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Research Article

AN OVERVIEW OF BIOGENIC SILICA PRODUCTION PATTERN IN THE LEAVES OF *HORDEUM VULGARE* L.

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ABSTRACT
Hordeum vulgare of the family Poaceae is very significant food crop that absorbs biogenic silica in its different parts through their roots and deposited in various cells. The plant cells which were purely silicified after biogenic silica deposition are called as phytoliths. Similarly, present study describes the different type of phytoliths produced by the *Hordeum vulgare* leaf. The most common type of phytoliths found in the leaves of *Hordeum vulgare* are prickle hairs, hair cell phytolith, hair base, rod shaped, epidermal long cell with sinuous margins, sub epidermal long cells, trapezoid, long trapezoid, tracheids, stomata, mesophyll cells and flat epidermal sheet phytoliths. These phytoliths are varied morphologically, morphometrically and also at the level of frequency distributions. These phytoliths provide mechanical support, hardness and strength to plant and also of taxonomically significant.

Keywords: *Phytolith, Silica, Hordeum vulgare, Poaceae*

INTRODUCTION
Phytolith research is an important aid to various disciplines of science like in Plant taxonomy, Plant physiology, Plant molecular biology, Agriculture science, Palaeobotany and Archaeology (Wang and Lu, 1993; Piperno, 2006; Lu *et al.*, 2007; Madella *et al.*, 2009, Chauhan *et al.*, 2011; Tripathi *et al.*, 2011, 2012 a, b, 2013).

Phytoliths are basically plant silica stones made up of silicon dioxide. Among all elements silica is regarded as one of the most beneficial element for the plants that protects them from various biotic and abiotic stresses (Tripathi *et al.*, 2012 a, b, c, 2013, 2014; Ma and Yamaji, 2006). Plants accumulate silica in the form of monosilicic acid, $Si(OH)_4$ and deposited in and between various types of the plant cells (Tripathi *et al.*, 2011, 2012 a,b,c,d).

It has been observed that comparatively, phytoliths are highly abundant in leaves followed by the inflorescence, leaf sheaths and stem (Chauhan *et al.*, 2011a, b; Tripathi *et al.*, 2012 a, b). Phytolith reference collection of various families and genera is carried out all over the world, however phytolith analysis of Poaceae plants has a core attention aspect among the phytolith researchers while In India it is lacking.

Therefore, in the present study we have selected *Hordeum valgaure* L. belonging to Poaceae family to analyze its phytolith production pattern in general.

Hordeum is a genus consist about 30 species of annual and perennial grasses, inhabitant of the temperate area all over the world. *H. vulgare* species is principally used as food crops for human beings and as a fodder for animals while it also have a major commercial importance (Purseglove, 1974).

The formation and deposition of phytoliths in various cereals including *Hordeum* was investigated earlier by several workers which may include like Blackman (1968, 1969), Blackman and Parry (1968), Hayward and Parry (1973), Hodson and Sangster (1989), Hutton and Norrish (1974), Jones and Handreck (1965), Kaufman *et al.*, (1972), Soni and Parry (1973).

Although the phytoliths of *Hordeum vulgare* inflorescence were described by Ball *et al.,* (1999); Ball *et al.*, (2009); Honain *et al.*, (2006) but no detailed study of *Hordeum vulgare* leaf phytoliths has been taken yet. Therefore, we have described the phytolith types, measurements and frequency distribution of *Hordeum vulgare* leaf in detail.

MATERIALS AND METHODS

The samples of *Hordeum vulgare* were collected from the Roxburgh Botanical Garden, Department of Botany, University of Allahabad. Leaves of *Hordeum vulgare* were subjected to a carefully washing in distilled water to remove the dust particles from the surface of the samples and were dried at 60^0C for 48 hrs. After removing the moisture from the samples, they were positioned 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 emerged whitish in color. Consequently the ash was treated with nitric acid (HNO_3) and rest were rinsed with distilled water and centrifuged. The residual biogenic silica was dried and mounted in Canada balsam using 0.001 gm ash/ slide and ten slides were prepared. Difference in frequency distribution of silicified cells as well as their shape and size was observed in ten slides under the area of 1.24 mm^2/ slide (Chauhan et al., 2011; Tripathi et al., 2012; 2013).

