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BIOGENIC SILICA (PHYTOLITH) PRODUCTION PATTERN OF COTYLEDONARY, FIRST TWO AND MATURE LEAVES OF *HOLOPTELEA INTEGRIFOLIA* PLANCH., SEEDLINGS

DHARMENDRA KUMAR[1], DURGESH KUMAR TRIPATHI[2]. AND DEVENDRA KUMAR CHAUHAN[1]*

Department of Botany, University of Allahabad, Allahabad[1]
Center of Advance study, Department of Botany, Banaras Hindu University, Varanasi[1, 2]
Email-dharmendra.dhuriya@gmail.com, dktripathiau@gmail.com, dkchauhanau@yahoo.com*
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ABSTRACT

Holoptelea integrifolia Planch. is a large deciduous tree belonging to family Ulmaceae. Silica is absorbed in the form of monosilicic acid from the soil through their roots and become deposited in the cells of cotyledons, first two leaves and mature leaves. Silica is one of the most beneficial elements for all biological forms. It enhances the yield and quality of certain crops and help plants to overcome various biotic and abiotic stresses.

Present study demonstrates the silica deposition pattern in cotyledons, first two leaves and mature leaves of *H. integrifolia*. In all three leafy forms silica is mostly deposited in the hairs, hair bases, tracheids, epidermal cells, and corkwarts. Hairs and hair bases are of different shapes and sizes in cotyledons, first two leaves and mature leaves of seedlings. Silicified hairs are unicellular, multicellular or glandular having smooth walls or armed with papillae. The highest frequency of silica deposition is found in mature leaves followed by first two leaves and cotyledons. These silicified hairs protect the plants against herbivores and check evaporation. They also inhibit the growth of pathogens and protect the delicate tissues from solar radiation.

Keywords : *Cotyledon, Hair, Hair base, Holoptelea, Phytoliths, Seedling, Silica.*

Introduction

Silicon is known as the second richest element on lithosphere which played many vital roles in several plant species (Epstein, 2009). However, it has not yet been rewarded the status of essential element, in spite of its physical, chemical and structural role to plants (Epstein, 2009). Silicon enhances the plant growth, check the excess of evaporation, and protect the plants from many bacterial, fungal and viral diseases (Ishiguro, 2001; Meyer & Keeping. 2001). It also acts as a defensive barrier against various biotic and abiotic stresses (Lux et al., 2002; Ma & Yamaji, 2006; Ma et al., 2006; Ma & Yamaji, 2008).

Silica increases the productivity of the many crop plants like wheat, rice and sugarcane (Lanning, 1966; Korndorfer & Lepsch, 2001; Epstein, 2009). It also enhances the size of the vascular bundles and improves the potency of the stem of crop plants. It also reduces or stops the toxicity of Manganese, Cadmium, Zinc, Iron and Aluminium (Mitani & Ma, 2005; Ma & Yamaji, 2008; Cunha & Nascimento, 2009; Tripathi et al., 2012a). It has been well documented that silicon reduces the negative effect of radiation damage by increasing the accumulation rate of anthocyanins, Flavonoids and Phenolic compounds in epidermal cells of the plants which further provide the strength

of plants against the UV-B resistance (Wenbin et al., 2003; Shen et al., 2010). Silicon also makes the nutrient imbalance between zinc and phosphorus in number of plant species (Epstein, 1999; Zsoldos et al., 2000, 2004; Ma & Takahashi, 2002; Ma, 2004; Tripathi et al., 2012b).

Silicon is absorbed by the plants through their roots in the form of soluble silicic acid (Si (OH) 4) and translocated to the aerial parts of the plants through xylem (Epstein, 2009). Silica is deposited in the different parts of plant i.e. roots, stems, leaves, reproductive parts and takes the characteristic shape of the cells. Some specific plant cells in which silica is generally deposited are called as silica cells or phytoliths. These phytoliths are morphologically varied and found abundantly in plants and greatly preserved in the soil after the decay of plants for thousands of the years. These cells are generally characterized as silica short cells, cork cells, bulliform cells, mesophyll cells, fibers, sclereids, tracheids, and trichomes (Rovner, 1971, 1983; Ollendorf, 1987; Piperno, 1988; Alexandre et al., 1997). Phytolith analysis has been proved its usefulness to solve the archaeological, geological problems as well as in tracing the history of cultivation of crop plants due to the structural dignity and potential of stability of phytoliths in the soil (Metcalfe, 1960; Twiss, 1987; Piperno, 1988).

