Research Article

Phytoliths as a Tool for the Identification of Some Chloridoideae Grasses in Kerala

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The phytoliths of eight genera including fifteen species of grasses under the subfamily Chloridoideae in Kerala were studied. Phytoliths were studied after chemical isolation. Every species was found to produce a diverse array of phytoliths. However the frequency assemblages of phytoliths, their size, and orientation in the epidermal layer appear to vary among the different species and hence can be used for the delimitation of the taxa. Consequently, an identification key following the International Code for Phytolith Nomenclature was developed for all the species studied.

1. Introduction

Silicon is deposited in plants as hydrated amorphous silica ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) through the polymerization of monosilicic acid ($\text{Si}(\text{OH})_4$) absorbed by roots from soil [1]. Silica deposited as inclusions within the cells are usually termed as phytoliths or silica bodies. Phytoliths have proved to be a potential tool in palaeoecological studies as they remain stable in the soil for even millions of years after plant tissues decay [2–12].

Depending upon the species of the plant, silica is deposited between the cells, within the cell walls, or even sometimes completely infilling the cells themselves. Silica bodies in the silica cells of grasses assume characteristic forms when the leaf is mature. Silica deposits in grasses have been well documented [13–17]. According to Metcalfe and Kaufman et al. [13, 18] silica deposits in grasses occur primarily in epidermal long cells, trichomes (hairs), specialized silica short cells, and as fillings within the bulliform cells of plant leaves. Phytolith shapes are found to be consistent within a species and hence they can provide significant taxonomic information [6, 19–21].

Metcalfe [13] considered the structure of silica bodies as one of the characteristics useful in plant identification. Since the publication of Metcalfe's work, the utility of phytoliths in plant taxonomy has been demonstrated in other publications. Studies made by Ollendorf et al. [14] have shown that

phytolith characters can be used to distinguish *Arundo donax* from *Phragmites communis*, two giant reed grasses that is otherwise difficult to identify from the field. Various studies have been made to distinguish phytoliths in cultivated crops from those of wild relatives of crop plants [22–24].

The present study is concerned with the identification of some Chloridoideae grasses in the vegetative stage based on the size, shape, frequency, and orientation of phytoliths in the leaf epidermis.

2. Materials and Methods

Plants included in the present study belong to the subfamily Chloridoideae of the family Poaceae, according to the classification made by Clayton and Renvoize [25]. In particular, a total of fifteen species of grasses under eight genera were selected for the present study (Table 1). Specimens were collected fresh from different parts of Kerala. Voucher specimens of collected plants are deposited in Calicut University Herbarium (CALI). Herbarium specimens collected from CALI were also utilized for the study.

Mature leaf blades were selected for the present study. The leaf blades were first washed in water and wiped dry. They were then cut into 1 cm^2 pieces and soaked in acid mixture (con. HNO_3 + con. H_2SO_4 in 1 : 1 proportion) for 15 days [15]. The mixture was then centrifuged and the

TABLE 1: Grasses included in the present study.

Si. no.	Latin name	Collection no.
(1)	<i>Chloris barbata</i> Sw.	127309, 127380, 6177
(2)	<i>Cynadon arcuatus</i> J.S. Presl. ex C.B. Presl.	127304
(3)	<i>C. dactylon</i> (Linn.) Pers.	113370, 11276
(4)	<i>Dactyloctenium aegyptium</i> (Linn.) P. Beauv.	113373, 8951, 3580
(5)	<i>Eleusine corocana</i> (Linn.) Gaertn.	124901, 521, 3819
(6)	<i>E. indica</i> (Linn.) Gaertn.	113343, 9479, 2667
(7)	<i>Eragrostis nutans</i> (Retz.) Steud.	127390
(8)	<i>E. tenella</i> (Linn.) Roem. and Schult	113375, 34419
(9)	<i>E. unioloides</i> (Retz.) Steud.	113383, 3587, 2565
(10)	<i>E. viscosa</i> (Retz.) Trin.	113351, 12406
(11)	<i>Leptochloa chinensis</i> (Linn.) Nees	127355
(12)	<i>Perotis indica</i> (Linn.) O. Kuntze	113381, 5803, 3986
(13)	<i>Sporobolus diander</i> (Retz.) P. Beauv.	113377, 1337
(14)	<i>S. piliferus</i> (Trin.) Kunth	127307, 2891, 4650
(15)	<i>S. tenuissimus</i> (Schrank) O. Kuntze	113357, 113375

TABLE 2: Different types of phytoliths observed and their abbreviations.