RESULTS AND DISCUSSION

Studies suggested that different types of cells are present in the plant leaves in which silica is finally deposited likewise these silicified cells are called as phytolihs. Figure 1 depicts the variety of phytoliths present in the leaves of *Hordeum vulgare i.e.* prickle hairs (1-9), hair cell phytolith (10), hair base (11), rod shaped (12,13), epidermal long cell with sinuous margins (14-17), sub epidermal long cells (18), trapezoid (19), long trapezoid (20), tracheids (21), stomata (22) mesophyll cells and flat epidermal sheet. These phytoliths are taxonomically very important in identifying plants at genus or at family level, beside this, silicified structures also play an important role to protect the plants in various ways, such as they affected the optical properties of the leaves after interaction with sun light and also improve the strength of plants (Goto *et al.,* 2009; Klancnik *et al.,* 2014). Klancnik *et al.,* (2014) suggested the considerable roles of the various silicified leaf structures found at near-surface area such as prickle hairs, cuticle and epidermis on the basis of redundancy investigations. Therefore, it may assume that phytolihs present in the leaves of *Hordeum vulgare* perform various roles to protect the plants in adverse environmental conditions. Another role of silicified cells in carbon sequestration is a new area of research which interestingly carrying out by phytolith researchers in all over the world. Carbon entrapped by the silicified cells is extremely resistant and decomposed in the soil for several thousands of years after plant decay which clearly reveal the significance of silicified cells (phytoliths) in the continuing biogeochemical sequestration of atmospheric CO_2 (Song *et al.,* 2013).

Stomata are very important internal machinery of plants by which plants performs various metabolic and physiological responsibilities to protect the plants as well as regulate the essential phenomena. It has also been reported that various silicified cells along with stomata may be a good indicator to detect the level of CO_2 in the paleo-atmosphere (Parr and Sullivan 2011; Song *et al.,* 2013). Similarly figure 1 of this study shows silicified stomata found in the leaves of *Hordeum vulgare* thus phytolith analysis of this study may also be helpful to detect the level of CO_2 in the paleo-atmosphere, if *Hordeum vulgare* leaf phytolihs were suspected in the soil samples of archaeological sites.

It has been recommended after the morphological observations of phytolihs that, their morphometry have also very important contributions in phytolith research because various studies revealed that morphology of different types of phytolihs in different plant species or genera were more or less similar however, size of these phytolihs may varied. Thus morphometrical study of each phytolith type may helps to discriminate the plants at different level (Tripathi *et al.,* 2011, 2012a, b; Madella *et al.,* 2009; Ball *et al.,* 1999; Ball *et al.,* 2009; Honain *et al.,* 2006). In this study we have also measured the size of each phytolith which are different among each other due to differences in their particular shapes (Table1). Measurements of phytoliths shows that long epidermal phytoliths are highest in length followed by the hair cell phytoliths, sub epidermal or rod-shaped, large-prickle phytoliths, stomata, long trapezoids, short epidermal phytoliths, small prickle phytoliths and short trapezoids. Beside stomata of the *Hordeum vulgare* leaf shows highest in width followed by the large-prickle phytoliths, small prickle phytoliths, long epidermal phytoliths, sub epidermal or rod-shaped, short epidermal phytoliths, hair cell phytoliths, long trapezoids and short trapezoids. These observations clearly depict the variations among the phytoliths of *Hordeum vulgare* that is because of variation in the original size of plant cells.

