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LS-8-P-2463 Visualization of silica phytoliths in Sorghum bicolor (L.) Moench. by fluorescence microscopy

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Silica phytoliths are microscopic biominerals of amorphous silica ($SiO_2 \bullet nH_2O$) formed by specialised plant cells. Their morphology is very variable among the species and they are often used as a taxonomic marker. They serve as a protection against herbivores and pathogens and support mechanical properties of plant tissues. Their structural features offer many possibilities of application also in industry and technology [1, 2]. However, small dimensions and optical properties of phytoliths obstruct their microscopic observations. Recent imaging techniques require lengthy preparation of samples, phytolith isolation, or a work with toxic chemicals. Tracking the changes in biomineralization process caused by various exogenous or endogenous factors thus might be complicated. Our study was focused on elaboration of a novel phytolith visualization technique by fluorescence microscopy that could facilitate further silica biomineralization studies.
Experiments were performed with *Sorghum bicolor* L. plants characteristic by regularly distributed silica phytoliths in root endodermal cells. Plants were grown for three days in hydroponics containing distilled water and sodium silicate solution in final concentration of 2.5 mol.dm^{-3}. Samples were prepared from seminal root basal regions 20 – 30 mm from the root-shoot junction. Rhizodermis and cortical tissues were mechanically removed and samples were placed on slides into a mounting solution of NaOH with pH adjusted to 12. Observations were performed by fluorescent microscope Axioskop 2 plus (Carl Zeiss) with excitation filter TBP 400 + 495 + 570 nm, dichroic beamsplitter TFT 410 + 505 + 585 nm and emission filter TBP 460 + 530 + 610 nm. Spectrofluorimetric properties were analysed by spectrofluorimeter FSP 920 (Edinburgh Instruments).
Phytoliths in *Sorghum* roots are formed as dome-shaped structures of amorphous silica nested in inner tangential walls of endodermal cells. Their diameter and axial dimensions ranged 8.51 ± 2.49 µm and 7.08 ± 1.17 µm, respectively. Number of phytoliths counted from 7 to 14 per cell. Silica phytoliths emitted blue fluorescent signal, which was clearly distinguishable from cell wall background and was time-stable for more than 10 minutes. Despite this fact, the fluorescence emission spectrum induced by 400 nm excitation wavelength did not show any remarkable peaks and reaches maximum at 475 nm.
Fluorescent visualization of silica phytoliths induced by basic pH provided suitable features for their localisation and morphological studies. Preparation of samples was quick and simple, and might be applicable for high throughput screenings.

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