# Diversity, Distribution and Frequency Based Attributes of Phytolith in *Arundo donax* L.

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**Abstact**

*Arundo donax* of the family Poaceae is economically very important plant which accumulates silica in the form of phytoliths. Silica bodies (phytoliths) protect the plant from various biotic and abiotic stresses. It also provides the mechanical support, stiffness and durability to the stem. Present paper describes the distribution, diversity and frequency of opal phytoliths in the leaves and stems of *Arundo donax*. A number of morphotype of phytoliths are present in the leaves and stem, which are very characteristic and are useful for the taxonomic identification of the plant. The most common type is the bilobate phytolith, other forms include the trapezoids, prickle micro hairs, long micro hairs, epidermal long cells, stomata, bulliform, parallelepipedal cells and knobbed spine phytoliths. Silica deposition is the most common in the epidermis or in the bundle sheath cells of the vascular bundles and in the subepidermal sclerenchyma.

**Key words**: *Arundo donax,* Epidermal cells, Phytolith, Silica, Monosilicic acid

## INTRODUCTION

Biogenic silica has been established to be advantageous for the growth and development of various plants [1,2]. it assist plants to overcome various biotic and abiotic stresses [3,4,5,6].The existence of opal silica in the plant tissues has structural and protective role against fungi, insects, and herbivores. Silica bodies are abundant and morphologically distinct as well as much durable in soil for thousand even millions of the year after death and decay of the plants [7,8,9.] The structural hierarchy and durability of this element in the form of phytoliths in different parts of the plant [10,11] as well as in soil has proven the possibilities to solve the archaeological problems and to identify the crop plants which were grown in our past [12,13]. Majority of archaeologist and palaeoecologist have been using this kind of study for the reconstruction of palaeoenvironments, palaeoclimate and the presence of forested vegetation, soil horizons as well as past plant- people relationship [14,15,16]. The mapping of Silica bodies (phytoliths) from different ecological Zones is very much beneficial to analyze the probable reasons of biodiversity depletion and to understand the relationship between human and Land [17,18,19]. *Arundo donax* L. belongs to the family Poaceae. It is a tall perennial cane like grass forming dense stands on disturbed sites, sand dunes, wetlands and riparian habitats. It is native to the Mediterranean region while in India growing in Kashmir, Assam, Uttar Pradesh, Bihar and Southern India. The hollow culms are used to manufacture baskets, mats, flutes, writing pen and printing papers. *Arundo donax* is a prolific accumulator of biogenic silica in its tissues. It takes soluble monosilicic acid from the soil, which subsequently deposits in the inter cellular or intra cellular spaces of the cell in the form of opal silica bodies called phytoliths. A little account of phytolith analysis of *Arundo donax* has reported along with *Phragmites communis* by Ollendorf et. al. [20]. Present study gives detailed analysis of diversity and distribution as well as frequency of phytoliths in the leaves and stem of *Arundo donax* which provide taxonomically significant features and strive to construct consistent a parameter for the identification of this genus.

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## MATERIAL AND METHODS

### Phytolith extraction

The samples were collected from the Roxburgh Botanical Garden Department of Botany, University of Allahabad, Allahabad. Intact specimens were cut from standing clumps of each part of the *Arundo donax* plants. Specimens were divided into two fractions leaf blade, and stem. Both the fractions were subjected to thoroughly washing in distilled water and dried at $60^0$C for 48 h. They were then placed in porcelain crucibles and reduced to ash in a muffle furnace at about 400°C to 500°C for 4-6 hours until the ash appeared whitish.

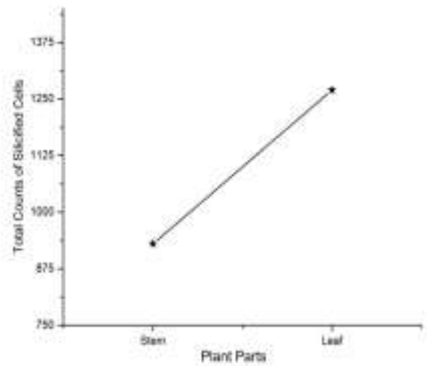


**Figure 1 Showing the silica concentration on the basis counts of silicified cells of *Arundo donex* stem and leaf blades.**

Subsequently the ash was treated with nitric acid ($HNO_3$). Residues were washed with distilled water and centrifuged. The remaining biogenic silica residue was dried.

Five slides of each sample (leaf blade and stem) were prepared with Canada balsam by taking .001 gm ash/ slide. Variation in the shape and size of silicified cells was observed along with their frequency in fixed observed area (1.24 $mm^2$)/ slide.

**Sample collection and preparation for transparency**

Small pieces of leaves were washed in distilled water and made transparent by using the technique of Stebbins, [21] to see the deposition of silica in different cells at different places of the leaves.

