Zinc tolerance of *Physcomitrella patens* evaluated by X-ray microanalysis

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Physcomitrella patens is a non-vascular, multicellular land plant and belongs to the bryophytes. Due to the simple morphology of the moss and its facile *in vitro* cultivation, *P. patens* has become a model organism in plant and molecular biology [1]. Most mosses are specialists that inhabit ecological niches; some even live on heavy metal enriched substrates (e.g. *Pohlia drummondii*, *Mielichhoferia elongata*, [2]). We wondered if this potential is unique to certain moss species and tested *P. patens* that normally occurs on uncontaminated soil for its Zn-sensitivity. Zn^{2+} is an essential cation for eukaryotic cells with harmful effects if applied at high doses.

P. patens was grown on agar plates enriched with either zinc-EDTA (0.1 mM, 1 mM, 10 mM, 100 mM) or zinc-chloride (0.1 mM, 1 mM, 5 mM). Higher concentrations of Zn^{2+} showed to be lethal.

Semiquantitative analysis of heavy metal uptake was performed with X-ray microanalysis (EDX) on a scanning electron microscope (SEM). Moss samples were air dried, mounted and carbon-coated. During sample preparation, great attention was paid to avoid contamination with the Zn-containing growth medium. Figure 1a shows a typical EDX-spectrum with a distinct Zn-peak (arrow). At least seven measurements per plant were taken (Figure 1b) and the results are shown in Figure 1c. Student`s T-test was applied to indicate significant value differences. The uptake correlated with the amount of Zn offered in a gradient manner. $ZnCl_2$ is more harmful to *P. patens* than Zn-EDTA: on $ZnCl_2$-plates, the plants survived up to a concentration of 5 mM whereas the highest Zn-level for the EDTA-plates was shown in the probes containing 100 mM Zn-EDTA. Low amounts of $ZnCl_2$ accumulated stronger within the plants than low amounts of Zn-EDTA.

On the cellular level, we tested heavy metal resistance by exposure to gradient Zn-solutions. After 48 h, plasmolysis of the cells in 0.8 M mannitol reflected their viability and was determined in the light microscope. Cells of *P. patens* gametophytes showed a high tolerance to zinc up to concentrations of 100 mM Zn-EDTA (Figure 2).

In summary, the hypothesis that *P. patens* is sensitive to heavy metals could not be proven. By contrast, we observed an interestingly high tolerance to both, $ZnCl_2$ and Zn-EDTA. Further experiments are underway to specifically locate Zn storages within the cells and to show the impact of other heavy metals such as copper and cadmium.

