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SHORT COMMUNICATION

Variation in phytoliths morphology of *Erianthus arundinaceus*

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Abstract

Five improved *Erianthus arundinaceus* clones were studied for variations in phytoliths present in leaves. Phytoliths were extracted by acid digestion and the morphology was studied by light microscopy. Phytoliths belonging to three classes viz., Eupanicoid, Festucoid and Chloridoid were identified in the samples. Variations in type and shape were observed among the clones studied. The results indicate that phytolith characteristics may be an important and reliable trait for clonal characterization in improved *Erianthus* clones and also there is a possibility of using this trait as a tool for the identification of species, hybrids and their parentage in sugarcane and their related genera.

Keywords: Phytolith; Plant stone; Sugarcane; *Erianthus arundinaceus*

Phytoliths, also referred as plant stones are plant silica formed inside cells. Phytoliths are studied by several researchers in Graminae family as a potential source of carbon sequestration, a trait for identification of species and clones and paleo-botanical studies. Phytoliths also provide a snap shot of past climate at the time of stable environments (Parr and Sullivan 2005; Piperno 2006). In the past, phytoliths were also termed as amorphous silica gel, plant opal, grass opal, biogenic opal, Opal-A, opal phytoliths, opaline silica, biogenic silica, and biogenic silicon opal (Smithson 1956; Smithson 1958; Geiss 1973; Kaufman et al.1979; Drees et al.1989). For any given plant species, higher the phytolith concentration and biomass greater the potential for carbon sequestration. Sugarcane and related species may also potentially provide a similar function. The first phytoliths reported were recovered by Darwin in a dust sample collected from the sails of the Beagle off of the coast of Africa (Darwin 1846). Phytoliths would tend to remain airborne longer than quartz based particulate. This prolonged suspension time would be potentially exacerbated by phytolith's non-spherical (*i.e*. tabular) shape. Amorphous silica is also being used in nanotechnology (Neethirajan *et al.*2009). According to Lu and Liu (2003), long and short cell phytoliths are identified in grass species. Phytolith has contributed significantly to the research on the taxonomy of monocotyledons especially the grass family. Wild sugarcane species, particularly, *Saccharum spontaneum* L. and the closely related genus *Erianthus* (old world species) are native to India and occur naturally in many parts of the country. They belong to the tribe Andropogoneae coming under the grass family Poaceae. These species include bushy type plants with very narrow leaves and absence of stem to tall plants with broad leaves and thick stem closely resembling sugarcane plants. *Erianthus arundinaceus* produces a wide array of morphologically distinct types of phytoliths, with considerable size variance within each type (Seikh et al. 2014). It is apparent that phytolith analysis and research is a relevant, multidisciplinary, very viable, and rapidly growing discipline in relation to sugarcane and its wild relatives. Studies indicated that the various species were found to produce diverse forms of phytoliths. But the size, orientation and the abundance vary among different species (Ollendorf et al. 1988; Bozarth, 1993; Jattisha and Sabu, 2012; Chauhan et al. 2011; El-Gazzar et al. 2013). Since the firm basic morphologic foundation reported by Twiss *et al.* (1969) based on C_3 and C_4 metabolic differences, the study of grass phytoliths has continued to develop over the ensuing decades. Twiss (1980, 1983, 1986, 1987, 1992 and 2001) continued to study grass phytoliths and their value as climatic indicators. Hence, the phytolith characteristics may be an important and reliable trait for clonal characterization and also there is a possibility of using this trait as a tool for the identification of species, hybrids and their parentage in sugarcane and their related genera. With this background, present study was made to record the variations in type and shape of phytoliths present in leaves of improved *Erianthus arundinaceus* clones.

Improved *Erianthus arundinaceus* clones selected from the germplasm collection were raised in three replications of ten rows of each clone at the Additional Land area of ICAR-Sugarcane Breeding Institute, Coimbatore, India. Twenty green leaves were collected from each clone in three replications at 12th month of the crop for studying the phytolith morphology. The collected leaf samples were washed, dried in shade and then in a hot air oven at 60°C. The dried leaves were pulverised in a Foss Tecator Cyclotec1093 Sample Mill. Ten grams of each sample was weighed into digestion tube and digested using nitric: perchloric acid mixture (6:2 ratio) (Kalra, 1998) in a PELICAN KEL PLUS Automatic Sample digestion System. The excess acid was removed by centrifugation at 2000 rpm in a Thermo Scientific Heraeus Biofuge Centrifuge. The residue was washed several times with distilled water and with 95% ethanol till the supernatant was clear. The residue was transferred to vials and stored for microscopic studies.

