International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Validation of *Setaria* (L.) P. Beauv Species Based on Palyonological and Anatomical Techniques

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Abstract

In present study external morphology, leaf epidermal anatomy and pollen structureof three species of genus *Setaria* (Poaceae) *viz*., *S. glauca*, *S. verticillata* and *S. viridis* were studied. The major emphasis was on the importance of palyno-anatomical characters used as an aid in plant systematics. Light microscopy (LM) and scanning electron microscopy (SEM) were used to study leaf epidermal anatomy and pollen structure. Palyno-anatomical analysis of genus *Setaria* shows variations within the species. 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: Palynology; anatomy; scanning electron microscopy; *Setaria* (Poaceae)

1. Introduction

The genus *Setaria* P. Beauv.ex belongs to the tribe Paniceae, subfamily Panicoideae, and includes approximately 100 species in tropical, subtropical and temperate regions throughout the world including seven species in Pakistan

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[1]. Inflorescence features provide good diagnostic characters in different groups of grasses which are helpful in distinguishing grasses at species and sub species level [2]. In *Setaria* different morphological features like texture of lemma, ligule shape, awn color etc. are used to distinguish different taxa [3, 4, and 5]. However, these morphological features are used to differentiate the species are somehow overlapping at specific and sub specific level.

Palyno-anatomical characterization has assumed a great significance in plant taxonomy and with the advancements in the microscopy has led to the effective use of parameters for taxonomic purposes. 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 was to assess the significance of pollen and leaf epidermal morphology for systematic applications in *Setaria*. Light microscope and SEM were employed for palyno-anatomical characterization and to correlate it with morphological feature in three different species of *Setaria* for this purpose.

2. Method

2.1 Morphology

To study the morphological features of *Setaria* spp. different field trips were conducted to collect plant specimens. The voucher specimens were deposited in the herbarium of Quaid-i-Azam University Islamabad, Pakistan (ISL). Five specimens per species were used for assessment of morphological characters, 3 to 5 readings were taken for each character of a representative species. Morphological characteristics were reconfirmed by using various Floras [6, 7, 8, 9, 10, and 11].

2.2 Leaf epidermal anatomy

Leaves from fresh and dried specimens were used for anatomical studies. Leaves samples were prepared according to the method of Cotton [12] who followed Clark's technique [13], but with a little modification [14]. In brief, the leaves were folded and soaked in lactic acid for few minutes to make them soft. After removing them from the lactic acid the epidermal layer was removed. Different epidermal characteristics including qualitative and quantitative features were determined using light microscope (Mejii- MX 5200H, Japan). The microphotographs of epidermal features are taken using Leica light microscope (LM).

2.3 Pollen study

2.3.1 Light microscopy

Pollen material from dried specimens was used to prepare slides by adopting the method [15]. Various features of pollen such as, shape, type, structure, diameter, exine thickness, P/E ratio and sculpturing were determined using light microscope (Mejii – MX 5200H, Japan).

2.3.2 Scanning electron microscopy

For pollen studied (sculpturing pattern) by SEM anthers were crushed in 45% acetic acid and one to two drops of material containing acetolysed pollen were mounted on metallic stubs with a fine pipette and coated with gold in vacuum coater and examined with a Jeol microscope (JSM 1200).

3. Result and discussion

3.1 Morphology

Inflorescence in different species has different color, size and shape that are used to differentiate the various species. The inflorescence had main stalk with shortened branching bearing spikes and bristles in *S. italica* [16, 17]. *S. glauca* does not have cluster of hairs at its rachilla, a representative characteristic in the most of the species. Bristles are arranged in whorls to make involucres in spikelet. The size and nature of the bristles are used to distinguish different species. The size, shape and nerves on the glumes are also diagnostic features. For example, in *S. viridis* the glume is equal in length as that of spikelet but in *S. glauca* and *S. verticellat*a it is smaller than spikelet. Number of nerves on upper glume can be used as a diagnostic character at specific level, for example *S. verticellata* is 5- nerved while *S. glauca* is 3- nerved. Fig. 1 represents three different species of *Setaria*.

In *S. glauca* upper glume is shorter while it is as long as spikelet in *S. italica* and *S. verticillata*, [1, 18]. Similarly, the length of anther and palea are also the diagnostic features. In *S. viridis*, the anther length is 0.3 to 0.6 mm whereas in *S. verticillata*, the anther is 0.7-1 mm long. In many species, the length of the lower lemma is analogous to spikelet length; however, *S. verticillata* has short palea while in *S. glauca* the palea is as long as the lemma. Different morphological characteristics of three different species of *Setaria* are given in Table 1.

