Silica Biominerals (Phytolith) Compound of Cultured Barley Plants  (*Hordeum Vulgare* L.)

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Abstract: The comparative analysis results of the morphological and chemical composition of biomineral elements (phytolith) of cultured barley (*Hordeum vulgare* L.) have been presented for the first time. On the example of the biserial “Pacific” variety related to var. nutans and line 342 (var. nigricans). The optical microscopy method has been used to identify 4 morphotypes of fossil plants. The distinctive sitespecific signs were established, manifested in the size multiblade fossil plants and the presence of a disc-shaped fossil plants from one variety not only in the ear, but in the stalk.

Keywords: barley, phytolith, biominerals

1. INTRODUCTION

One of the most important interdisciplinary problems in biology is the relationship of mineral (nonliving) and organic (living) world. Today to research the biological role of evolutionary origin of the minerals, the various taxa of plants and animals from diatoms to humans are being explored.

Barley is one of the oldest cereal plants cultivated in all agricultural areas of the world. A huge range and the Millennium cultivation of barley has determined a large inter-and intra-species diversity of the genus *Hordeum* L.

It is believed that to understand the evolution processes it is necessary to study biomineralization in plants, since the volume of the metabolism of mineral-forming elements (silicon, calcium, barium, strontium) is comparable to biogeochemical cycles prokaryotes.

Such studies are important not only from the point of view of fundamental knowledge about the role of fossil plants and of the biological cycle of different forms of silicon. The practical interest is determined mostly by the possibility of creating biomimetic and other materials with desired properties using biotechnology [1].

Previously it has been shown that silicon formation is found in all taxa of plants, ranging from red algae and lichens, the representatives of which (e.g., Chondrus crispus) have proteins similar to proteins of partially biocritical sponges, diatoms and higher plants [2, 3]. Speaking about the distant taxa, the differences in phytolith compound are obvious.

The hypothesis about the existence of intervarietal differences within the same species such as barley culture has been confirmed with the help of comparative methods of study of
phytolith profile of different varieties of crops plants in the present work. (Hordeum vulgare L.)

2. MATERIALS AND METHODS

2.1 The object of research and sample plant selection
As the object for the experiment a two-rowed spring barley of “Pacific” variety has been chosen related to the variety var. nutans (yellow ears) and two-rowed breeding line 342 - var. nigricans (black ears). It has been obtained by intervarietal hybridization with followed individual selection in the laboratory breeding of grain and cereal crops in Primorsky agriculture research Institute (Temiryazevsky, Ussuri Region, Primorsk Territory). For the study the barley fossil plants, specimens varieties of the Pacific and line 342 in the full ripeness phase have been collected grown in a breeding nursery in 2014. The phytolith preparations have been obtained according to the modified method [4]. The samples of stems and ears (20 g) have been washed with the distilled water, then burned in enameled ceramic crucible, covered with a lid in a muffle furnace in ambient atmosphere for 4 hours at 450 °C.

The ashes have been placed in a plastic test tube and kept successively in 10% HCl solution and concentrated HNO₃ (JSC "Neva-Reaktiv", Russia) 20 min., periodically shaking lightly test tube. It has been washed many times using distilled water, and after that the last wash water has been poured out (0,5ml remained in test tube). Ready-made preparations were used for microscopic research.

2.2. Microscopic examination
The samples were studied on a light microscope Axio Imager 2 (Zeiss, Oberkochen, Germany) at magnification of 100 to 630 times. Glass slides and cover glass were used (JSC BioVitrum, Sankt-Peterburg, Russian Federation). Using the program Axio Vision 4.2 (Zeiss, Oberkochen, Germany) the length and width of the visible image of each particle were measured. The narrowest sections of the formless parts were considered. The measurements were carried out according to the two-dimensional images, the real sizes objects may differ.

2.3. Raman-spectroscopy
Spectra have been obtained on a Raman spectrometer RNX1 (Kaiser Optical Systems, USA) combined with an optical microscope Morphologi G3-ID (Malvern, United Kindom). The preparations have been placed on a plastic slide. Laser excitation has been used at 50 × magnification at 785 nm wavelength, laser exposure is 1 second, the number of savings is 50. The laser has been switched on in the high-power mode, corresponding to 16 mV in this device configuration [5].

