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ORIGINAL ARTICLE

HABITAT SPECIFICITY OF PHYTOLITH PRODUCTION IN *ACROSTICHUM AUREUM L.*

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Abstract: *The mangrove fern species Acrostichum aureum L. grown in different habitats namely, mangrove region of Sundarbans, and mesophytic regions of Kolkata and Nadia are chosen to study the habitat specificity of phytoliths (biogenic silica bodies) production in the plant. Five types of phytoliths (Type I, II, III, IV and V) are identified from the extracted leaf lamina of Acrostichum. The observation shows the types and percentage of phytolith distribution are more or less identical in the Acrostichum of Sundarbans and Kolkata whereas discriminating result is noticed for the specimen of Nadia. This discrimination suggests that the production of phytolith in different parts of plant depends on some ecological factors.*

Key Words*:* Phytolith, *Acrostichum aureum*, Sundarbans, Kolkata, Nadia.

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1. INTRODUCTION

Acrostichum aureum L. belonging to the family Pteridaceae is commonly known as mangrove fern or golden leather fern and occur in tropical and subtropical areas across the world. This large understory fern thrives well in swamps, salt marshes, low hammocks, and canal margins in mangrove forests and other wetlands. *The plant* is halophytic, but generally requires fresh water for it to become established and grow optimally [1]. It does not grow in areas where soil salinity exceeds 50 ppt, nor does it grow on arid coastlines. Several authors reported that *A. aureum* grows optimally on somewhat elevated grounds in mangrove forests that are well protected from frequent tidal influx and have high rainfall, which tends to desalinate upper soil layers.

The biogenic opal silica bodies in plant cells are amorphous form of silicon dioxide, commonly known as phytolith (Greek *phyto* = plant, *lithos* = stones). They occur in different plant parts namely, stems, leaves, roots, and inflorescence etc. Phytoliths forming silica is actually carried up from ground water as monosilicic acid [$Si(OH)_4$][2] then converted and eventually deposited as solid opaline silica bodies (SiO_2, nH_2O) in epidermal cell walls, cell lumen, intercellular spaces of the cortex and other cells of the growing plants. In many plant taxa, distinctive forms that retain cell shape are formed after organic tissue has decayed or burned [3]. In other taxa, silica deposition produces less distinctive masses, difficult to characterize by shape or size. Phytoliths are produced in plants through two basic mechanisms:

A. Non situational pattern that is formation within specialized silica accumulating cells having little relationship to the shape of the parent cell, referred to as idioblasts [4], or
B. Incidental silicification pattern, i.e., the formation in the cellular and intercellular spaces of plants which typically take on the shape of the cell [5].

When the plant body parts are degraded, the phytoliths can be preserved for a prolonged period of time in soil. By this way, phytoliths provide valuable information about vegetation in Archaeology and Palaeoecology [6-12]. They can also be used for crop processing [13].

Organic carbon occluded within phytoliths is called phytOC [14] that can be used for radiometric dating [15] and monitoring vegetation and climate change [16]. Particular plant taxon or plant part can be identified by phytolith characters which can also help in identification of a plant species or its part [17-19]. Phytoliths also have many biological functions such as mechanical support, defence against herbivores and pathogens [20], metabolism [21].

German microbiologist Ehrenburg was the first to study phytoliths systematically [22]. These silica bodies are known to occur at least sporadically within all major groups of vascular plants [23, 24], but have been most intensively studied in the monocotyledons (commelinids and Orchidaceae; [25]. Epidermal silica bodies have proved useful for diagnostic and systematic purposes in monocotyledons [25, 26] but remain to be studied in many other groups particularly in pteridophytes.

Piperno [23] summarized what was so far known of phytolith production and silicification patterns in different plant groups. She incorporated 4 families and 13 genera of pteridophytes and gymnosperms, 21 families and 52 genera of monocotyledons (exclusive of grasses), 45 families and 125 genera of dicotyledons. Later on, Pearsall [27] added more data, 11 families and 27 genera of pteridophytes and gymnosperms, 28 families and 115 genera of monocotyledons (exclusive of many hundred grass genera), 103 families and 475 genera of dicotyledons.

'Silicification' is common in many cell types within the gymnosperms, resulting in cellular fragments or well-preserved cellular replicas of epidermal cells, stomata, tracheids, parenchyma cells and endodermal cells. Hodson *et al.* [28] presented a useful overview of the literature on gymnosperm, and report on the abundance of silica produced in 42 species in the Araucariaceae, Cupressaceae, Pinaceae, Taxaceae, and Taxodiaceae.