Table 1 Comparison of morphological characteristics of 3 species of *Setaria*

Features	*S. glauca*	*S. verticellata*	*S. viridis*
Culm	Spreading to erect	Geniculate at base	Decumbent
Habit	Annual	Annual	Annual
Length of leaf (cm)	7-30	5-22	6.5-25
Sheath	Glabrous	Scabrid	Sparsely hairy
Width of leaf (mm)	5-7	5-14	3-8
Ligule length (mm)	2	3	1.2-2
Inflorescence	Panicle	Panicle	Panicle
Length of inflorescence (cm)	9.5	12	13
Spikelet	Ovate	Plano-convex	Elliptic oblong
Shape of lower glume	Broadly ovate	Broadly ovate	Ovate
Nerves on lower glume	3-nerved	3-nerved	3-nerved
Upper glume	Ovate	Elliptic	Ovate
Upper glume nerves	5-nerved	7 nerved	5-nerved
Length of lemma (mm)	1-3	1.5-2.3	1-3
Palea	Elliptic	Elliptic	Ovate
Length of anther (mm)	1-1.5	0.7-1.1	0.3-0.6
Caryopsis (mm)	1.3-1.5	1	0.9-1

3.2 Leaf epidermal anatomy

The leaf epidermal anatomy is helpful to identify different taxa. Different species of the genus *Setaria* can be distinguished on the basis of leaf epidermal anatomy. Abaxial and adaxial leaf epidermal anatomy of four species having morphological resemblances was studied [19]. More than five cells are fused together in a row in the costal region in *S. glauca*, and *S. viridis*, whereas four-five cells are arranged in a row in the costal region in the *S. verticillata*. Silica bodies are similar in shape on both the abaxial and adaxial surfaces in the genus *Setaria*, but number of rows of silica bodies and size of silica bodies are different in different species. *S. glauca* and *S. viridis* have dumb-bell and nodular shaped silica bodies (Fig. 2a, c) while *S. verticillata* has dumb-bell to cross-shaped silica bodies (Fig. 2b). Silica bodies alternating with cork cells are present in the costal regions in all the species of *Setaria* except *S. plicata* where they are observed in both the costal and intercostal regions [20]. In the genus *Setaria* most useful diagnostic feature is the shape of silica bodies, which is helpful at specific level. Various epidermal anatomical features of three different species of Setaria are mentioned in Table 2.

Fig. 1 Three different species of *Setaria* (a) *S. glauca* (b) *S. verticellata* and (c) *S. viridis*

Table 2 Comparison of leaf epidermal anatomy of three species of *Setaria*

Features	*S. glauca*		*S. verticellata*		*S. viridis*	
Epidermis	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Papillae	Absent	Absent	Absent	Absent	Absent	Absent
Long cell wall shape	Markedly sinuous	Undulating wall	Markedly sinuous	Markedly sinuous	Markedly sinuous	Markedly sinuous
Microhair length (µm)	32-65	50-92	40-90	36-80	42-60	40-53
Stomata length (µm)	26-33	41-57	40	40	20-25	15-21
Silica bodies	Saddle shaped	Cross shape	Cross shape	Cross shape	Cross shape	Cross shape
Subsidiary cells	Dome shape/ triangular	Dome shape/ triangular	Low Dome shape	Dome shape	Low Dome shape	Dome shape
Costal short cells	Long rows	Long rows	Paired	Not paired	Long rows	Long rows
Prickle	Absent	Present	Present	Present	Present	Present

Resemblance between grass phylogeny and morphological patterns in silica bodies has been used for a long time by agrostologists [20, 21]. Macro-hairs and micro-hairs are found on adaxial and abaxial surface in all the species of the genus *Setaria*. The presence or absence of micro-hairs is widely used as a taxonomic character [22]. Micro-hairs are morphologically classified into two major types, i) the chloridoid type, with relatively short, broad and thick walled cap cells, and ii) the panicoid type with relatively long, narrow and thin walled and elongated cap cells with very long basal cells [23, 24]. These morphological types also have taxonomic significance. The prickles were found to be absent on the abaxial surface in *S. glauca*, and present on both abaxial and adaxial side of *S. viridis*, *S. intermedia, S. italica* and *S. verticillata*. So the presence of prickles only on adaxial surface of *S. glauca* distinguishes it from the rest of *Setaria* species [25]. Prickles are found in all the species of *Setaria* except *S. sphacelata* in which pickles are absent on both sides [26, 16]. Stomata are arranged in rows in the intercostal

