LS.6.P153
Application of Rhodamine B as a potential marker for silicon in cell walls of higher plants

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Keywords: phytoliths, Rhodamine B, root, silica bodies, silicon, *Sorghum bicolor* L.

Silicon is the second most abundant element in earth's crust. Even though it is not considered as an essential element in plant nutrition it is actively taken up by many plant species [1]. Within the plant organism, silicon is mainly deposited into endodermal and epidermal cell walls, and cuticle layer [2, 3]. Besides adjusting cell wall mechanical properties silicon provides also many other benefits and increases plant stress resistance [4].

Grasses are often capable to precipitate silicon into amorphous hydrated silica bodies, also called phytoliths. These microscopic structures are morphologically specific among the species [3, 5]. Their localisation and identification within intact plant tissues and cells is quite difficult because of their transparency and small sizes. In our work we tested application of Rhodamine B, a fluorescent dye used also for diatom silica thecae staining [6], as a possibility for visualisation of silica bodies and silicon cell wall deposits in plant organism by fluorescence microscopy.

Experiments were focused on endodermal silica bodies in *Sorghum bicolor* L. roots because of their known localisation and rapid development [7]. Plants in control variant were cultivated for three days in hydroponics containing distilled water with addition of Na_2SiO_3 as a source of silicon. The RhB variant consisted of control medium enriched with Rhodamine B. Transversal sections of primary seminal roots were prepared from the zone 2 – 3 cm from the root base. Prepared sections were additionally stained with toluidine blue in ethanol (TB) to suppress the fluorescence of lignin. Observations were performed with a fluorescent microscope Axioskop 2 plus (Carl Zeiss) with excitation filter TBP 400 + 495 + 570, dichroic beamsplitter TFT 410 + 505 + 585 and emission filter TBP 460 + 530 + 610 (wavelengths are listed in nm).

Unstained control variant root sections did not show any specific signal and emitted blue-green fluorescence of cell walls, presumably caused by a presence of ferulic acid [8]. Centrifugal parts of xylem cell walls and inner tangential endodermal cell walls displayed enhanced blue fluorescent signal, probably as a consequence of lignin deposition [8, 9]. Endodermal silica bodies also possessed weak blue signal but it might represent a reflection of the endodermal cell wall fluorescence. After additional TB staining the blue-green fluorescence of cell walls was suppressed and no relevant signal was received. In RhB variant without additional TB staining the cell walls emitted strong red fluorescence lacking any specificity within the root anatomical structures. Additional staining with TB suppressed the non-specific red fluorescence after Rhodamine B treatment. The inner tangential endodermal cell walls remained the only structures displaying red fluorescent signal. Anyhow, the endodermal silica bodies did not show any positive signal. According to X-ray analysis of *Sorghum* root transversal sections, the only silicon accumulation occurred in endodermal cells [10]. The deposition of silicon into the inner tangential walls of endodermis is reported also in many other species [2, 3, 4, 11]. On the other hand, the presence of silicon within the silica body is obvious and thus proposing a question whether the mechanism of silicon cell wall deposition and silica body formation is the same.

Application of Rhodamine B with additional toluidine blue staining is a potential method for fluorescence visualisation of silicon deposits within the plant cell walls. Anyhow, the adjustment of this method needs further investigations and testing. On the other hand, staining of silica bodies in *Sorghum* root endodermis by Rhodamine B did not give any satisfying result and thus requires the development and application of another technique.

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12. This study was supported by grants 1/0817/12 by Slovak grant agency VEGA and by Slovak Research and Development Agency under the contract No. APVV-0140-10.