Occurrence and characterization of pteridophytic phytoliths have been reported from *Equisetum* [23], *Trichomanes* [23, 24], *Adiantum* and *Selaginella* [23], *Christella*, *Ampelopteris*, *Cyclosorus* [12]. Taxonomically diagnostic silica bodies have been reported in the following ferns and lycophytes *Selaginella* [23, 29], *Isoetes* [30], *Equisetum* [31] and Pteridaceae. Within Pteridaceae, silica bodies have been reported from *Afropteris*. Several other authors have also reported thickened epidermal cells that may in fact be silica bodies, which found within Pteridaceae. Nyer [32] found 'thickened epidermal' cells in nine species of *Adiantum*. Wagner [33] has been used the elongate epidermal idioblasts as a taxonomic character to distinguish species in *Adiantum*. Kao et al [34] employed histochemical staining and scanning electron microscopy (SEM) with Energy Dispersive x-ray Spectrometry (EDS) in *Pteris grevilleana* to confirm that Wagner's idioblasts are indeed silica bodies. Recently, silica bodies are evaluated as a systematic character in the fern family Pteridaceae [35]. From this review, it is apparent that, insufficient data are available on pteridophytes to draw any conclusions about phytolith production pattern therefore it needs more research [36].

In present study an attempt has been given to study biogenic opal silica bodies (phytoliths) production in *Acrostichum aureum* growing in three different places namely, Sundarbans, Kolkata, and Nadia of West Bengal. The objective of the investigation is to study the occurrence and characterization of opal crystals in leaf tissues from *Acrostichum aureum* grown under varying habitats and to observe the relation of environment on phytolith production, if any.

2. MATERIALS AND METHODS

Silica bodies were extracted from leaf fragments that had been removed from fresh and herbarium specimens. Phytolith extraction method by Piperno *et al*. [24] was followed with some alterations. For the present study, the leaves of *Acrostichum aureum* are chosen from three different habitats. One is from brackish to saline swamp area of mangrove vegetation in Sundarbans. The other two specimens are collected from Kalyani of Nadia district and from eastern bypass area near Ruby Hospital in Kolkata city.

Lamina of mature leaves of *Acrostichum* were collected and washed thoroughly several times with distilled water. Samples cut into few square centimetre including veins, costae and leaf margins. About 1gm of each of the plant materials were taken in test tubes. Allow samples to dry at hot air oven at the temperature of 60-80ºC. This drying process continues for few days. Then the samples were digested with concentrated nitric acid (HNO_3). Digested materials were washed with distilled water by centrifugation at 2000 rpm for 10 minutes. This method removes more or less all plant tissues except silica. Finally permanent slides were prepared from silica pellets with the help of polyvinyl alcohol as fixative and Canada balsam as mounting media. 5 slides were prepared for each sample. The prepared slides were observed under compound microscope and photographs were taken by Zeiss Axioskop 2 microscope. All the slides have been kept at the repository of Pteridology-Palaeobotany Section, Department of Botany, University of Kalyani.

3. RESULTS

Microscopic studies of the prepared slides show opal silica bodies of biogenic origin are present in all the collected mature leaf lamina of *Acrostichum aureum* from three different sites.

From the observation, it is clear that altogether five types of phytoliths (Type I, II, III, IV and V) [Plate I] are deposited on leaf epidermal cells of *Acrostichum aureum*. All the types are three-dimensional, hyaline with smooth surfaces but most of the types with wavy outline seems like epidermal cells.

Irregular multifaceted phytoliths are identified as Type I. They are 20 to 60 µm in size. Multifaceted forms are 3 dimensional, have an irregular outline and have multiple facets or folds (Plate I, Figure 1).

Type II phytoliths is of jigsaw types. This type is generally with good clean edges and some pitting, and specimens are 20 to 57 µm in size. Jigsaw phytoliths are flat plates with wavy, undulating edges (Plate I, Figure 2-3).

Type III is classified as elongate phytoliths. Elongate phytolith forms are well preserved and are 30 to 70 µm in size. These phytoliths are flat rectangular plates, some with pitted surfaces and others with smooth surfaces (Plate I, Figure 4-5).

Polyhedral phytoliths are present as Type IV. They have a plate-like appearance of 4 to 6 approximately rounded sides, range in size from 25 and 30 µm and the majority have smooth surfaces (Plate I, Figure 6).