Si. no.	Type	Abbreviation
(1)	Associated epidermal polygonal cells	AEP
(2)	Associated long cells	ALC
(3)	Associated papilla	AP
(4)	Associated short cells	AS
(5)	Bulliform elements	BE
(6)	Stomatal complex	SC
(7)	Subepidermal elements	SEE
(8)	Simple bilobate	SB
(9)	Complex bilobate	CB
(10)	Nodular bilobate	NB
(11)	Quadralobate	Q
(12)	Normal saddle	S
(13)	Elongate	E
(14)	Fan	F
(15)	Horned towers	HT
(16)	Macrohairs	LH
(17)	Microhairs	MH
(18)	Prickle hairs	PR
(19)	Papilla	P

Abbreviations for lobate phytoliths are followed by C, F, or X denoting concave, fattened, or convex margins.

acid decanted. The residue was then suspended in distilled water and again centrifuged. This process was repeated upto 4 cycles in order to completely remove the acid from the residue. The final residue was then suspended in rectified spirit and stored in storage vials for further study.

A drop of the phytolith alcohol mixture was placed on a clean slide and warmed under the spirit lamp. Then a drop of crystal violet stain was applied and again warmed. Excess stain was removed by suddenly dipping the slide and taking

out from water. This was warmed again and the slide was mounted permanently in DPX (Distyrene Plasticizer Xylene) for further observations and photography.

For observations and photography Motic Digital Microscope with image analyser was used. Observations and photography were taken under oil immersion objective (100x). Measurements were taken from surface view of phytoliths using Motic Image Analyzer software. Various features of phytoliths noted include length, width, nature of outer margins, shape, and so forth.

In addition to measurement, frequency of phytolith assemblages was also noted. About 1000 phytoliths from each species were counted and frequency determined. For both frequency and measurement range, average and standard error were calculated.

Photographs of different types of phytoliths observed for different species are also provided. Species taken for the present study and their collection number are shown in Table 1. The serial numbers of the species shown in this table are used to denote the name of the species in Tables 3 and 4.

3. Results

Data obtained from the frequency of phytolith assemblages as well as measurements were utilized for the preparation of an identification key for grasses upto species level. Length and width dimensions and frequency of about 1000 phytoliths were measured from each species. The present study on phytoliths applies the rules of International Code for Phytolith Nomenclature [26]. Silica bodies were found in two forms—articulated (associated with other cells) and isolated. Various types of phytoliths observed and their abbreviations used are given in Table 2.

3.1. Articulated Forms. These are the associated forms of phytoliths found joined either with the same type or different types of cells. These include the following subcategories.

TABLE 3: Measurements (in μm) of phytoliths.