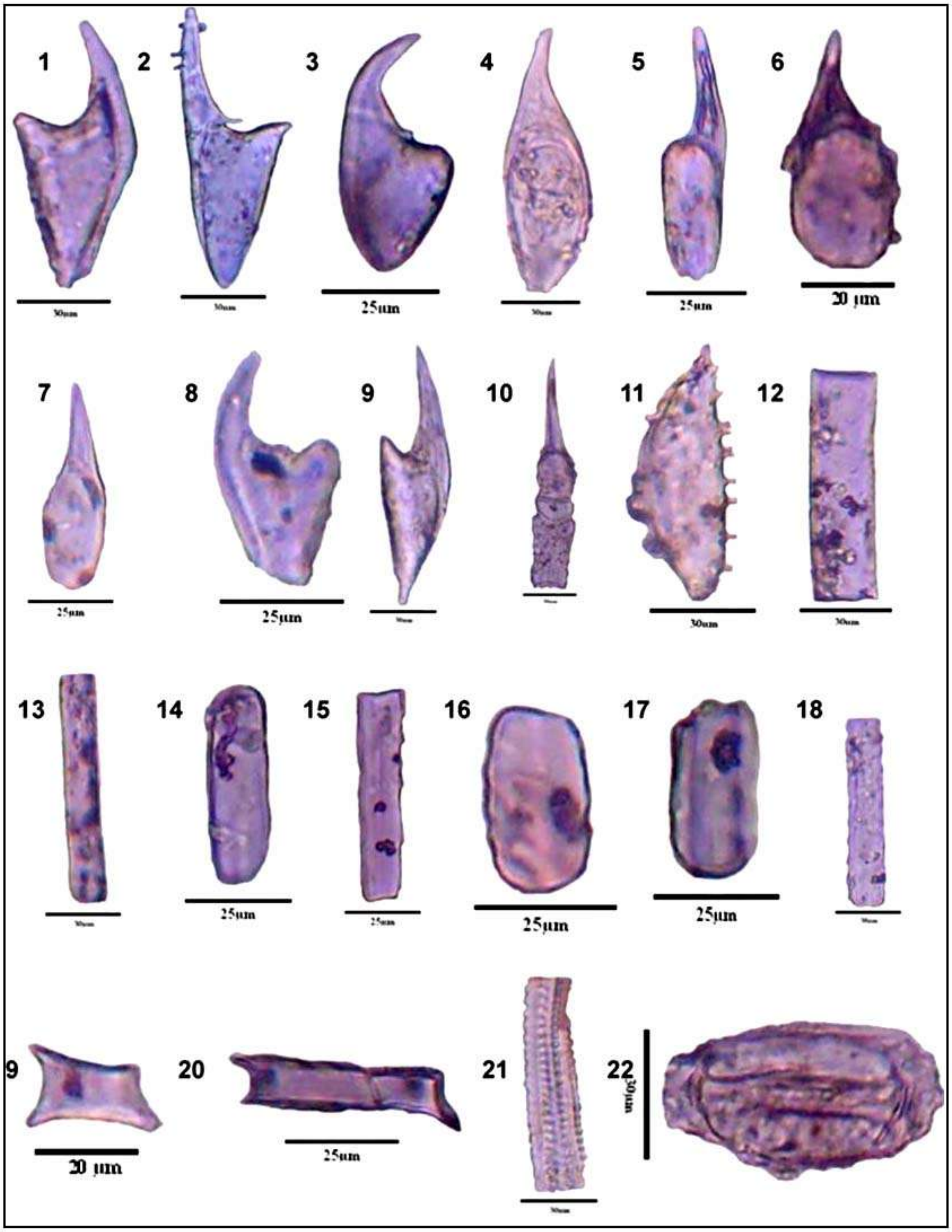

Figure 1: Different type of leaf phytoliths of *Hordeum vulgare:* prickle hairs (1-10), hair cell phytolith (10), hair base (11), rod shaped (12, 13), epidermal long cell with sinuous margins (14-17), sub epidermal long cells (18), trapezoid (19), long trapezoid (20), tracheids (21), stomata (22).