Etched polyhedral phytoliths are recognized as Type V. This type is with good clean edges, and specimens are 20 to 40 µm in size (Plate I, Figure 7-9). Etched forms consist of a flat plate, with a hollowed top surface which gives an etched appearance.

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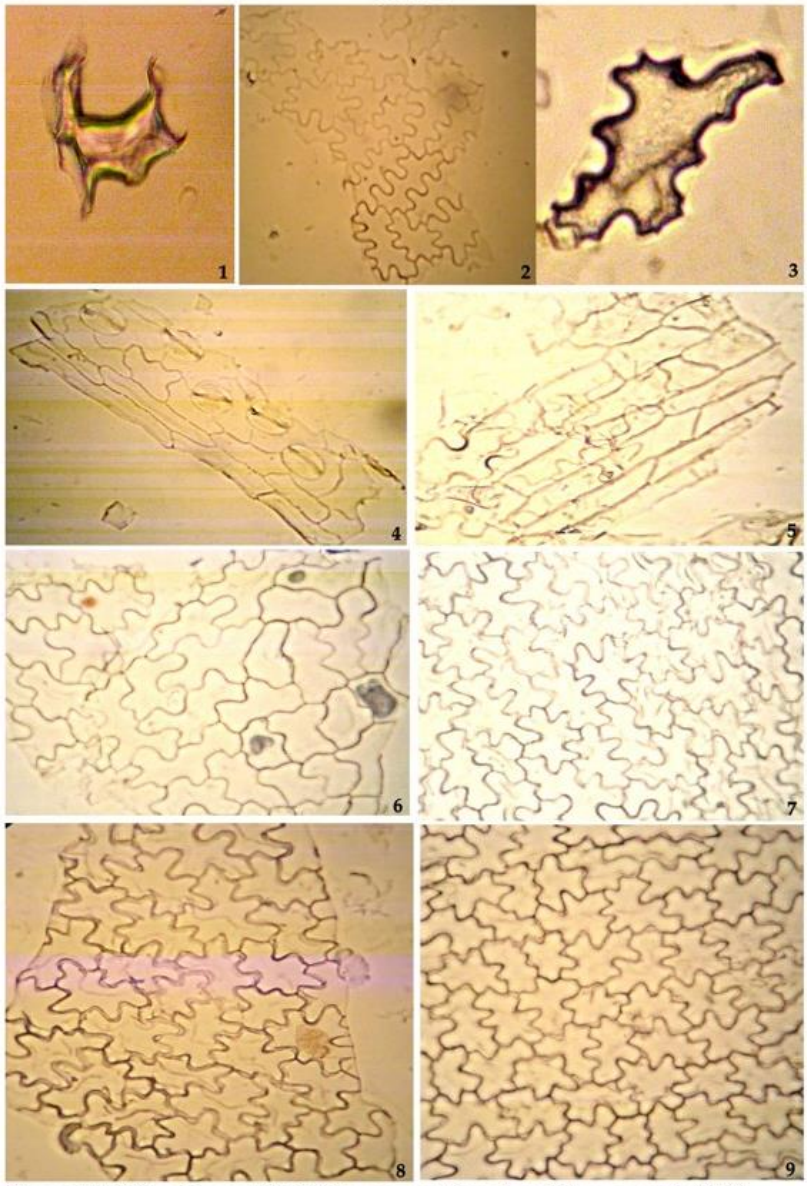
PLATE I



Figures 1-9: Different types of phytoliths recovered from *Acrostichum aureum* L. (x600)
1. Type I 2. Type II 3. Type II(enlarged view)x700 4.-5. Type III 6. Type IV 7.-9. Type V

Plate I - Figure 1-9: Different types of phytoliths recovered from lamina of *Acrostichum aureum* L. (x 600); Figure 1: Type I; **Figure 2:** Type II; **Figure 3:** Type II (enlarged view x700); **Figure 4-5:** Type III; **Figure 6:** Type IV; **Figure 7-9:** Type V.

