

Spheroid Echininate Symmetric Phytolith Assemblage in Sago Palm (*Metroxylon sagu* Rottb.) Leaflets

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Abstract: The Arecaceae family of plants accumulates a substantial amount of silicon, which helps to mitigate the harmful effects of stress caused by heavy metals, salts, and drought. This also leads to the formation of phytoliths in all parts of the palm. In this study, phytoliths from several palm leaflets were compared. In order to determine the relative abundance of phytoliths, slides containing 250 phytoliths each were observed by microscope. The samples were subjected to incineration at 550°C, ultrasonic treatment, 0.1 mol/L HCl treatment, and distilled water washing. Sago palm (*Metroxylon sagu*) phytoliths were identified as spheroid echinate symmetric morphotypes with diameters of <5 µm (1.2%), 5–10 µm (27.8%), 10–15 µm (48.8%), 15–20 µm (20.4%), and >20 µm (1.9%). These spheroid phytoliths were relatively larger than those of other palm species such as *Phoenix roebelenii*, *Phoenix canariensis*, and *Pritchardia pacifica*. The phytolith assemblages of sago palms were also distinguished by their symmetrical spheroidal shape and sharp spines. Meanwhile, *Corypha umbraculifera* and *Washingtonia robusta* produced both spheroid and ellipsoid phytoliths. *Caryota maxima*, *Butia yatay*, *Dypsis lutescens*, and *Dypsis decaryi* formed three types of phytoliths: spheroid, ellipsoid, and conical. *Areca catechu*, *Cocos nucifera*, *Chamaedorea elegans*, and *Chamaedorea cataractarum* produced both ellipsoid and conical phytoliths. Phytoliths of *Hyophorbe verschaaffeltii* were identified as conical morphotypes only. We have proposed a schematic genealogical tree for Arecaceae based on the description (morphotypes and size distribution) of their phytoliths, using the results of this study.

Keywords: Arecaceae, conical, echinate, phylogenetic tree, spheroid

Introduction

Several plants, especially rice plants, vigorously absorb silicon to protect their metabolisms and body against disease and herbivores by accumulating phytoliths in their whole bodies (Belton et al., 2012; Katz 2015). Although the correlation between siliceous solution species and the final silica

structure on both microscopic and macroscopic scales is still unclear (Belton et al., 2012), Nakamura et al. (2021) recently found the mechanism of phytolith formation in rice plants and reported that cellulose nanofibers were essential as a scaffold for silica accumulation for phytoliths between the epidermal cell wall and the cuticle layer, which

Yoshida et al. (1962) called the “cuticle-silica double layer.”

Sago palms (*Metroxylon sagu* Rottb.) produce phytoliths—spheroid echinate symmetric phytoliths (Fenwick et al., 2011; Bowdrey, 2014)—recommended for use by the International Committee for Phytolith Taxonomy (2019) and Witteveen et al. (2022). Fenwick et al. (2011) described the phytoliths of *M. sagu* as having a mean maximum diameter of 14.09 μm . Baba et al. (2020) also reported a similar diameter of *M. sagu* collected from Philippines. The characteristics of spheroid echinate and conical phytoliths are useful for the identification of palms. Witteveen et al. (2022) reported that the appearance, diameter, and conical projection (spine) length of phytoliths were good indexes to identify palms in Amazonia. Under a microscope, we tried to observe the appearance (morphotypes) of phytoliths collected from Southeast Asia and Japan and determine the frequency distribution of the diameter of phytoliths as the first step of the characterization of *M. sagu* phytoliths, as compared with other palms. Finally, we presented a schematic genealogical tree for several Arecaceae using morphotypes and size distribution in order to elucidate the characteristic status of *M. sagu* in the Arecaceae family.