**Data analysis**

Statistical analysis of the observed data was analyzed by using Origin 8.1 software. The mean and standard deviation (SD) was calculated for the frequency analysis of each morphometriy.

## RESULTS

**Silica concentration**

Counting of silicified cells in leaves and stem reveal that highest silica percentage is present in leaf blade followed by the stem (Figure-1).

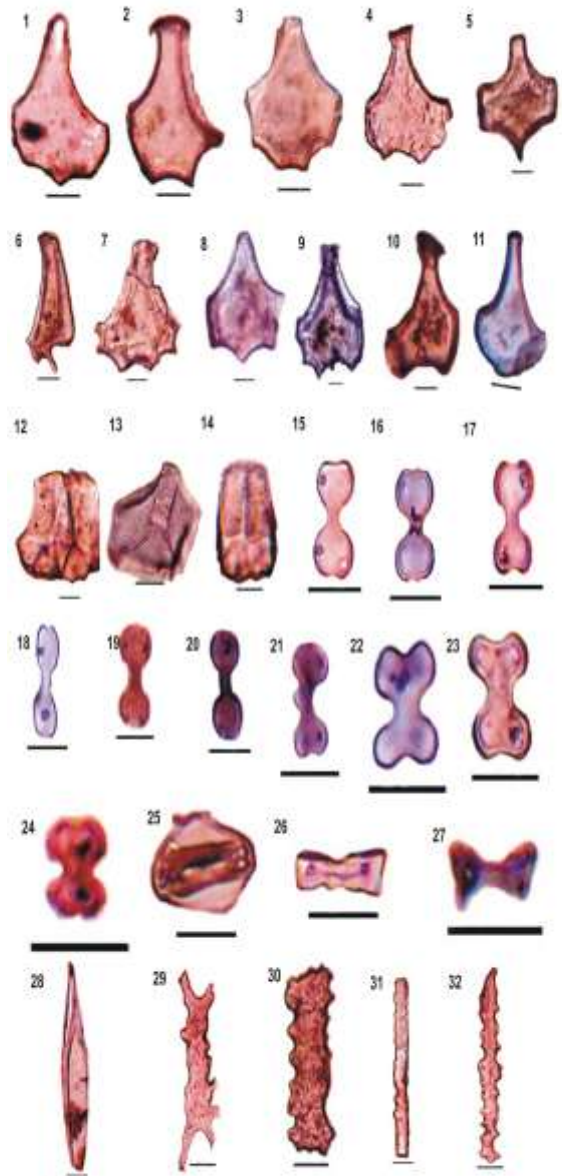


**Figure 2 Various phytoliths of *Arundo donax* leaf blade (bulliform phytoliths (1-13), parallelepipedal bulliform (14), bilobate (15-20), bilobate with nodular shank (21), cross (22-24), stomata (25), trapezoid (26, 27), micro hairs (28), elongate spiny with concave end (29), elongate spiny with pavement (30), elongate sinuous (31, 32).(Scale bar-20µm).**

**Silicification in leaf blades**

Silica deposition pattern generally occurs in the cellular or intercellular spaces of the plant tissues and is very common in the leaves of Poaceae family. The leaves of *Arundo donax* show well preserved silicified epidermal long cells, bulliform cells, hair bases, prickle micro hairs, trapezoids, bilobates, crosses, epidermal short cell phytoliths, elongated smooth, elongated sinuous with concave ends (Figure 2, 1-32). The lumen of the bulliform cells are frequently filled with silica forming a cast which shows significant variation in its shape and size. The base of the bulliform cells show 2-3 lobes of concavity (Figure 2, 1-15), which is a very characteristic and feature of this plant. In the transparency of the leaf blades bulliform cells, mostly arranged near the midrib region of the leaves were noticed. Such type of silicification in these specialized cells of that particular area of the leaf blade plays a very important role in the folding of the leaves during drought and stress conditions. A number of hair cells mostly placed near the margin area of the leaves protect the plant from the insect and pest attacks (Figure 2, 28). Epidermal long cell phytolith are produced by the silicification of the long cells of leaf the blades (Figure 2, 31&32). Silicified trapezoid phytoliths having variations in their shape and size are also found (Figure 2, (26 & 27). Parallelepipedal bulliform phytoliths are present in the leaf blade (Figure 2 (14). These phytoliths usually occur in the row of 1-3 above the veins on both the surfaces of leaf blades. Among all types of phytoliths found in the leaf blades of *Arundo donax*, the highest frequency belongs to the bilobate phytoliths (Figure 3). However, their shape and size vary and shank of the bilobate shaped phytoliths may be long, short or with nodular spines (Figure-2, (15-21).