Phytolith extracts were quick-mounted in distilled water and viewed in an optical microscope. Whole slides were scanned at 100X to find clusters of particles, which were then scanned at 400 X to determine the character of the individual particles. Representative and especially significant phytoliths and other bio-silica bodies in each slide mount were noted. Images of typical or frequent and/or distinctive phytoliths were digitized as black-and-white camera mounted on the microscope and

Table 1: Classification of *Erianthus* phytoliths

S.No	Phytlithic types	Shape	Clone No.
1	Eupanicoid	Halter-shaped had a smooth, dumb-bell-shaped outline and opaque	IK76-81 x GC 04-16
		Cylindric sulcate tracheid	IK76-81 x CoS 8408-04-20
2	Festucoid	Circular crenate	IK76-81 x GC04-09, IK 76-81 x CoS 8408- 04-20
		Elongate spiny and elongate concave ends	IK76-91 9 x SF 06-41
3	Chloridoid	Crenate variant Mixtures of keeled and conical short cell forms	IK76-93 9 x SF 06-48

connected to a computer. 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 present paper represents only a preliminary study towards developing an identification key for *Saccharum* germplasam based on the foliar phytolith characteristics. The phytoliths identified in the five *Erianthus* clones were grouped into three classes. A halter-shaped phytolith belonging to the class eupanicoid sub family phytoliths was observed in abundant (Fig. 1). The halter-shaped phytolith was predominant and had a smooth, dumbbell-shaped outline and opaque. This class was reported to be abundant in the three tribes Andropogoneae, Paniceae, and Maydeae in the division (Hubbard 1948). Brown (1984)included Isachneae and Oryzeae in the Panicoid group; their silica bodies of these families are modified dumbbells which cannot be distinguished from other Panicoid forms where they occur as discrete particles in the plant parts.

Cylindric sulcate tracheid (Tracheid) type was found more in clone number three (Fig. 1a). Among the eight Festucoid class types reported, only circular crenate was observed in the samples examined (Fig.1b notched or scalloped, dented with the much rounded). Among the five types in Elongate class reported, elongate spiny and elongate concave ends types were observed in the *Erianthus* clones (Fig.2 and 3). Chloridoid class was designated as battle axes with double edges because saddle-shaped bodies are distinctive and common in atmospheric dust originating which could not be observed in the samples examined. While adjusting the microscope to focus through crenate phytoliths, a faint purple band was visible (Fig.4) along the long axis of the cellin some shorter near-round forms suggesting that these may be another crenate variant or related form even though there is no edge development. These rondels may represent a distinct short cell phytolith form, a large conical variant, or perhaps an intermediate morphologic type between conical and crenate forms. Mixtures of keeled and conical short cell forms as well as weakly formed crenate cells were observed (Fig.5).These grass phytolith morphologic classes were first observed and reported by Twiss et al. 1969).The morphologic differences observed in the short cell phytoliths in these samples might be due to metabolic differences as well genetic architecture (Taiz and Zeigler 2002).

Observations on various classes, types and variants in the leaf phytoliths in the *Erianthus* clones studied indicates that the phytolith morphologic forms can form a potential trait in identifying the species and tracing the genetic base of sugarcane hybrids (Krishnan et al. 2000; Jattisha and Sabu 2012; Naskar and Bera 2018; Bhat et al. 2018). Brown studied and reported phytolith morphologic forms from a variety of voucher specimens and evaluated phytolith size relative to water availability (1984, 1986a, 1986b). It was also found that the expression of some genes and phytolith production were directly related. The deposition of opal silica in the fruit rinds of