Materials and Methods

1. Samples and phytolith identification

Palm species that were the focus in this study were dominant in tropical areas or useful for humans. Phytoliths in the leaflets of *Metroxylon sagu*, *Phoenix canariensis*, *Phoenix roebelenii*, *Pritchardia pacifica*, *Corypha umbraculifera*, *Washingtonia robusta*, *Butia yatay*, *Dypsis lutescens*, *Dypsis decaryi*, *Cocos nucifera*, *Areca catechu*, *Caryota maxima*, *Chamaedorea elegans*, *Chamaedorea cataractarum*, and *Hyophorbe verschaffeltii* were collected from Southeast Asia and Japan. The leaflet samples were air-dried, oven-dried at 105°C, and incinerated in an electric furnace at 550°C for 6 hr. Parts of the incinerated samples were treated with 0.1

mol/L HCl solution, washed with distilled water in a centrifuge tube, and centrifuged at 7000 rpm and 5 min several times. These samples were mounted onto a slide glass using a suitable permanent fixative polystyrene (MGK-S) (Matsunami) and covered with a glass for observing under a microscope. The phytoliths were examined under transmitted light using a Meiji Tech MT-5000 light microscope at 400x magnification, and their photographs were taken using a Cannon EOS Kiss X5 (Okazaki et al., 2020a, b). The morphology of phytoliths was written according to the descriptions of spheroid, ellipsoid, and conical morphotypes (Witteveen et al., 2022).

2. Quantitative variables of phytoliths

The total phytolith weight of oven-dried leaflet samples was determined in duplicate (Baba et al., 2020). Quantitative measurement of the maximum diameter of phytoliths was performed using an eyepiece graticule with a 1.25 μm tolerance. One hundred fifty phytoliths were identified as spheroid, ellipsoid (longer diameter), and conical (base diameter) morphotypes; they were measured to obtain the maximum diameter data for each morphotype, and their distribution frequency was determined. A schematic genealogical tree for several Arecaceae, based on the similarity index of morphotypes and size distribution, was drawn in accordance with the example of Doi and Okamura (2011).

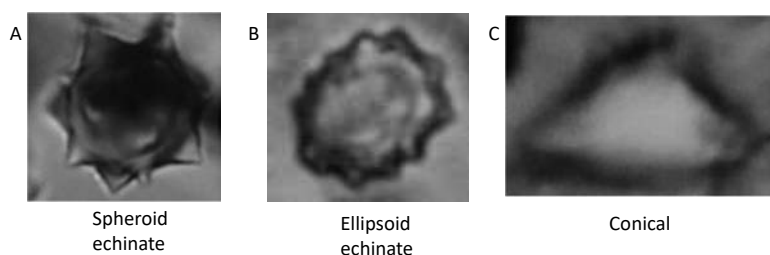
Results

1. Weight of phytoliths in sago palm leaflets

The weight of incinerated *M. sagu* leaflets collected from Pangasugan, Leyte, in 2023 was 10.3% of the total dry weight of leaflets, which was relatively smaller than that of *M. sagu* sampled from the same sago field of Pangasugan in 2019 (Baba et al., 2020) (Table 1). The spheroid echinate symmetric morphotype (Fig. 1A) in *P. canariensis* leaflets showed 5.5% of the total dry weight of leaflets. *Butia yatay* and *Cocos nucifera* produced ellipsoid (Fig. 1B) echinate phytoliths, which exhibited, respectively, 7.5 and 2.8%

Table 1. Phytolith weight percent of palm leaflets

Palm	Phytolith dry weight %
<i>Metroxylon sagu</i>	10.3
<i>Phoenix canariensis</i>	5.5
<i>Caryota maxima</i>	7.5
<i>Butia yatay</i>	2.8
<i>Cocos nucifera</i>	12.9
<i>Chamaedorea elegans</i>	5.4

**Fig. 1.** Spheroid, ellipsoid, and conical morphotypes of phytoliths

of the total dry weight of leaflets. On the other hand, *Caryota maxima* and *Chamaedorea elegans* formed conical morphotypes (Fig. 1C), respectively, of 12.9 and 5.4% of the total dry weight of leaflets.