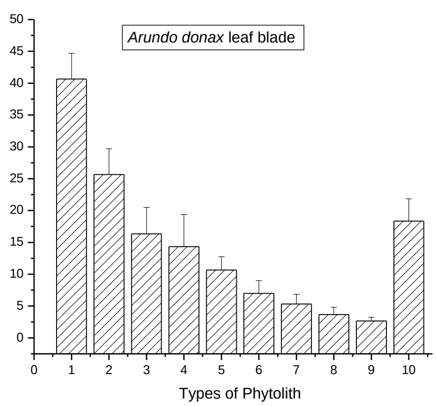


**Figure 3 Showing the frequency of various phytoliths of *Arundo donax* leaf blade. (1- bilobate, 2-cross,3-trapezoid, 4-bulliform, 5-micro hairs, 6-elongate sinuous, 7-parallelepipedal cells, 8-bilobate nodular shank, 9-elongate spiny pavement and concave end,10-others.)**

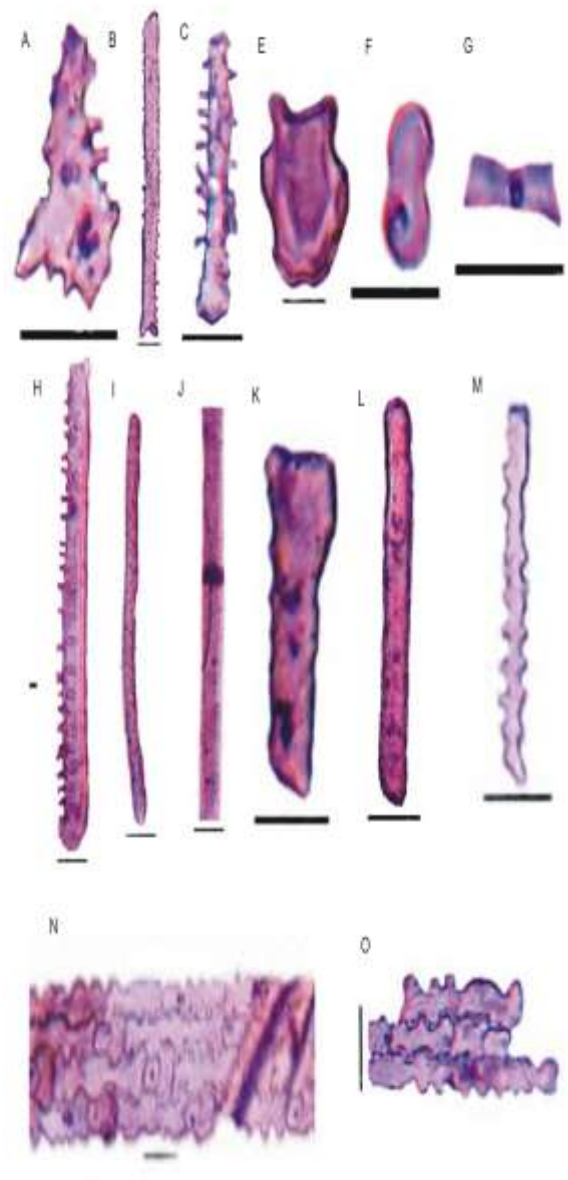


**Figure 4 Showing the various phytoliths of *Arundo donax* stem (trianguloid with spines (A), elongate spiny (B, H), knobbed spine phytolith (C), shield shaped phytolith (E), saddle (F), trapezoid (G), elongate smooth (I-L), elongate spiny with pavement (M), epidermal flat sheet phytoliths. (Scale bar-20µm).**

**Table 1 Showing the minimum and maximum length and width of leaf and stem phytoliths**

| Types of phytolith | Leaves | | Stem | |
|---|---|---|---|---|
| | Length in (µm) | Width in (µm) | Length in (µm) | Width in (µm) |
| Bilobate | 15-27.5 | 10-15 | × | × |
| Dumb-bell | × | × | 15.5-20 | 10-15 |
| Cross | 12.5-20 | 12.5-17.5 | × | × |
| Trepezoid | 7.5-15 | 12.5-22.5 | 7.5-15 | 12.5-22.5 |
| Bulliform | 50-75 | 30-62.5 | × | × |
| Micro hairs | 45-105 | 20-37.5 | 10-22.5 | 7.5-12.5 |
| Parallelepipedal | 17.5-62.5 | 10-20 | × | × |
| Nodular bilobate | 22.5-62.5 | 20-30 | × | × |
| Flat epidermal | 17.5-27.5 | 10-15 | 20-27.5 | 15-20 |
| Elongate spiny | 20-30 | 12.5-20 | 22.5-30 | 7.5-20 |
| Knobbed phytolith | × | × | 25-75 | 12.5-20 |
| Elongate smooth | × | × | 15-62.5 | 10-20 |

### Silicification in stem

The stem of *Arundo donax* also produces a large number of phytoliths having taxonomical significance (Figure 4). It is quite interesting that some epidermal and hypodermal cells possess knob like projections and are very common in the stem of the plants (Figure 4, A & C). These phytoliths provide an interlocking system to give mechanical support to the stem.