Shape Dimension		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
SB	L	—	—	—	—	—	—	—	13.2 $\pm$ 0.5	—	—	17.5 $\pm$ 0.6	14.3 $\pm$ 0.5	20.9 $\pm$ 1	12.1 $\pm$ 0.3	12.6 $\pm$ 0.6
		Avg $\pm$ SE														
	Range	—	—	—	—	—	—	—	10.2–14.5	—	—	10–21.1	8.3–20.9	11.9–29	8.6–15.4	9.1–17
	W	—	—	—	—	—	—	—	5.8 $\pm$ 0.31	—	—	8.1 $\pm$ 0.2	6.2 $\pm$ 0.2	8.1 $\pm$ 0.2	7.2 $\pm$ 0.2	5.3 $\pm$ 0.3
		Avg $\pm$ SE														
	Range	—	—	—	—	—	—	—	4.5–7.6	—	—	5.8–10.9	4.3–9	6.7–10.4	4.8–8.7	4.4–9.1
E	L	—	—	48.5 $\pm$ 3.6	—	—	60.6 $\pm$ 7.2	—	59.4 $\pm$ 5.7	—	—	—	—	—	—	—
		Avg $\pm$ SE														
	Range	—	—	36.1–66.8	—	—	44.8–89.7	—	58.2–72.4	—	—	—	—	—	—	—
	W	—	—	11 $\pm$ 0.6	—	—	11.8 $\pm$ 1.1	—	11.9 $\pm$ 0.7	—	—	—	—	—	—	—
		Avg $\pm$ SE														
	Range	—	—	8.6–15.7	—	—	11.2–16	—	11.5–13.6	—	—	—	—	—	—	—
S	L	11.9 $\pm$ 0.5	7.98 $\pm$ 0.2	8.03 $\pm$ 0.2	11.69 $\pm$ 0.2	8.4 $\pm$ 0.7	7.9 $\pm$ 0.3	7.2 $\pm$ 0.3	—	—	11.7 $\pm$ 0.9	—	—	9.5 $\pm$ 0.2	—	5.7 $\pm$ 0.3
		Avg $\pm$ SE														
	Range	7.5–18.1	5.7–9.9	4.8–10.5	8.7–13.4	5.9–10.5	6.3–10.7	5.9–10	—	—	9.5–13	—	—	8.5–10.8	—	3.1–7.9
	W	12.98 $\pm$ 0.4	9.29 $\pm$ 0.36	8.58 $\pm$ 0.17	10.6 $\pm$ 0.1	6.5 $\pm$ 0.5	10.3 $\pm$ 0.4	7.9 $\pm$ 0.8	—	—	7.8 $\pm$ 0.7	—	—	4.8 $\pm$ 0.3	—	7.3 $\pm$ 0.3
		Avg $\pm$ SE														
	Range	8.8–17.3	5.4–16.2	5–11.3	8.9–15.8	5.3–9.9	8–13.4	2.8–14.4	—	—	6.3–9.6	—	—	3.4–6.2	—	5–10.1
SC	L	—	—	—	—	—	28.6 $\pm$ 0.6	23.4 $\pm$ 0.5	19.3 $\pm$ 0.3	22.7 $\pm$ 0.6	26.3 $\pm$ 0.7	—	—	29.01 $\pm$ 2.7	—	—
		Avg $\pm$ SE														
	Range	—	—	—	—	—	25.8–31	21.7–26.6	16.7–21.9	19.1–32.1	20.7–30.9	—	—	17.3–35.8	—	—
	W	—	—	—	—	—	21.1 $\pm$ 1.2	16.1 $\pm$ 0.5	12.5 $\pm$ 0.3	16.4 $\pm$ 0.6	17.98 $\pm$ 0.4	—	—	17.7 $\pm$ 1.7	—	—
		Avg $\pm$ SE														
	Range	—	—	—	—	—	16.4–26	14.5–17.9	10.5–15.5	12.5–24.5	15–21.8	—	—	10.4–23	—	—
PR	L	—	—	43.4 $\pm$ 4.3	—	—	—	—	29.8 $\pm$ 1.3	—	—	—	—	—	—	43.7 $\pm$ 2.1
		Avg $\pm$ SE														
	Range	—	—	24.2–67.5	—	—	—	—	24.6–32.5	—	—	—	—	—	—	30.1–58
	W	—	—	18.8 $\pm$ 2.7	—	—	—	—	21.7 $\pm$ 1.4	—	—	—	—	—	—	23.7 $\pm$ 2.01
		Avg $\pm$ SE														
	Range	—	—	7.4–34.5	—	—	—	—	16.5–25.7	—	—	—	—	—	—	11.7–36.2

TABLE 4: Frequency (in percentage) of phytoliths.