2. Relative abundance of phytoliths of spheroid, ellipsoid, and conical morphotypes

Spheroid echinate, ellipsoid echinate, and conical morphotypes (Fig. 1) were identified (Table 2). Symmetric spheroid echinate phytoliths were

Table 2. Morphotype distribution of palm phytoliths

	Spheroid	Ellipsoid	Conical
<i>Metroxylon sagu</i>	○		
<i>Phoenix canariensis</i>	○		
<i>Phoenix roebelenii</i>	○		
<i>Pritchardia pacifica</i>	○		
<i>Corypha umbraculifera</i>	○	○	
<i>Washingtonia robusta</i>	○	○	
<i>Caryota maxima</i>	○	○	○
<i>Butia yatay</i>	○	○	○
<i>Dyopsis lutescens</i>	○	○	○
<i>Dyopsis decaryi</i>	○	○	○
<i>Areca catechu</i>		○	○
<i>Cocos nucifera</i>		○	○
<i>Chamaedorea elegans</i>		○	○
<i>Chamaedorea cataractarum</i>		○	○
<i>Hyophorbe verschaffeltii</i>			○

Phytolith diameter: 5–15 μm

produced by *M. sagu*, *P. canariensis*, *P. roebelenii*, and *P. pacifica*. However, *P. roebelenii* and *P. pacifica* contained both symmetric spheroid echinate and spheroid echinate phytoliths (Witteveen et al., 2022). The ellipsoid echinate phytoliths with diameters of 5–15 μm were from *C. umbraculifera* and *W. robusta*, which also contained

small amounts of spheroid echinate phytoliths. On the other hand, the conical morphotypes (Morcote-Ríos et al., 2016; Witteveen et al., 2022) were produced by *C. maxima*, *B. yatay*, *D. lutescens*, *D. decaryi*, *A. catechu*, *C. nucifera*, *C. elegans*, *C. cataractarum*, and *H. verschaffeltii*. Therefore, *C.*

maxima, *B. yatay*, *D. lutescens*, and *D. decaryi* consisted of three types of morphotypes: spheroid, ellipsoid, and conical. Apart from these, *H. verschaffeltii* was comprised of the conical morphotype only.

3. Diameter distribution frequency of phytoliths

The diameter distribution of phytoliths is shown in Fig. 2. *M. sagu* phytoliths were identified as having spheroid echinate symmetric morphotypes with diameters of <5 μm (1.2%), 5–10 μm (27.8%), 10–15 μm (48.8%), 15–20 μm (20.4%), and >20 μm (1.9%). Almost all of the palm phytoliths sampled were concentrated in diameters of 5–10 μm , except the phytoliths of *W. robusta* and *C. elegans*. In the cases of *D. lutescens* and *C. maxima*, their phytoliths with diameters of 5–10 μm accounted for approximately half of the total phytoliths.

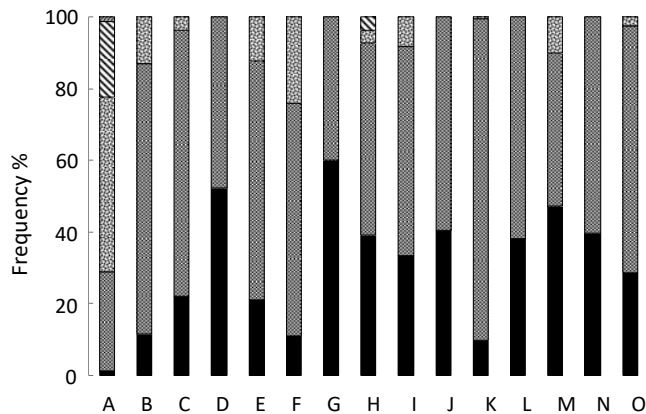


Fig. 2. Size distribution frequency of phytoliths from palm leaflets

■ < 5µm ■ 5-10µm ■ 10-15µm ■ 15-20µm ■ >20µm

A: *Metroxylon sagu*, B: *Phoenix canariensis*, C: *Phoenix roebelenii*, D: *Pritchardia pacifica*, E: *Corypha umbraculifera*, F: *Caryota maxima*, G: *Washingtonia robusta*, H: *Butia yatay*, I: *Dypsis lutescens*, J: *Dypsis decaryi*, K: *Areca catechu*, L: *Cocos nucifera*, M: *Chamaedorea elegans*, N: *Chamaedorea cataractarum*, O: *Hyophorbe verschaffeltii*

