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Phytolith characterization of some taxa of Poaceae- A preliminary study

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Abstract: Silica deposited intracellularly and over plant cell wall with calcium, nitrogen etc. constitutes phytolith. The chemistry of phytolith development and shape is affected by the cellular environment and diversity, respectively. The studies on phytolith morphometry have gained importance in the last few years as a novel method of taxonomic identification of the Poaceae family. The present study was conducted to list the shapes of phytoliths in *Dichanthium annulatum*, *Heteropogon contortus* and *Imperata cylindrica*. Total nine types of phytoliths were found in the three species studied. Bilobate or dumb-bell and elongated shaped phytoliths were found in all the three grasses, whereas Dendritic, Nodular bilobate and Trapezoid were found in two species each.

Keywords: Morphotypes; phytoliths; Poaceae; silica.

1. Introduction

Phytoliths or plant stones are minute microscopic structures of variable 3D shapes and sizes that are formed when monosilicic acid [$Si(OH)_4$] is taken up by roots in polymerized form and precipitated as hydrated silica (opal-A; $SiO2*nH_2O$) (Piperno, 2006). They occur in various plant families as depositions in inter or/and intracellular spaces in all plant tissue types contributing up to 20% of dry weight (Hodson *et al.*, 2005). Phytoliths gives structural scaffold to the plant and protect them from various stresses (Mazumdar, 2011) and make them indigestible by herbivores (defense mechanism) and offer the plant tissues resistance to flames of fire. Due to specific and consistent shape within plant species, phytoliths are an important tool for taxanomic identification and classification of species (Raven, 1983; Piperno, 1988) and as they are persist even after the plant decay, this stability is vital in palaeoecological studies (Piperno, 1988).

The history of Phytolith research includes four stages- Discovery and exploratory stage, Botanical phase, Ecological phase and the Modern period. The phase of Modern period (1978-present) includes archaeological and paleo-environmental research. The Phytoliths are mainly composed of Silicon dioxide (non-crystalline), Water (about 7% by mass), Carbon, Nitrogen, etc (less than 5% by mass). These immobilized elements permit radiometric dating which is helpful in reconstructing primitive vegetation patterns. Most of the phytoliths are transparent, but some of them may be light brown or opaque. The refractive index of siliceous phytoliths ranges from 1.41-1.47 and specific gravity ranges from 1.5- 2.3. The identification of phytoliths is based on morphology which is helpful in identification of the taxonomic differences of phytolith production. The phytoliths are helpful in archaeology to reconstruct plants present at a scene where rest of the plant parts is burnt. The phytolith content provides an overview of the economic importance of the plants and their pattern and sizes reflect the environment at a particular period of time (Piperno, 1988).

The Poaceae family includes the true grasses, which may be cereal crop species,

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bamboos and several weeds that found origin in the tropical rain forests. The grasses of Poaceae family are characterized by high phytolith production. The present study was conducted to find out various types of phytoliths present in some of the common grasses of Punjab.

2. Material and methods

2.1 Study site

The present study was conducted in Punjab plains. The sampling area is by large plain in topography with an average altitude of 237 m amsl and mean annual rainfall between 90 and 115cm.

2.2 Methodology

The whole grass specimens were collected and identified by comparing with grass specimens in the laboratory and confirmed with the help of local floras. For The segments of fresh leaves were dipped in solution of lactic acid and chloral hydrate in a molar concentration of 3:1. The leaves were then dried and softened in water. The leaves were incubated in clearing solution in an oven at 70° C. The cleared segments were wet mounted and examined under a microscope. Thereafter, the cleared segments were stained with Methyl red dye for silica bodies. After treatment of the cleared segments with sulphuric acid and nitric acid, silica bodies were isolated and recovered by centrifugation. Surface views of epidermal preparations were used for measuring dimensions of the silica cells. The direct measurements were made under the microscope. Phytoliths were classified according to the International Code of Phytolith Nomenclature (Madella *et al*., 2005).

3. Results

From the processed specimens, silica bodies of different shapes were identified (Table 1).

Table 1. Types of phytoliths found in the studied grasses along with abbreviations used.

Morphotype	Abbreviations
Bilobate	BL
Clavate	CL
Dendritic	DT
Elongated	EL
Nodular bilobate	NB
Polylobate	PL
Rectangular	RT
Rondel	R
Trapezoid	TZ

The most common phytolith type was bilobate or dumb-bell. Usually dumb-bell shaped phytoliths were arranged in rows on the adaxial surface of the leaves on both sides of the veins (Table 2). Other important phytolith types like Dendritic, Nodular bilobate, Elongated and Trapezoid were found in two species each.

Table 2. Frequency (%) of phytolith morphotypes of the grasses

Phytolith shape	Grasses		
	Dichanthium annulatum	*Heteropogon contortus*	*Imperata cylindrica*
Bilobate	33.3 ± 2.7	25.0 ± 2.2	50.0 ± 3.9
Clavate	13.3 ± 1.9		-
Dendritic	20.0 ± 2.2	18.8 ± 1.6	-
Elongated	6.7 ± 1.1	31.3 ± 2.6	20.0 ± 1.9
Nodular bilobate	20.0 ± 1.8		20.0 ± 2.5
Polylobate	6.7 ± 1.0		-
Rectangular	-	6.3 ± 1.3	-
Rondel	-	6.3 ± 1.3	-
Trapezoid	-	12.5 ± 1.7	10.0 ± 1.7

The values given are mean ± standard error; n=5.

A diverse distribution of phytolith morphotypes was evident in the three grasses (Table 3). Bilobate was the most abundant phytolith morphotype in all the three grasses. Elongated type was rare in *Dichanthium annulatum*, abundant in *Heteropogon contortus* and common in *Imperata cylindrica*. Rectangular and rondel phytolith types were rarely distributed in *Heteropogon contortus* only. Similarly, clavate (common distribution) and polylobate types (rare distribution) were only found in *Dichanthium annulatum*.

Table 3. Distribution of phytolith morphotypes in the three grass species

Phytolith shape	Grasses		
	Dichanthium annulatum	*Heteropogon contortus*	*Imperata cylindrica*
Bilobate	A	A	A
Clavate	C	-	-
Dendritic	C	C	-
Elongated	R	A	C
Nodular bilobate	C	-	C
Polylobate	R	-	-
Rectangular	-	R	-
Rondel	-	R	-
Trapezoid	-	C	R

The values given are mean ± standard error; n=5.

4. Discussion

A total of nine types of phytolith morphotypes were found in the three grasses discussed in the present study. The phytolith morphotypes present in the three grasses are also reported by Shakoor *et al.* (2014) and Hari Babu *et al.* (2015). Presence of dumb-bell shaped morphotypes is the characteristic feature of members of Andropogoneae tribe of Poaceae (Mercader *et al*., 2010; Shakoor *et al*., 2014) and was present in all the three grasses.

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