Phytolith type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AEP	—	—	—	—	—	—	0.4 ± 0.2	—	—	—	—	—	—	—	—
ALC	5.6 ± 0.3	3.6 ± 0.5	4.3 ± 1.1	1.8 ± 0.5	10.7 ± 0.9	15.7 ± 0.8	28.5 ± 1.4	64 ± 1	67.2 ± 1.4	31.2 ± 1.8	0.1 ± 0.1	0.5 ± 0.2	6.4 ± 0.9	—	2.3 ± 0.4
AP	0.4 ± 0.2	—	1.4 ± 0.4	—	—	—	—	—	—	—	—	—	—	—	—
AS	—	—	1.4 ± 0.6	0.5 ± 0.3	—	—	—	2.7 ± 0.2	—	—	—	0.5 ± 0.2	0.2 ± 0.1	—	0.3 ± 0.2
BE	—	—	—	0.3 ± 0.2	—	—	—	—	—	—	—	—	3.1 ± 0.8	—	—
SC	0.4 ± 0.2	—	—	—	11.9 ± 0.9	3.6 ± 0.3	22.8 ± 0.6	12.3 ± 0.6	16.4 ± 0.8	40.7 ± 1.3	—	—	9.6 ± 0.9	—	1 ± 0.2
SEE	—	—	—	—	0.8 ± 0.3	—	0.3 ± 0.2	—	—	0.4 ± 0.2	1 ± 0.3	—	0.6 ± 0.2	—	—
SBC	—	—	0.3 ± 0.2	0.7 ± 0.2	—	4.7 ± 0.5	5.2 ± 0.5	—	—	1.4 ± 0.4	79.7 ± 0.7	12.6 ± 1.5	6 ± 0.9	51.4 ± 0.8	6.5 ± 0.3
SBF	—	—	—	—	—	0.5 ± 0.2	6 ± 0.8	2.8 ± 0.3	2 ± 0.3	0.3 ± 0.2	—	54.1 ± 1.8	4.9 ± 0.6	15.8 ± 0.5	4.5 ± 0.6
SBF/C	—	—	—	0.1 ± 0.1	—	1.3 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	2.9 ± 0.7	1.3 ± 0.4	3.5 ± 0.5	8.5 ± 0.7	1.2 ± 0.4	16.4 ± 0.7	3.3 ± 0.4
SBF/X	—	—	—	—	—	—	—	—	—	1 ± 0.2	6.7 ± 0.3	11.9 ± 0.7	—	0.6 ± 0.3	0.6 ± 0.2
SBX	—	—	—	—	—	—	—	—	1.2 ± 0.1	—	—	6.8 ± 0.4	—	—	—
SBX/C	—	—	—	—	—	—	—	0.5 ± 0.2	0.6 ± 0.2	0.8 ± 0.2	—	1.5 ± 0.2	—	1.5 ± 0.2	2 ± 0.2
CBC	—	—	—	—	—	—	—	—	—	—	0.6 ± 0.2	—	—	—	—
CBF	—	—	—	—	—	—	—	—	—	—	—	1 ± 0.4	—	—	—
CBF/C	—	—	—	—	—	—	—	—	—	—	—	0.5 ± 0.2	—	—	—
E	0.2 ± 0.1	—	8.8 ± 0.7	2.1 ± 0.5	2.9 ± 0.3	6.9 ± 0.4	0.8 ± 0.3	7 ± 0.5	5.5 ± 0.6	4 ± 0.6	2.1 ± 0.5	0.4 ± 0.2	4.3 ± 0.5	—	3.1 ± 0.3
F	—	—	—	—	—	—	—	—	—	0.2 ± 0.1	—	—	0.4 ± 0.4	0.5 ± 0.2	—
HT	—	—	—	—	—	—	—	—	—	—	—	—	—	0.7 ± 0.3	—
LH	—	—	0.1 ± 0.1	0.6 ± 0.3	0.3 ± 0.2	3.7 ± 0.5	—	—	0.7 ± 0.3	1 ± 0.4	—	—	20.1 ± 1.8	—	0.4 ± 0.2
MH	—	—	—	—	—	—	0.5 ± 0.2	—	—	0.1 ± 0.1	—	—	—	—	—
NBF	—	—	—	—	—	—	—	—	—	0.4 ± 0.2	—	0.4 ± 0.2	—	0.9 ± 0.3	—
NBF/C	—	—	—	0.1 ± 0.1	—	—	—	—	—	—	—	—	—	—	—
P	—	—	2.4 ± 1	—	—	—	—	—	—	—	—	—	—	—	—
Q	—	—	—	0.1 ± 0.1	0.1 ± 0.1	—	0.4 ± 0.2	—	—	—	—	—	—	1.3 ± 0.4	—
S	89.4 ± 0.7	94.8 ± 0.5	75.5 ± 2.6	93.2 ± 0.8	57.2 ± 1.2	60.7 ± 0.9	18 ± 1.1	1.5 ± 0.2	1.5 ± 1	10.4 ± 1	—	0.4 ± 0.2	39.5 ± 1.3	10.2 ± 1.2	71.3 ± 1.1
PR	4 ± 0.5	1.6 ± 0.2	5.8 ± 0.9	0.5 ± 0.3	15.8 ± 0.6	—	15.9 ± 1.1	7.5 ± 0.3	3.5 ± 0.4	6.2 ± 0.8	6.3 ± 0.6	0.6 ± 0.2	3.6 ± 0.7	0.7 ± 0.2	5 ± 0.3
ST	—	—	—	—	0.4 ± 0.2	—	—	—	—	0.2 ± 0.1	—	—	—	—	—