Various studies have been reported on the diversity, frequency and morphometrical distribution of phytolith in different parts of plants, different species of plants or even at genus level. However, no study has been yet reported to analyze or distinguish the phytolith production from seedling stage of plants to its mature stage. Therefore, this study is aimed to analyze the nature of phytolith or silica deposition in *Holoptelea integrifolia* from seedling stage to mature leaves.

Material and Methods

Sample collection

Seeds of *H. integrifolia* were collected from the Vindhyan region of central India and germinated in the laboratory. The cotyledonary leaves, first two leaves were collected from the seedlings of *H. integrifolia,* however; mature leaves were collected from the mature trees. 1.00 gm. dry weight of cotyledonary leaves, first two leaves and mature leaves were taken for silica determination.

Extraction of silicified cells

Phytolith extraction from the cotyledons, first two leaves and mature leaves of *H. integrifolia* was performed by using dry ash technique (Twiss et al., 1969). Small pieces of leaves of plants were individually hand washed several times in distilled water with dilute HCl to wipe out any dust particles. The leaves were then placed in a ceramic crucible and ashed for at least 6 hours at 4000 C, in a muffle furnace. The ash was treated with Schulze's solution to remove the organic material. We have used 0.001gm ash for the preparation of microscopic slides mounted in Canada balsam and studied five slides of each sample for data analysis. We have counted number of silicified cells in an observed area (1.24 mm2) of each slide.

Data Analysis

For the statistical representation of the collected data we have used Sigma plot 10.0 and Origin 8.1 software. Further, Mean and SD were analyzed for the frequency distribution of each phytolith types.

Results and Discussion

Silica concentration

Different parts of the plant used for biogenic silica determination viz. cotyledons, first two leaves and mature leaf are shown in Fig. 1(a). On the basis of quantification of silicified cells found in different stage of *H. integrifolia,* the highest silicification was found at mature leaves followed by the first two leaves and cotyledons (Fig. 1(b)) however, similar results related to the silica concentration were also found by simple silica extraction method (Table-1). This suggest that highest silica concentration is in the mature leaves because mature leaves evaporate the highest amount of water followed by the others (Tubb et al., 1993; Epstein, 1999; Ma, 2004). This fact has been well supported in the earlier studies (Tubb et al., 1993; Epstein, 1999; Raven, 2003; Ma, 2004) that the highest silica concentration is found in major transpiration parts of the plant.