1. D. Lang et al., Trends Plant Sci. **13** (2008) p542.
2. H. Stummerer, Österr. Bot. Z. **118** (1970) p189.
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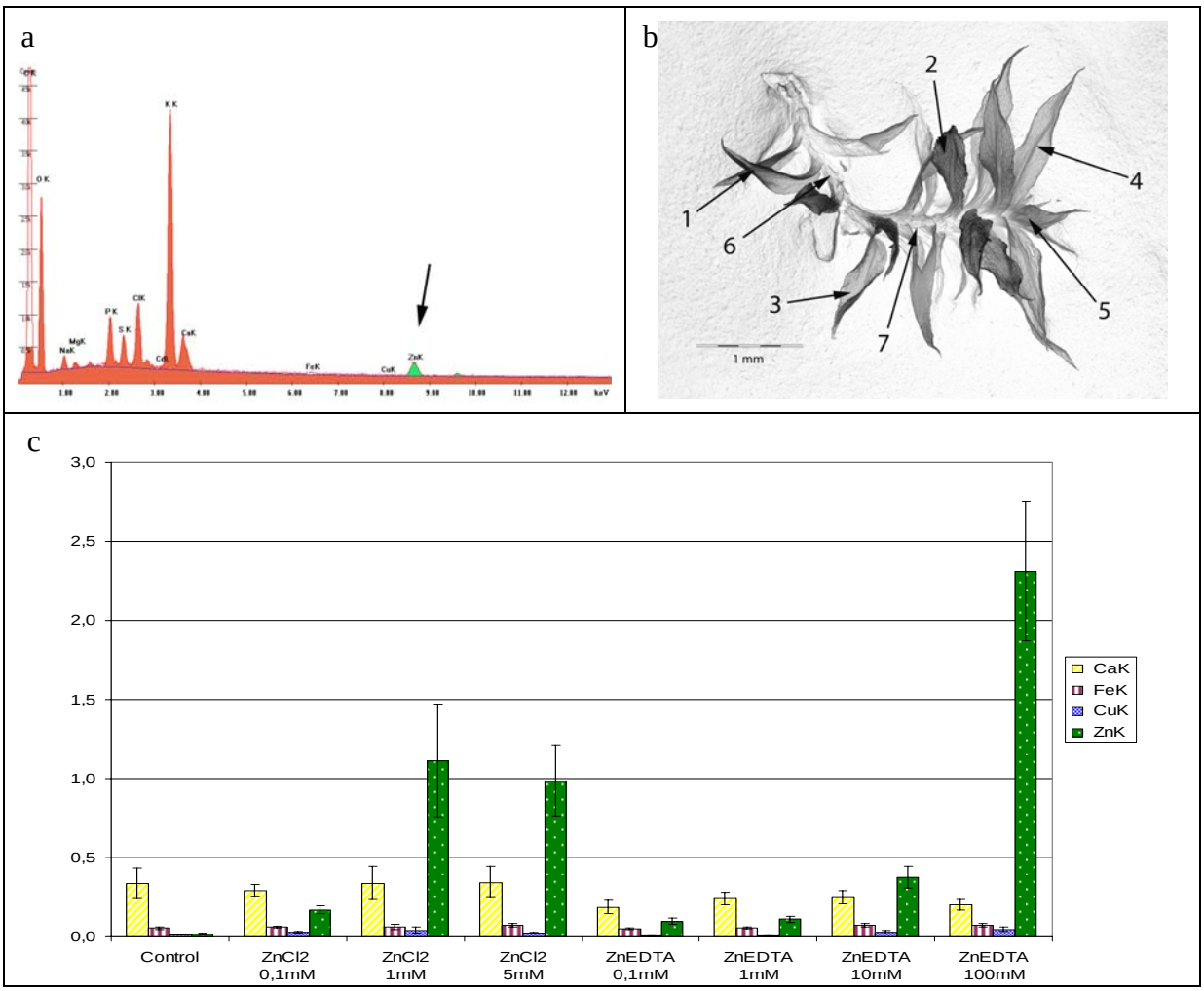

Figure 1. X-ray microanalysis of *P. patens* grown on gradient $ZnCl_2$ and Zn-EDTA plates. **a** Typical EDX-spectrum showing a distinct Zn peak (arrow). **b** SEM picture of gametophyte; numbers indicate EDX measurements per plant. **c** Gradient accumulation of Zn in the plants from both, $ZnCl_2$ and Zn-EDTA plates; leveling of $ZnCl_2$ occurs at 1 to 5 mM. Uptake of Zn-EDTA is less at low concentrations and EDX shows highest zinc amounts in plants from 100 mM Zn-EDTA plates.

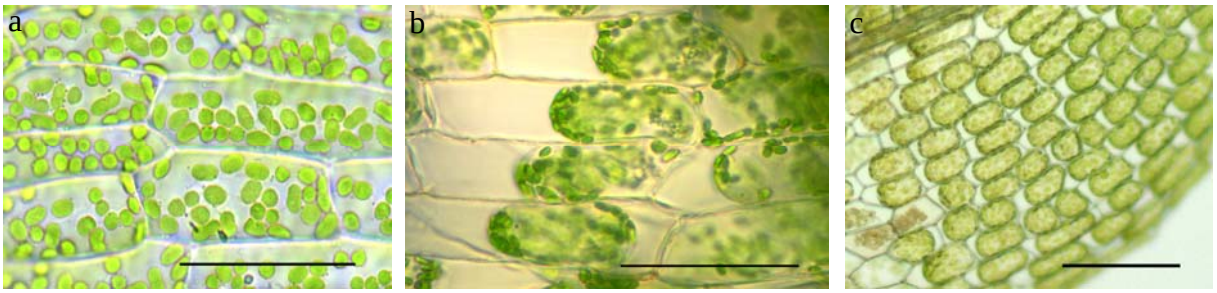

Figure 2. Resistance experiments of *P. patens* gametophyte: cells are exposed to gradient Zn-EDTA concentrations for 48 h; subsequent plasmolysis in 0.8 M mannitol reflects the viability of cells. **a** Control cells in water. **b** Control: plasmolysed cells. **c** 100 mM Zn-EDTA: the majority of cells is alive after 48 h and plasmolyses. Bar: **a, b**: 500 µm, **c**: 1000 µm.

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