FIGURE 1: Associated long cells of (1a) *Dactyloctenium aegyptium*, (1b) *Eragrostis tenella*, (1c) *E. unioides*, (1d) *E. viscosa*, (1e) *Sporobolus diander*; (2) associated short cells of (2a) *Cynadon arcuatus*, (2b) *Eragrostis tenella*; (3) fan cells of (3a) *Cynadon arcuatus*, (3b) *C. dactylon*, (3c) *Perotis indica*; (4) elongate cells of (4a) *Cynadon dactylon*, (4b) *Eleusine corocana*, (4c) *Eragrostis tenella*, (4d) *E. unioloides*, (4e) *E. viscosa*, (4f) *Perotis indica*, (4g) *Sporobolus diander*, (4h) *S. tenuissimus*; (5) stomatal complex of (5a) *Cynadon dactylon*, (5b) *E. unioloides*, (5c) *E. viscosa*, (5d) *Sporobolus diander*; (6) (6a) subepidermal elements of *Dactyloctenium aegyptium*; (7) (7a) horned tower of *Sporobolus piliferus* (8) prickly hairs of (8a) *Dactyloctenium aegyptium*, (8b) *Eleusine corocana*, (8c) *Eragrostis tenella*, (8d) *E. viscosa*, (8e) *Sporobolus tenuissimus*, (8f) *S. diander*.

FIGURE 2: 1–6: Simple bilobates (1) Bilobate with concave margins. (1a) *Eleusine indica*, (1b) *Eragrostis tenella*, (1c) *E. viscosa*, (1d) *Leptochloa chinensis*, (1e) *Perotis indica*, (1f) *Sporobolus diander* (1g) *S. piliferus* (1h) *S. tenuissimus*; (2) bilobate with flattened margins. (2a) *Eragrostis tenella*, (2b) *E. viscosa*, (2c) *Perotis indica*, (2d) *Sporobolus diander*, (2e) *S. piliferus*; (3) bilobate with flattened/concave margins. (3a) *Eleusine indica*, (3b) *Perotis indica*, (3c) *Sporobolus diander*, (3d) *S. piliferus*, (3e) *S. tenuissimus*; (4) Bilobate with flattened/concex margins. (4a) *Perotis indica*, (4b) *Sporobolus piliferus*; (5) (5a) Bilobate with convex margins of *Perotis indica*; (6) (6a) Bilobate with convex/concave margins of *Sporobolus diander*; (7) (7a) complex bilobate with concave margins of *S. piliferus*; (8) (8a) Complex bilobate with flattened margins of *Eleusine indica*; (9) (9a) nodular bilobate with concave margins of *Sporobolus diander*; (10) (10a) quadralobate phytoliths of *S. piliferus*; (11) saddles. (11a) *Chloris barbata*, (11b) *Cynadon arcuatus*, (11c) *C. dactylon* (11d) *Dactyloctenium aegyptium* (11e) *Eleusine corocana* (11f) *E. indica*, (11g) *Eragrostis viscosa*, (11h) *S. diander*, (11i) *S. tenuissimus*.

(1) *Associated Epidermal Polygonal Cells*. Polygonal shaped cells of the epidermal cells with smooth or sinuate margins. These were observed only in *Eragrostis nutans* with a frequency below 1%.