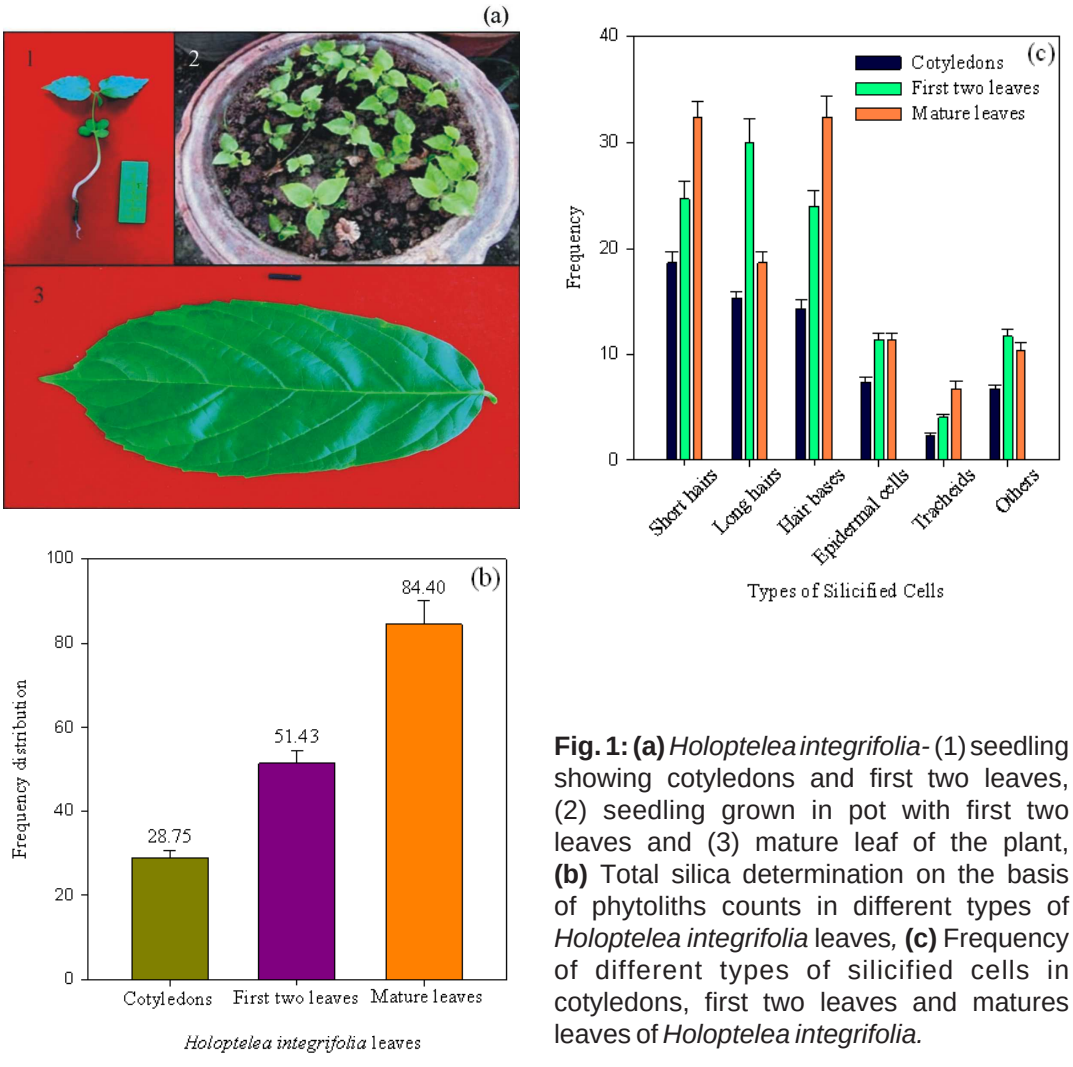

Fig. 1: (a) *Holoptelea integrifolia*- (1) seedling showing cotyledons and first two leaves, (2) seedling grown in pot with first two leaves and (3) mature leaf of the plant, **(b)** Total silica determination on the basis of phytoliths counts in different types of *Holoptelea integrifolia* leaves, **(c)** Frequency of different types of silicified cells in cotyledons, first two leaves and matures leaves of *Holoptelea integrifolia.*

TABLE 1– Approximate silica percentage from different leaves of *H. integrifolia.*

Leaves type	Dry weight (gm)	Crucible weight (gm)	Weight of crucible with sample (gm)	Weight of ash with crucible (gm)	Weight of Ash (gm)	Approximate silica %
Mature leaves	1.00	25.75	26.75	25.765	0.015	1.50
First two leaves	1.00	25.75	26.75	25.759	0.009	0.90
Cotyledons	1.00	25.75	26.75	25.755	0.005	0.50