3. RESULTS

Four morphotypes were discovered in preparations of barley fossil plants: elongated multi-blade (two subtypes), needle-shaped, disc-shaped, conical (Fig. 1, 2, table 1). Other phytoliths were formless (smooth or rough) with rounded and/or sharp edges. The discovered morphotypes of fossil plants coincide with previously studied [6-8]. Using light microscopy phytoliths were mostly colorless and with varying degrees of transparency. The significant number of shapeless fossil plants stems was of reddish color.
Fig. 1. The phytolith preparation – typical view of ear (A) and stem (B) barley of “Pacific” variety in optical microscope.
Fig. 2. The phytolith preparation – typical view of ear (A) and stem (B) barley Line 342 variety in optical microscope.

Table 1

| Phytolith Morphotype | “Pacific” | Line 342 |
|----------------------|-----------|----------|
| Multiblade with mild blades (ear) | ![Image](multiblade_mild_blades.jpg) | ![Image](multiblade_prominent_lobes.jpg) |
| Multiblade with prominent pointed lobes (ear) | ![Image](multiblade_prominent_lobes.jpg) | ![Image](multiblade_prominent_lobes.jpg) |
**Multilobe phytolith.** Barley ears are characterized by straight, curved and curved multiblade phytoliths. The blades are mainly pointed protrusions, rarely with rounded or shapeless ridges. They can be placed symmetrically or in a random order. Barley samples were detected interconnected in large aggregates (Fig. 3).

![Image](image_url)

**Fig. 3.** Barley ears examples of multiblade units fossil plants in line 342(A) and “Pacific” variety (B)
The multiblade phytoliths in stems were not identified in medications. Multiblade phytoliths in ears were divided in two subtypes: with mild blades and large, pointed lobes (see table 1). Single opaque inclusions in fossil plants were observed.

**Disk-shaped phytolith.** Disc-shaped phytoliths were discovered in the ears of all varieties, and the stem of only one. They have the form of flattened, slightly elongated disk. The edges of the disks were smooth or ribbed. Some of these phytoliths had opaque inclusions of unknown chemical compound. And some phytoliths ears had flattened edging up to 6-7 µm in width around the edge of the disk. Disc-shaped phytoliths were located between the multiblade phytolith ears (see Fig. 3) or near the multiblade phytolith , and in the stems of preparations – one by one.

**Needle phytolithes.** The largest number and diversity of needle fossil plants was discovered in the ears. The needle phytoliths were not found in the barley stems. Typical needle phytolith has a thickened base, resembling a nail head or mace, and a long body merging into a pointed top. These phytoliths can often be straight, curved, and very rarely less sharply curved in the middle or at the base.

**Strobilaceous phytolithes.** Strobilaceous phytoliths have been detected only in the barley ears. They represent truncated cones of irregular shape with single inclusions.

Fig. 4. Barley ears examples of multiblade units fossil plants of “Pacific” variety (A-E) and line 342 (F-J).

The average size of all studied fossil plants morphotypes can be compared (table 2). However, it should be noted that the line 342 multiblade phytolith are longer on average by 14-18%.

| phytolith morphotype | Pacific (µm) | Line 342 (µm) |
|----------------------|--------------|---------------|
|                      | W            | L             | W              | L             | W              | L             |
| Multiblade           | 16,76±5,53   | 16,06±4,50    | 56,79±29,02    | 65,09±43,04   |
| Disk-shaped (ear)    | 11,24±1,43   | 11,09±3,33    | 15,64±2,4      | 15,40±5,3     |
| Disk-shaped (culm)   | 10,70±3,08   | 10,78±3,18    | 13,18±3,49     | 13,28±3,49    |
| Strobilaceous        | 13,20±3,42   | 13,01±3,44    |                |               |
It was established with Raman spectroscopy method that phytolith consists of silicon dioxide (Fig. 5). The spectra show bands approximately 476 cm\(^{-1}\) and 1052 cm\(^{-1}\) of amorphous silica and the band around 514 cm\(^{-1}\) of microcrystalline silica. There were traces of inorganic sulfate and organic matter surrounded by some of the particles. The differences in the chemical composition were not established with this method.

|       | L       | 15,45±3,30   | 15,52±3,37   |
|-------|---------|--------------|--------------|
| Needle| W       | 12,64±3,60   | 13,04±3,65   |
|       | L       | 320±300,06   | 330±298,16   |

Note: W – width, D – length, «-» – non detected

4. Conclusion

The intraspecific differences of barley fossil plants were revealed between cultured varieties var. nutans and var. nigricans, which can be determined by different morphological characteristics and biochemical adaptation abilities of plants. This fact brings into question the verification level phytolith analysis when only specific, generic and other taxonomic levels are considered. This point of view requires much deeper evidence. The hypothesis about the existence of intervarietal differences in barley phytolith composition has been confirmed.

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