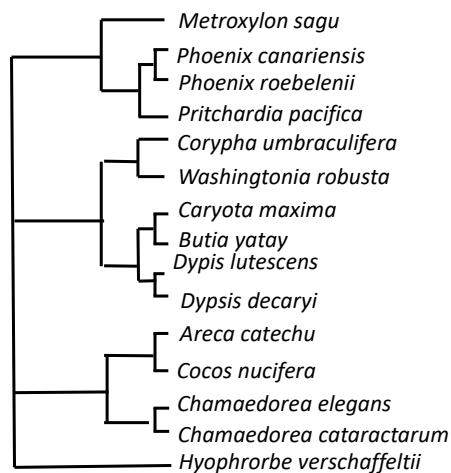


Fig. 3. Schematic phylogenetic tree for Arecaceae based on the description of their phytoliths

4. Schematic genealogical tree based on morphotypes and size distribution

Figure 3 shows a schematic phylogenetic tree for Arecaceae based on the morphotypes and the size distribution of their phytoliths using the results of this study according to similarity index (Doi and Okamura, 2011). The palm phytoliths studied were classified broadly into three groups based on morphotypes: spheroid (Fig. 1A), ellipsoid (Fig. 1B), and conical (Fig. 1C). High percentages of spheroid morphotypes were found in the order of *M. sagu*, *P. canariensis*, *P. roebelenii*, and *P. pacifica*. The system diagram of the palm phytoliths of *M. sagu* is worthy of special

mention, based on the morphotype characteristics (spheroid echinate) (Table 2) and high percentages of phytoliths that are larger than those of other Arecaceae (Fig. 2). *M. sagu*, *P. canariensis*, *P. roebelenii*, and *P. pacifica* were distinguished by their spheroid morphotype only. *C. umbraculifera* and *W. robusta* were categorized by the phytolith mixture of spheroid and ellipsoid morphotypes. The ellipsoid morphotype was closely related to the ellipsoid morphotype of *C. maxima*, *B. yatay*, *D. lutescens*, and *D. decaryi*. Meanwhile, the phytoliths

of *A. catechu*, *C. nucifera*, *C. maxima*, *C. elegans*, and *C. cataractarum* were characterized by both the ellipsoid and conical morphotypes and subdivided by the other groups. *H. verschaffeltii* contained the conical morphotype only.

Discussion

Silicon is not an essential element for plants, according to the definition (Ma et al., 2001; Mitani-Ueno and Ma, 2021). However, it effectively protects the whole body against disease and herbivores. Palms can absorb silicon and make phytoliths in the whole body. Fenwick et al. (2011) concluded that it is possible to make use of morphological criteria to distinguish between some taxa of palms on the basis of leaf phytoliths. Morcote-Ríos et al. (2016) proposed a taxonomic distribution of phytolith morphotypes in Amazonian palms that included globular echinate symmetric; globular echinate; globular echinate elongate; globular echinate with dense, short projections; reniform echinate; globular echinate with long, acute projections; conical; and conical with acute basal projections. We identified the phytolith morphotypes by a microscope using 400x magnification, although Huisman et al. (2018) subdivided morphotype categories based on differential interference contrast (DIC) microscopy

using 630x magnification. The variability of Amazonian palm phytolith morphotypes was described (spheroid—12, conical—11, and no phytoliths—1) and proposed a quick guide to identify spheroid and conical palm phytoliths (Witteveen et al., 2022). We followed the quick guide of Witteveen et al. (2022) to describe the palms sampled.

The mean values of the maximum diameter of *M. sagu* morphotypes in leaflet samples were larger than those of the other species investigated. *M. sagu* phytoliths are strongly characterized by spheroid echinate symmetric features.

Conclusion

This study shows that *M. sagu* produces spheroid echinate symmetric phytoliths and is clearly distinguished by the characteristic morphotype and distribution of diameter in its leaflets.

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