Silicified cell analysis

Silica deposition in various cells was observed in cotyledons, first two leaves and mature leaves of *H. integrifolia*. In the mature leaves, it is deposited in the epidermal cells, trichomes, trichome bases etc. (Fig. 2). Further, trichomes are generally unicelled to multicelled; bulbous long hairs (Fig. 2a-2d), peg like hair bases (Fig. 2e), armed hair (Fig. 2f), multicellular hair (Fig. 2g) and bulbous base short hairs (Fig. 2h-2k), peltate disc of glandular trichomes (Fig. 2p, 2q) are found, these silicified hairs are about 50 to 300 ìm in size. The bulbous base of hairs show laminations in silicification (Fig. 2l, 2m), epidermal cells near the trichome bases are also silicified (Fig. 2r), foot cell (Fig. 2n) and thick silicified poral rim surrounded by epidermal cells (Fig. 2o). In the first two leaves of *H. integrifolia* pattern of silicification is similar to mature leaves. Silicification occurs in epidermal cells, trichome, trichome bases, tracheids and mesophyll cells while the frequency of trichome and trichome bases is less than that of the mature leaves (Fig. 1c). A number of short and long hairs with bulbous base cells, sheet phytoliths of epidermal cells with peltate disc of glandular hairs (Fig. 3k, 3l) were silicified and also found in first two leaves of *H. integrifolia.*

In addition silicification in cotyledons of *H. integrifolia* were also found similar to first two leaves however, the size and frequency of silicified cells were less than first two and mature leaves. Some of the silicified cells are bicellular hairs (Fig. 3a), bulbous hair (Fig. 3b, 3c), hooked hair (Fig. 3d), multicellular hair (Fig. 3e), hair base with epidermal cells (Fig. 3f), cylindrical sulcate tracheids (Fig. 3g), silicified tracheid from vein ending (Fig. 3h), flat sheet phytoliths of epidermal cells (Fig. 3i) and cork wart like structures (Fig. 3j).

Fig. 1(c) demonstrates the quantification of each type of phytoliths present in the leaves of each stage of plants and it shows that in mature leaves short hairs with bulbous base and hair bases depict their highest frequency followed by the long hairs, epidermal cell and tracheids. While, long hairs were more in first two leaves followed by the short hairs, hair bases, epidermal cells and tracheids. Cotyledons and mature leaves illustrate similar pattern of silica deposition.

Results of this study further suggested that the preference of silica deposition in plants varies according to their growth and development. The process of silica deposition starts from the cotyledons and culminates in the mature leaves of the plants. Various types of silicified cells were present in all stages of plant leaves.

It is a well established fact in the phytolith studies that grasses of the Poaceae family are one of the most abundant silica accumulating plants and produces very characteristic phytoliths which might be very much valuable in the taxonomical, palaeo-ecological as well as archaeological research (Piperno, 1988; Epstein, 2009; Tripathi et al., 2011). Tripathi et al., (2013) characterized the phytoliths of two *Sorghum* species on the basis of their characteristic shape, similarly Chauhan et al., (2011) also discriminate the phytoliths as well as the variation of silica concentration