regions on both surfaces in all the species of *Setaria* (Table 2). There are two subsidiary cells and are mostly low-dome shaped, dome shaped or triangular shaped. Stomata occur in well-defined bands in intercostal zones in poaceae and they may be classified according to the shape of subsidiary cells [27, 28]. There is a great diversity in the long cells of the species of the genus *Setaria*. For instance, *S. glauca* and *S. viridis* possess almost rectangular shaped, non-sinuous to markedly sinuous walls (Fig. 2a, c) and *S. verticillata* possesses thin sinuous walls. In *S. glauca*, long cells are with thin deeply undulate sinuous walls, which may be silicified. 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 [29].

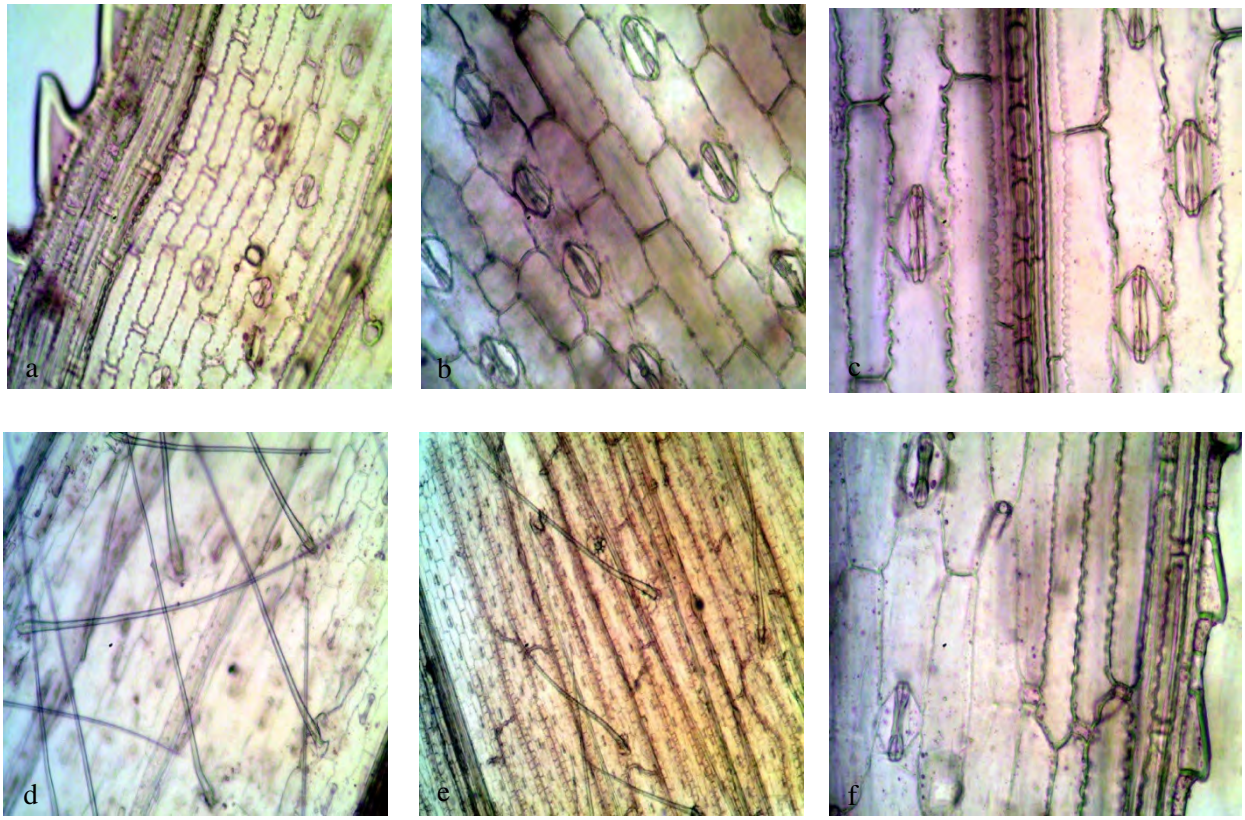

Fig. 2 (a) abaxial surface of *S. glauca,* (b) abaxial surface of *S. verticellata,* (c) abaxial surface of *S. viridis,* (d) adaxial surface of *S. glauca*, (e) adaxial surface of *S. verticellata,* (f) adaxial surface of *S. viridis*