The knobbed phytoliths are highly silicified followed by the trapezoids, elongate spiny and with pavement, saddles, elongate smooth, and epidermal flat sheet phytoliths.

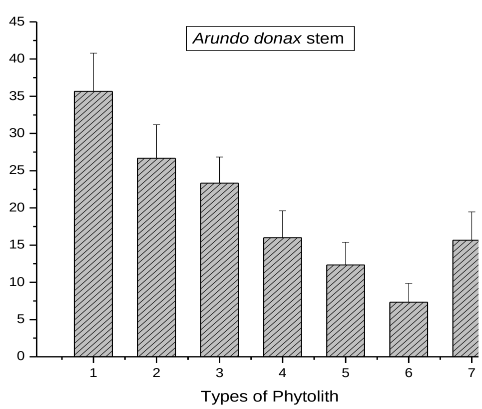


**Figure 5 Showing the frequency of various phytoliths of *Arundo donax* stem.(1-knobbed spiny phytoliths, 2-trapezoid,3-elogate spiny and with pavement, 4-saddle, 5-elongate smooth, 6-epidermal flat sheet phytoliths, 7- others)**

## DISCUSSION

It has been reported that the transpiration system is responsible for the heavy deposition of Si in plants [3,6]. Raven [22] also suggested that the highest silica deposition in plants is found in major transpiration parts of the plant. In this present study we have also found that the highest silica percentage in the leaf blades followed by the stem on the basis of counts of silicified cells in leaves and stem, (Figure-1).

Present study focuses on the diversity, distribution and frequency of phytoliths in the leaves and stem of *Arundo donax*. The leaf blade of this species produce a variety of phytoliths in which bilobates denote their utmost occurrence followed by the cross shaped phytoliths, trapezoid, bulliform cells, micro hairs, elongate sinuous, parallelepipedal cells, bilobate nodular shank, elongate spiny pavement and concave end, (Figure 3). In the stem, epidermal cells, fibers and hypodermal cells show silica accumulation. Some long cells of the ground tissue appear rod shaped with a knobbed spine (Figure 4, A, C) like process which interlocks with adjacent cells to provide mechanical support to the stem. Phytoliths having knobbed spines are the most common in stem, it is also a notable point that these cells appeared in the highest amount followed by the trapezoid, epidermal cells, rod shaped, saddles, sinuous walled cells and shield shaped hypodermal cells (Figure 5).

Over a century plant physiologist, taxonomists and archaeologists have been investigating silica deposition pattern in the various plant families and tried to know their immense role in the archaeological, palaeobotanical, taxonomical and physiological aspects [23,24,25]. From the last few decades a majority of archaeologist have also been using the living plant phytoliths to know the past plant people interactions as well as palaeoclimate and palaeoenvironment [26,27,28].

A number of crops produce very distinct types of phytolith which are very useful in the identification of these crops. Rosen [29] identified wheat and Barley on the basis of wave pattern of the long cell walls of the glumes, Pearsall et al.[30], Zhao et al [31] and Houyuan et al. [32] identified rice on the basis of bulliform phytoliths while maize can be identified on the basis of their cross [8]. In order to use phytoliths as a tool in taxonomic identification, environmental reconstruction as well as past plant- people relationship, it is very necessary to recognize morphological and quantification variations of phytoliths of different species of grasses. On the other hand certain grasses produce phytoliths which are not very characteristic and are produced by a number of grasses [7], thus various phytolith frequencies in addition to characteristic shape of any one or more phytolith of different organs of the plants can be a good tool for identification of grasses.

Present study provides some interesting results regarding *Arundo donax* such as highest frequency of bilobate phytoliths along with the concavity of bulliform phytoliths in leaf blade while knobbed spine phytoliths which is present only in the stem with their highest frequency can act as notable characters for the identification of *Arundo donax*. It is quite interesting that some phytoliths which are present in leaves and stem of *Arundo donax* have very much similarity in their shapes and sizes (Figure 2 &4, Table-1). These above parameters would be useful for the researchers seeking identification of *Arundo donax* phytoliths recovered from the soil samples of the Archaeological sites and is also helpful for the Taxonomists.

## CONCLUSION

Taxonomically considerable phytoliths are known to occur especially in the family Poaceae. This systematic study will be further enhancement of this knowledge and will also helpful to palaeobotanist and archaeologist in reconstructing the vegetation of plants. On the other hand it can also possible to use the given parameters to resolve taxonomic problems because taxonomists have been regularly using characters of silica bodies of grasses to identify them.

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