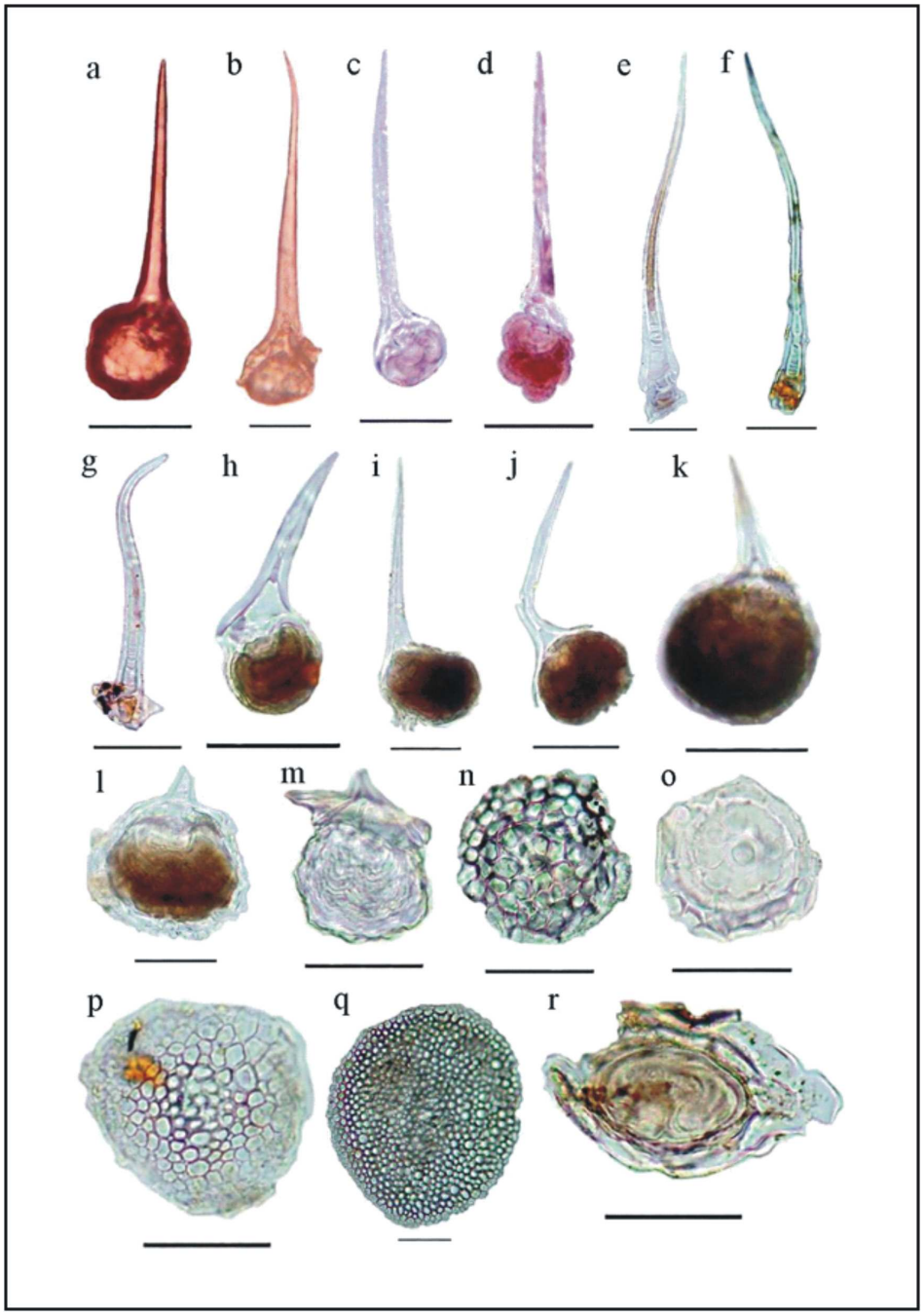

Fig. 2: Types of silicified cells of mature leaves i.e. Bulbous long hairs (a-d), peg like hair base (e), armed hair (f), multicellular hair (g), bulbous base short hairs (h-k), laminated silicification (l, m), foot cell (n), poral rim (o), peltate disc of glandular trichomes (p, q) and epidermal cells near trichome base (r) [Scale bar= 50 µm].