(2) *Associated Long Cells*. These are associated long cells of the epidermis either with stomata or prickly hairs. These are very common and are found in almost all the species. Their frequency in *Eragrostis unioides* is greater than 60%.

(3) *Associated Papilla*. These are protrusions from the epidermal cells. Sometimes 2 or more papillae are seen associated together. These were observed only in *Chloris barbata* and *Cynadon dactylon* with a frequency less than 2%.

(4) *Associated Short cells*. These are long cells associated with one or more short cells. Frequency of this type was found to be always less than 3%. However they were observed in six species.

(5) *Bulliform Elements*. These are silicified bulliform cells associated with each other. These were observed only in two species, that is, *Dactyloctenium aegyptium* and *Sporobolus diander*, with a frequency below 4%.

(6) *Stomatal Complex*. These are silicified guard cells joined together. This is a common type and were observed in nine species studied. The highest frequency of about 40% was observed in *Eragrostis viscosa*.

(7) *Subepidermal Elements*. These are silicified mesophyll or other cells present beneath the epidermis. These were observed in five species but their frequency was less than 1%.

3.2. *Isolated Forms*. Phytoliths remain isolated without association with other cells. These include the following.

(8) *Simple Bilobate*. These are phytoliths with two equal lobes connected by a shank. Their margins can be concave, convex or flattened or the two lobes of the same phytolith can have different margins. At least one type of simple bilobate phytoliths is represented in all the species except *Chloris barbata* and *Cynadon arcuatus* where they were found to be totally absent.

(9) *Complex Bilobate*. These are phytoliths with 3 equal lobes. Their margins may be concave or flattened. This is a rare type in the subfamily and their frequency falls below 2%. They were observed only in *Leptochloa chinensis* and *Perotis indica*.

(10) *Nodular Bilobate*. These are bilobate phytoliths with a projection or a small lobe between the two major lobes. Margins can be concave or flattened. Convex margins are found to be totally absent in the subfamily.

(11) *Quadralobate*. They are characterised by almost similar length and width dimensions and in having four lobes. They were observed only in four species with a frequency of less than 2%.

(12) *Normal Saddle*. Saddle shaped phytoliths are arranged vertically on the epidermis. They are present in almost all the members of Chloridoideae except one, that is, *Leptochloa chinensis*.

(13) *Elongate*. These are silicified long cells of the epidermis with echinate or sinuate walls. They are represented in all the species except two. However their frequency does not exceed beyond 10%. The highest frequency of 8.8% was observed in *Cynadon dactylon*.

(14) *Fan*. These are silicified bulliform cells of the epidermis. They were found in *Eragrostis viscosa*, *Sporobolus diander* and *S. piliferus* with a frequency less than 1%.

(15) *Horned Towers*. Cylindrical short cells with two projections on one side. This type was observed only in *Sporobolus piliferus* with a frequency of 0.7%.

(16) *Macro Hairs*. These are elongated hairs present on the epidermis. They were observed in eight species studied. Their frequency in most cases falls below 5%. However in *Sporobolus diander* it is 20%.

(17) *Microhairs*. These are formed as a result of silicification of slightly curved two-celled blunt hairs of the epidermis. This type is rare and was observed in *Eragrostis nutans* and *E. viscosa*. Their frequency is less than 1%.

(18) *Prickle Hairs*. These are formed as a result of silicification of sharply pointed and slightly curved structures on the epidermis. This is a common type found in all the species except *Eleusine indica*. Highest frequency of about 15% was observed in *Eleusine corocana* and *Eragrostis nutans*.

(19) *Papilla*. Protrusions from the epidermis seen isolated. This is very rare in the subfamily and was observed only in *Cynadon arcuatus* with a frequency of 2.4%.

All the dimensions of phytoliths were studied. For taking measurements surface view of phytoliths were considered. Measurements of phytoliths of each species are shown in Table 3. and frequency assemblages in Table 4. Photographs of different types of phytoliths are given in Figures 1 and 2.

Key for the identification of species based on the frequency, size, and shape of phytoliths.