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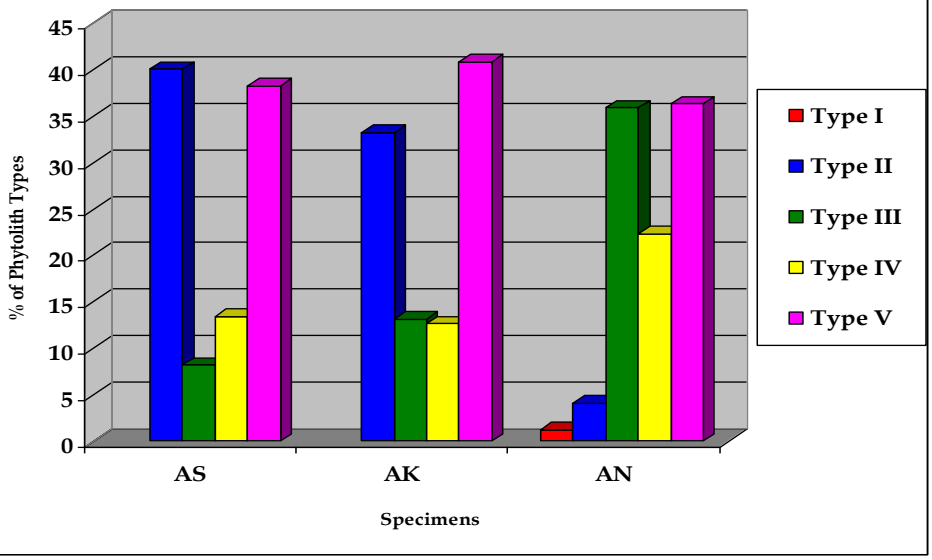
The frequency distribution of each of the types present in three different specimens is calculated which is given in Table 1. The values are also represented graphically in Figure 1.

Table 1: Frequency of the phytolith types extracted from three specimens

Specimen	Type I	Type II	Type III	Type IV	Type V
AS	---	40.1	8.2	13.41	38.33
AK	---	33.26	13.17	12.68	40.89
AN	1.2	4.13	35.91	22.39	36.37

AS: *Acrostichum aureum* from Sundarbans, AK: from Kolkata, AN: from Nadia

Figure 1: Frequency distribution of phytolith types of *Acrostichum aureum* L. growing in different habitats



AS: *Acrostichum aureum* from Sundarbans, AK: from Kolkata, AN: from Nadia

4. DISCUSSION

There are currently two basic approaches to phytolith systematic, the description of phytolith shape and distribution within the species. In traditional botanical taxonomy, an unidentified plant specimen is classified to species by comparing it to type specimen housed in a herbarium or to specimens previously identified by comparison to the type. Criteria used to make the comparison vary and typically include morpho-anatomical features, and increasingly chemical and chromosomal characteristics. The process of identifying phytoliths shares similarities and demonstrates differences with the classification process.

From the present study, the identification of *Acrostichum aureum* L. could be possible on the basis on phytolith types. It is worth mentioning that Sundue [35] pointed out total absence of phytoliths in the Ceratopteroid clade of fern including *Acrostichum danaeifolium* during the study of silica bodies and their systematic implications in Pteridaceae (Pteridophyta). But the present species that is *Acrostichum aureum* grown under saline condition as well as fresh water condition is well in conformity by occurrence of epidermal silica bodies in good percentage. However, at the same time, it must be mentioned here that the present study is in preliminary stage, for authentic identification more in-depth study of the specimens collected from different places is suggested.

The other objective of the present work is to compare the distribution pattern of phytolith assemblages from *Acrostichum aureum* grown under varying habitat. The differential pattern of phytolith production is evident from the Table 1 and Figure 1. The etched polyhedral phytoliths (Type V) is present in high frequency in all the specimens that is for Sundarbans (38.33%), Kolkata (40.89%) and Nadia (36.37%). Same result is for the Type IV (polyhedral). Considerable observation is noticed for the type II and III. Type II is present in negligible amount (4.13%) in the sample of Nadia but present in good frequency in other two materials, for Sundarbans it is 40.1% and 33.26% for Kolkata. But totally contrasting result is for Type III. It is to be noted that Type I is present only in the specimen of Nadia and totally absent in other two specimens.

5. CONCLUSION

By these significant observations it can be presumed that the production of phytoliths on leaf epidermal cells is controlled by ecological condition where the plants are grown. Here, it is clear that the phytolith types and their distribution pattern more or less same in case of *Acrostichum* plant grown at Sundarbans region and eastern bypass area of Kolkata but it differs variably from the plants collected from Nadia. This result suggests though now the bypass area of Kolkata is away from mangrove ecosystem, the area was a part of the same ecosystem which also established by palaeopalynological works. So, the edaphic factors of the bypass area have not been so changed. However, further data are required with more species from diverse ecological regimes to establish effect of ecology on phytolith production as well as to identify species specific distinctive form of phytoliths as a taxonomic tool.

6. CONFLICTS OF INTEREST

There are no conflicts of interest among the authors of this article.

7. ACKNOWLEDGEMENT

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