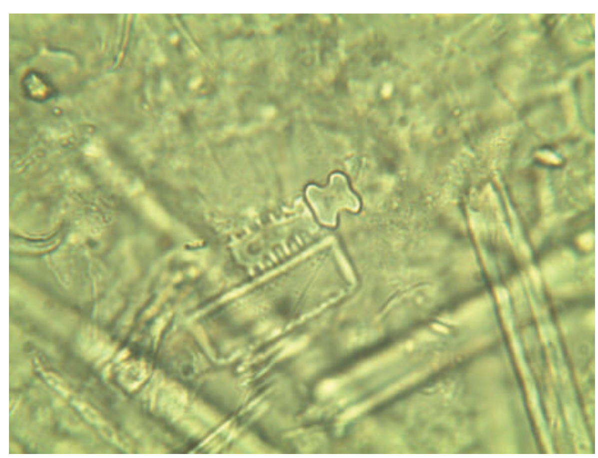

Figure 1. Crosses

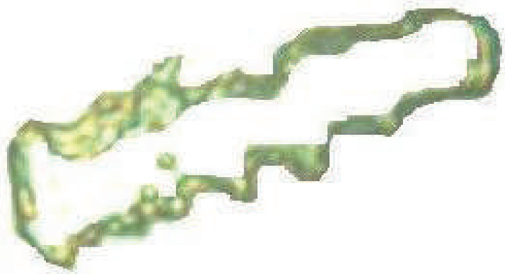

Figure 1a. Cylindric sulcate tracheid

Figure 1b. Crenate

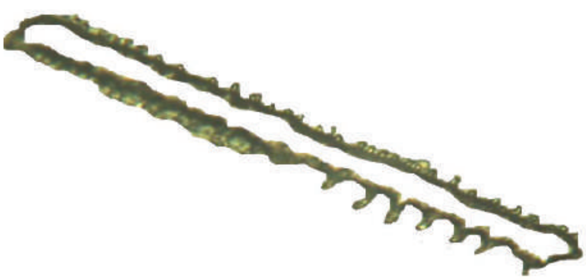

Figure 1c. Elongated

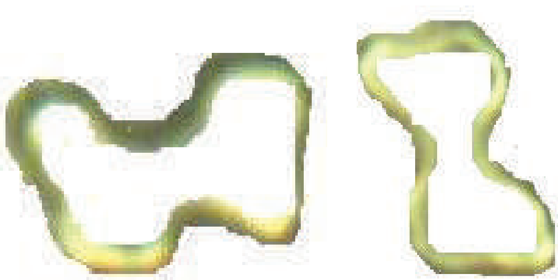

Figure 1d. Saddles

Figure 1e. Crosses

Cucurbita was found to be linked to the hard rind (Hr) genetic locus (Piperno et al. 2002) while locus tga1 was shown to control deposition of silica in teosinte glumes (Dorweiler and Doebley 1997). However, to adopt phytolith morphology