4. Key to the Species

(1) Saddles less than 1%, simple bilobates dominant with a frequency of 90–96%...2.

(1) Saddles greater than 1%, simple bilobates usually not dominant, if so, frequency below 80%...3.

- (2) Simple bilobates with concave margins dominant with frequency greater than 70%...*Leptochloa chinensis*.
- (2) Simple bilobates with flattened margins dominant with frequency less than 50%...*Perotis indica*.
- (3) Saddles the most dominant type, simple bilobate phytoliths rare, if present only less than 1%...4.
- (3) Saddles usually not the most dominant type, if so simple bilobates 5% or greater...8.
- (4) Average length of saddles 11-12 μm ...5.
- (4) Average length of saddles 7-9 μm ...6.
- (5) Hook shaped prickles present, average width of saddles about 10.6 μm ...*Dactyloctenium aegyptium*.
- (5) Hook shaped prickles absent, straight sharply pointed structures seen, average width of saddles about 12.9 μm ...*Chloris barbata*.
- (6) Saddles constitute about greater than 75% of the total phytoliths...7.
- (6) Saddles constitute less than 60% of the total phytoliths...*Eleusine corocana*.
- (7) Sharply pointed structures rare, less than 2% saddles about 95%...*Cynadon arcuatus*.
- (7) Sharply pointed structures present at about 5%, saddles about 76%...*Cynadon dactylon*.
- (8) ALC and SC together constitute more than 50% of the total phytoliths...9.
- (8) ALC and SC usually very less and will not exceed 20%...12.
- (9) Average length of SC about 26 μm ...*Eragrostis viscosa*.
- (9) Average length of SC less than 25 μm ...10.
- (10) Proportion of ALC and SC nearly 1:1...*Eragrostis nutans*.
- (10) Proportion of ALC and SC is about 4:1...11.
- (11) SC about 12.5 μm wide, outline more or less rounded...*Eragrostis tenella*.
- (11) SC about 16 μm wide, outline somewhat V-shaped, not rounded...*Eragrostis unioides*.
- (12) Frequency of saddle shaped phytoliths greater than 60%...13.
- (12) Frequency of saddle shaped phytoliths less than 50%...14.
- (13) ALC and SC together constitute more than 15% of the total phytoliths...*Eleusine indica*.
- (13) ALC and SC together constitute less than 5% of the total phytoliths...*Sporobolus tenuissimus*.
- (14) Frequency of simple bilobate phytoliths greater than 65%, average width about 12.09 μm ...*Sporobolus piliferus*.

- (14) Frequency of simple bilobate phytoliths less than 15%, average width about 20.9 μm ...*Sporobolus dianther*.

5. Discussion

The present study of grasses from the Indian subcontinent underscores what previous investigations have shown already elsewhere—the Poaceae family produces a diverse array of phytolith shapes yet certain shapes of phytoliths can consistently appear at least at the subfamily level.

Saddle shaped phytoliths characterises the subfamily Chloridoideae. Of the 15 species studied saddle shaped phytoliths were found to be the dominant type in seven species. Saddles were not observed only in *Leptochloa chinensis*. Highest frequency of saddles was noticed in *Chloris barbata*, *Cynadon arcuatus*, *Cynadon dactylon*, and *Dactyloctenium aegyptium*. In *Leptochloa chinensis*, *Perotis indica*, and *Sporobolus piliferus* bilobate phytoliths were found to be the dominant type. Associated long cells and stomatal complex were found in almost all the species studied. However their frequency was found to supersede in species of *Eragrostis*. More species has to be studied before this character could be attributed as diagnostic to the genus.

Frequency of phytolith assemblages and measurements of phytoliths are found to be consistent within a species and have been useful for developing the key. Even though phytolith multiplicity and redundancy occur in grasses, frequency assemblages reveal that a particular morphotype dominate over the other in a given species. Hence the present study shows that characteristics of phytoliths can be used as a tool for the identification of the species in the vegetative stage.

A brief survey on 15 species of Chloridoideae grasses has proved the usefulness of phytoliths in the identification of grasses. The present paper represents only a preliminary study towards developing an identification key for all South Indian grasses based on the foliar phytolith characteristics.

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