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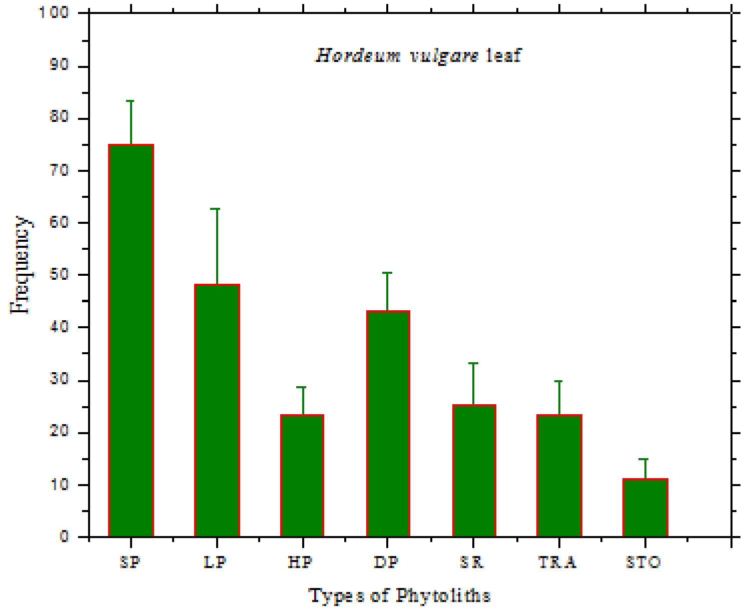

Figure 2: Frequency of different types of phytoliths of *Hordeum vulgare* leaves: small prickle phytoliths (SP), large-prickle phytoliths (LP), hair cell phytoliths (HP), long epidermal phytoliths with sinuous margins (DP), short epidermal phytoliths (SR), trapezoids (TRA), stomata (STO).

Table 1: Measurements of different phytoliths of *Hordeum vulgare* leaves

Phytolith Types	Length(μm)	Width(μm)
Small prickle phytoliths	35.6± 11.43	16.87± 2.39
Large-prickle phytoliths,	75.5± 15.2	19.37±4.26
Hair cell phytoliths,	118.75±19.4	10±3.53
Long epidermal Phytoliths	200±25	17.5±2.5
Short epidermal Phytoliths	44.16± 6.29	13.12±4.2
Sub epidermal or rod-shaped	82.5± 13.9	13.3±3.81
Long trapezoids	48.33±10.10	10±2.5
short trapezoids	16.25±3.2	7.5±2.5
Stomata	50.83±6.29	24.3±6.02

Furthermore, in recent years of phytolihs research, some issues of phytolih quantification i.e. phytolihs counts are important or not for discrimination of plants and its species are gaining much interest between the phytolihs researcher and also have a matters of interesting debates (Strömberg, 2009a, b; Alexandre and Bremond, 2009). In this study quantification of *Hordeum vulgare* phytoliths were also performed which deals highest frequency of short prickle phytoliths followed by the long prickle, hair cell, epidermal long cell phytolith with sinuous margins, short epidermal rod shaped, trapezoid and stomata (figure 2). Chauhan *et al.*, (2011) and Tripathi *et al.*, (2012) successfully reported the variation of frequency distribution in each type of phytoliths in *Arndo donax* and two species of *Sorghum*.
Previous studies of *Hordeum vulgare* were mainly focused on their inflorescence phytolith which did not address combined issues like morphology, morphometry and frequency of phytoliths and also does not

discussed the leaf phytolith production pattern (Blackman, 1969; Hutton and Norris, 1974; Hayward and Parry, 1973; Bennett, 1982; Hodson and Sangster, 1988, 1989; Ball *et al.*, 1993). This study documented the phytolith production pattern of *Hordeum vulgare* leaf along with their morphology, morphometry and frequency distribution. Further this study may be helpful to resolve some physiological, archaeobotanical and taxonomical issues, however detailed study of other parts of *Hordeum vulgare* as well as comparison among the *Hordeum* species are needed.

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