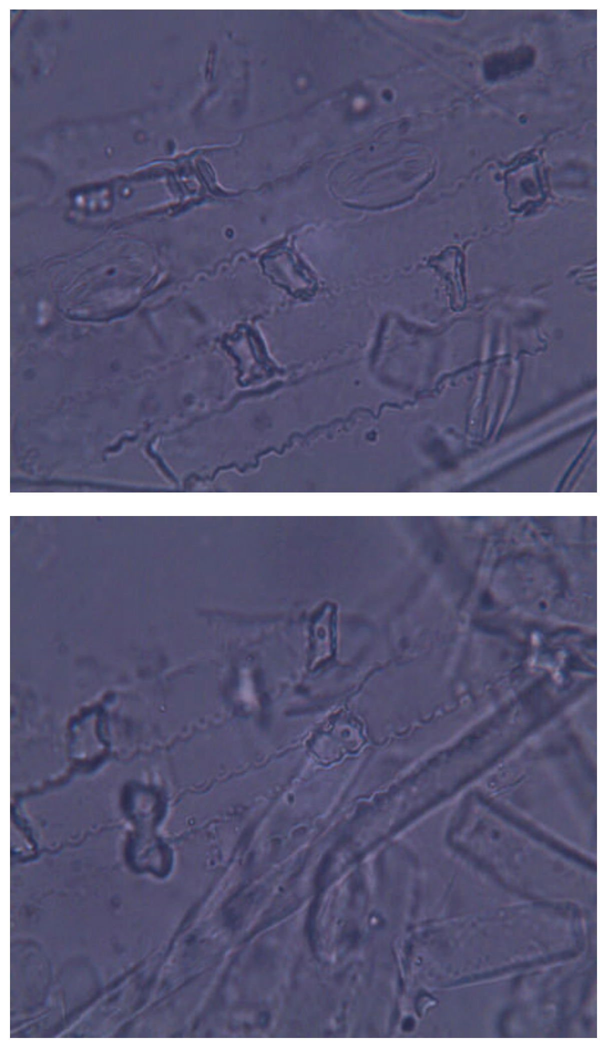

Figure 2. Saddle

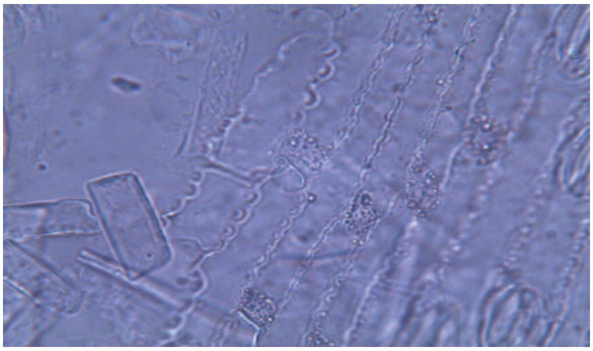

Figure 3. Elongate types

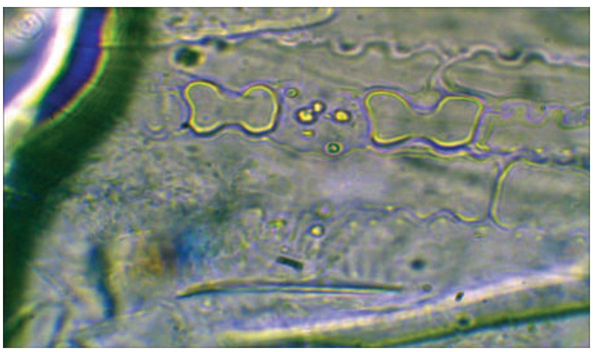

Figure 4. Saddle shape

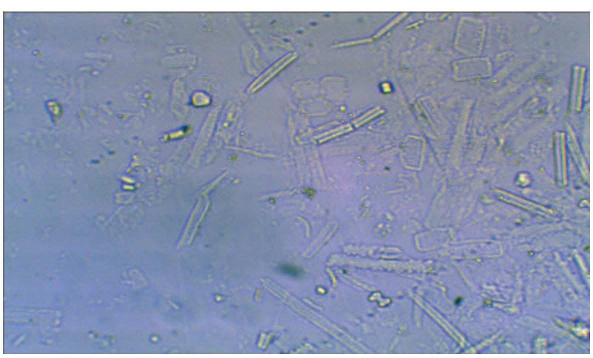

Figure 5. Mixtures of keeled and conical short cell forms

as a tool to identify and classify sugarcane and related species and hybrids, a detailed database on phytolith morphology has to be created and phytolith classes and types specific to various species and hybrids has to be established. The morphology of phytoliths from all plant parts (roots, stem, leaves, and reproductive structures) should also be documented with morphometric (size dimensions) as well as frequency data of each phytolith type in each species, which will serve as a better tool for the classification of sugarcane and related genera. Information on the factors influencing the phytolith morphology like plant maturity, intraspecific variation, the rate of leaf transpiration, tissue type *etc*. should be studied through experiments.There is an urgent need to understand the effect of these variables before one could utilize the full potential of phytoliths as diagnostic markers in plant genetic resource classification, preservation and utilization.

An idendification key for the species based on the frequency, size, and shape of phytoliths can be developed in future

Phytoliths present in the leaves of five *Erianthus arundinaceus* clones were studied. Phytoliths belonging to three classes *viz*., Eupanicoid, Festucoid and Chloridoid were identified with morphological variations. The results indicate that phytolith class and morphology may be an important and reliable trait for clonal characterization and identification of species, hybrids and their parentage in sugarcane and their related genera. However, this requires further studies and development of a database of phytolith morpho-types in different sugarcane and related genera and their hybrids, different factors influencing the shapes and sizes of phytoliths and the presence and morphology of phytoliths in different plant parts.

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