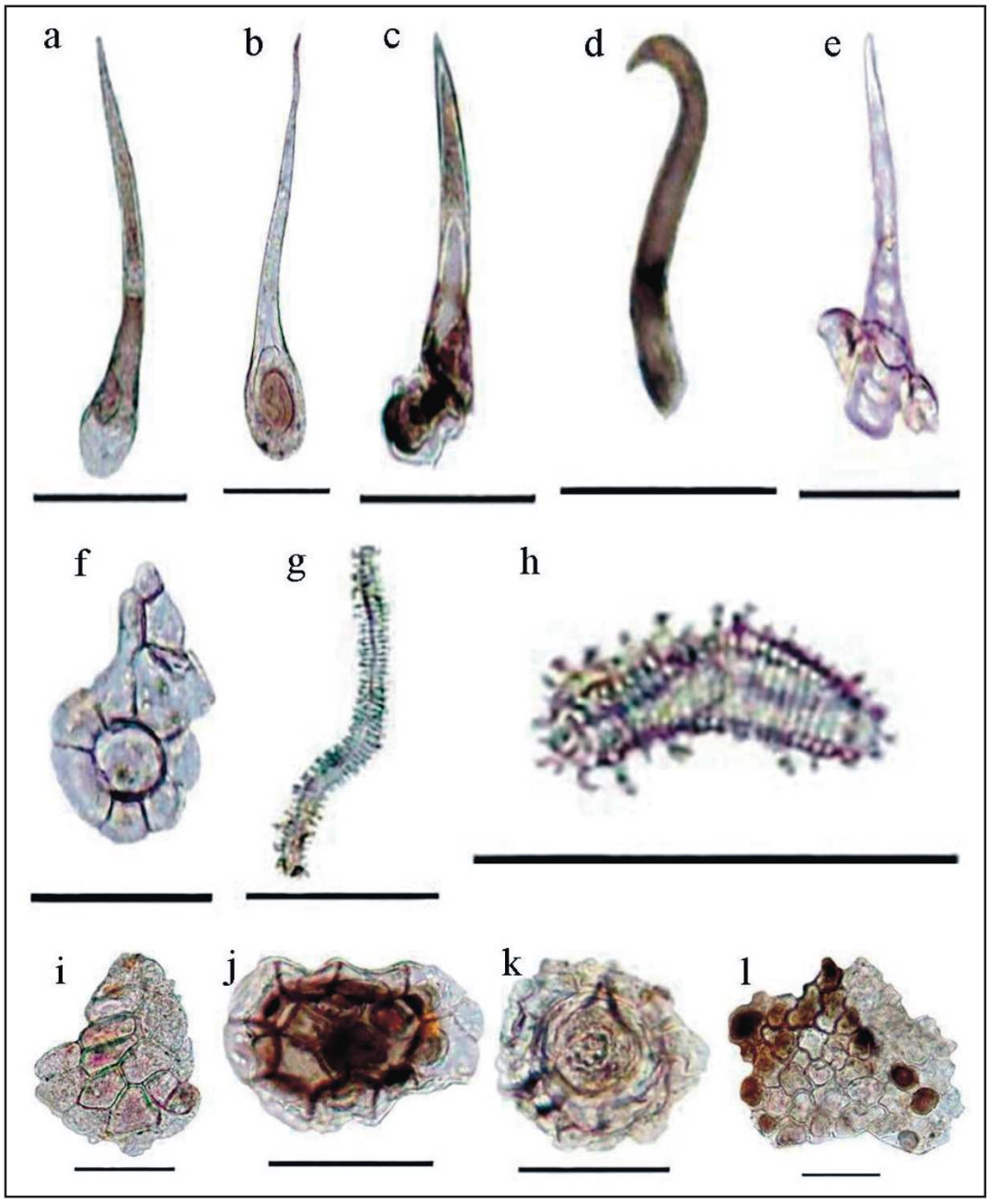

Fig. 3: Silicified cells of cotyledons (a-j) and first two leaves (k, l) i.e. bicellular hair (a), bulbus hair (b, c), hooked hair (d), multicellular hair (e), hair base with epidermal cell (f), cylindric sulcate tracheid (g), silicified tracheid from vein ending (h), flat sheet phytoliths of epidermal cell (i), cork wart (j), short hair with bulbous base (k), sheet phytolith of epidermal cells with peltate disc of glandular hairs (l) [Scale bar= 50 µm].

in different parts of *Cynodon dactylon.* Pearsall et al., (1995) and Zhao et al., (1998) also distinguished rice species on the basis of bulliform phytoliths. On the other hand only few studies have been made to study the silica or phytoliths deposition in plants of the family Ulmaceae (Kealhofer & Piperno, 1998; Wallis, 2001). The plants of the family Ulmaceae accumulate silica in very small amount and phytoliths produced in this family are not much characteristic (Kealhofer & Piperno, 1998; Wallis, 2001). Silica is generally accumulated in the various types of hairs and hair bases and these silicified hairs reflect solar radiation, protect the delicate tissues of leaves in hot, dry and open habitats and also protect the leaves from various forages, bacterial attacks as well as from the herbivores. In mature leaves silicified hairs act as a glass wool and create a physical barrier to insect feeding.

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