-30)	31.3 (25-37.5)	25 (20-30)	25.8 (25-26.5)
Equatorial diameter (µm)	45 (40-50)	31.3 (27.5-35)	23 (16-30)	35 (30-40)	25 (22.5-27.5)
P/E Ratio	0.38	0.88	1.36	0.71	1.03
Exine thickness (µm)	0.95 (0.8-1.1)	0.65 (0.4-0.9)	0.85 (0.4-1.3)	0.8 (0.4-1.2)	0.65 (0.2-1.1)
Pollen fertility (%)	75.38	79.59	96.49	89.18	82.92
Shape in equatorial view	Spheroidal-subspheroidal	Semi-angular	Angular	Circular	Angular

Figure 6. *Setaria verticillata* endoporus pollen.

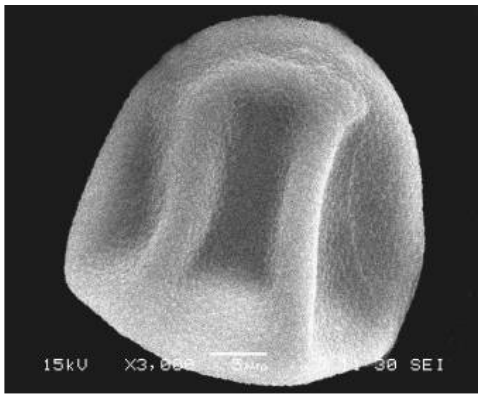

Figure 7. *Setaria italica* pollen cavity.

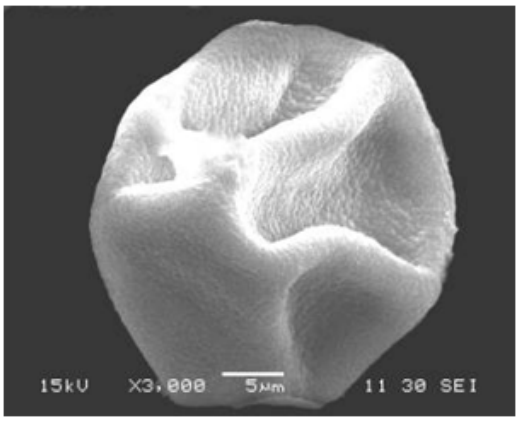

Figure 8. *Setaria glauca* equatorial view.

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