3.3 Palynology

The size of pollen grains ranged from 26.55 to 36.55 µm in polar view. Pollen of *S. verticillata* appears to be the smallest (26.55µm) whereas that of *S. glauca* appears to be the larger in size i.e., 35.55µm (Table 3). These results are in agreement with the findings [30]. The size ranged from 26.65 to 34.88 µm in equatorial diameter. *S. viridis* has the smallest pollen whereas *S. glauca* has the largest pollens (Fig. 3). *Hordeum vulgare* has monoporate pollen grains with 3 µm in diameter [31]. P/E ratio ranged from 1.0 to 1.13. *S. verticellata* shows the lower value whereas *S. viridis* shows the higher value (Table 3). Exine thickness ranged from 0.2 to 1.5 µm. *S. viridis* has scabrid type sculpturing while *S. glauca* and *S. verticellata* have rugulate type sculpturing. Most of the species have circular pollen grains in equatorial view. Pollen fertility ranged from 81.45 to 96.42% in genus *Setaria*. The highest value of pollen fertility is found in *S.viridis* as 96.42% and the lowest value is in *S. glauca* as 81.45% (Table 3)

Table 3 Comparison of pollen study of three species of *Setaria*

Features	*S. glauca*	*S. verticellata*	*S. viridis*
Shape in Equatorial view	Circular	Circular	Circular
Polar diameter (µm)	35.55	26.55	27.54
Equatorial diameter (µm)	34.88	26.65	24.97
P/E ratio	1.02	1.00	1.13
Sculpturing	Rugulate	Rugulate	Scabrate
Pore diameter (µm)	2.97	2.0	1.8
Exine thickness(µm)	1.5	0.2	1.23
Pollen Fertility (%)	87.55	81.45	96.42

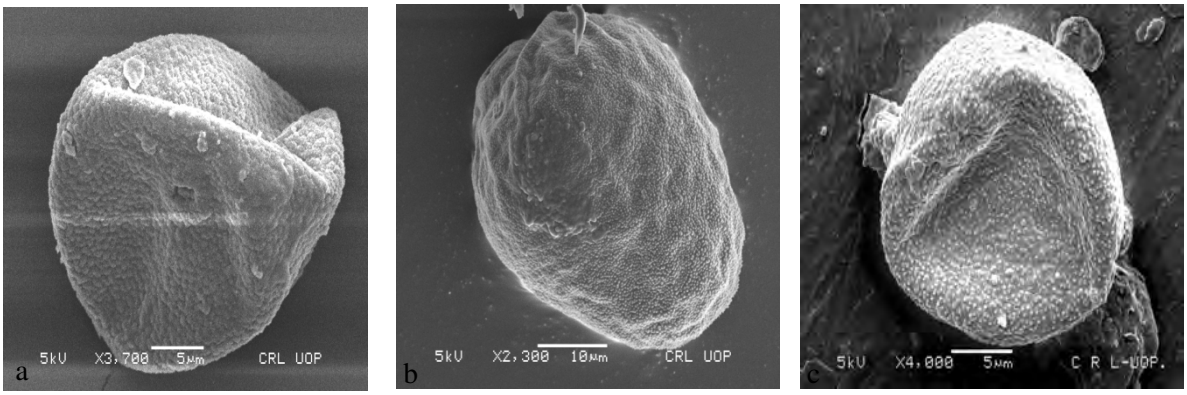

Fig. 3 Pollen morphology of three species of *Setaria* studies by SEM (a) *S. glauca* (b) *S. verticellata* and (c) *S. viridis*

4. Conclusion

The studies revealed that foliar epidermal studies assist in identification and differentiation of different *Setaria* species. Different anatomical characters such as absence or presence of prickles on the abaxial and adaxial side, shape of silica bodies and size of stomatal complex are of prime importance in delimiting different taxa at the species and generic level. Palynological studies can provide more information to identify different closely related species of plant species.

5. Acknowledgments

Higher Education Commission (HEC), Pakistan is highly acknowledged for providing financial support to conduct the research.

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