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Zinc and copper uptake in *Physcomitrella patens*: Limitations and effects on growth and morphology

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ABSTRACT

Bryophytes are well-studied model systems for the investigation of uptake mechanisms and pollutant tolerance of plants. The moss *Physcomitrella patens* is among the most intensively studied organisms with regard to physiological and cellular pathways; however, direct ecological studies on metal stress are rare in this species. We conducted growth experiments on copper and zinc containing media for five and ten weeks, respectively in order to investigate the element and anion dependent metal uptake by energy—dispersive X-ray spectroscopy on a scanning electron microscope. We compared the uptake of metals to the growth and morphology of leaves and protonemata of *P. patens*. The metal content of both tissues was strongly correlated with availability of free metal cations. Differences between copper and zinc uptake included the limitation of zinc uptake found after five weeks for both tissues. No further sequestration of metal occurred after week five. The content of up to 1.3 wt% zinc and 0.4 wt% copper in the dry biomass correlated negatively to leafy gametophyte growth and to cell length in both tissues. No such correlation to protonema growth or cell width was found. The shortening of protonema cells exposed to zinc did not necessarily lead to decreased protonemal growth. This indicates a compensation of the shorter cells by increased cell division of the protonema filaments.

1. Introduction

Research on the impact of metals and bryophytes has come a long way together. Metal contaminated habitats have always been colonized by certain moss and liverwort species regardless of whether naturally occurring or man-made (Adlassnig et al., 2013; Baumbach and Schubert, 2008). Many studies investigated the toxicity of metals and the tolerance of plants but differences in tolerance are always dependent on plant strategy (Baker, 1981), species and metal. In bryophytes, Tyler (1990) reported decreasing toxicity for Hg > Cu > Cd > Pb > Zn, regarding zinc as the least harmful of the investigated metals.

Unlike flowering plants, mosses use rhizoids for surface adhesion and do not possess a root system for nutrient uptake or metal avoidance. Therefore, the avoidance via e.g., the casparian strip is impossible. In fact, mosses take up nutrients by surface adsorption—hence also toxic metals. This characteristic led to their widespread use as bioindicators and in biomonitoring of aerial metal deposition (Frahm, 1998; Spagnuolo et al., 2011; Uyar et al., 2009). However, suitable candidates for bioindication or biomonitoring need additional requirements. To enable estimates on environmental metal concentrations, the organism has to show a linear correlation in uptake and conservation of the level of metal-uptake. Therefore, the organism should not actively accumulate, avoid or segregate them.

To further complicate the matter, uptake of metals depends both on the plant and metal species, the availability of the metal in the substrate, and it further may be limited by a physiological maximum within the plant. Such an upper limit would lead to an underestimation of high metal concentrations in the environment (Boquete et al., 2011). Therefore, experiments under controlled conditions are necessary to investigate metal uptake behavior of a single species. In this study, we analyzed the metal uptake and its effects on cell morphology in the protonemata and leaves of the well-established model moss *Physcomitrella patens* (Cove and Knight, 1993). As *P. patens* naturally inhabits non-metal sites, knowledge about metal uptake in *P. patens* is limited. However, in a previous study on metal tolerance and its influence on growth, *P.*

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patens showed a remarkable tolerance toward zinc and copper. A shift to increased protonema formation could be observed suggesting that these cells could superiorly handle the metal induced stress (Sassmann et al., 2015). Growth reduction and increased protonema formation were best explained by free metal cation concentrations but questions remained concerning metal uptake and changes in metal content of this important model organism. Here, we aim to investigate the following hypotheses (H1 – H4):

- There is a limited uptake capacity, resulting in a saturation curve, regardless of the offered concentrations. As copper is considered more toxic to mosses than zinc, *P. patens* will endure lower copper concentrations and use its homeostasis mechanisms to avoid uptake of copper rather than zinc, resulting in lower overall concentration of copper in the organism (H1).
- As a non-metal adapted plant, *P. patens* cannot segregate metals after the uptake and will therefore store them in a concentration dependent manner. Neither accumulation nor segregation will occur over time (H2).
- *P. patens* showed augmented protonema growth under metal stress. Protonema cells are structurally different to the leafy gametophor, suggesting differences in metal uptake. We postulate higher metal tolerance, and significantly higher metal concentrations in the protonema tissue (H3).
- Growth reduction and morphological changes are best explained by metal uptake. As chelated metals are likely to be unavailable for *P. patens*, we postulate that uptake itself is therefore best explained by the concentrations of free metal cations in the media (H4).

To test these hypotheses, we analyzed the relative metal content using energy dispersive X-ray spectroscopy on a scanning electron microscope. This technique allowed to measure differences in the metal accumulation between protonema and leaf tissues. In addition, we investigated the influence of metal bioavailability and the effect of elevated metal levels. The data were compared to growth parameters and morphological changes (i.e., cell size and shape).

2. Material and methods

2.1. Plant material and cultivation

To investigate metal uptake behavior of a non-specialized moss, we chose *P. patens* (Hedw. [Lind.] *Funariaceae*; Fig. 1), a model organism to explore plant physiology (Cove, 2000; Reski and Cove, 2004). *P. patens* gametophytes are formed by protonemata (Fig. 1a) consisting of single cell filaments referred to as “protonema”, and the leafy moss plant (Fig. 1b,c; referred to as “gametophor”) well

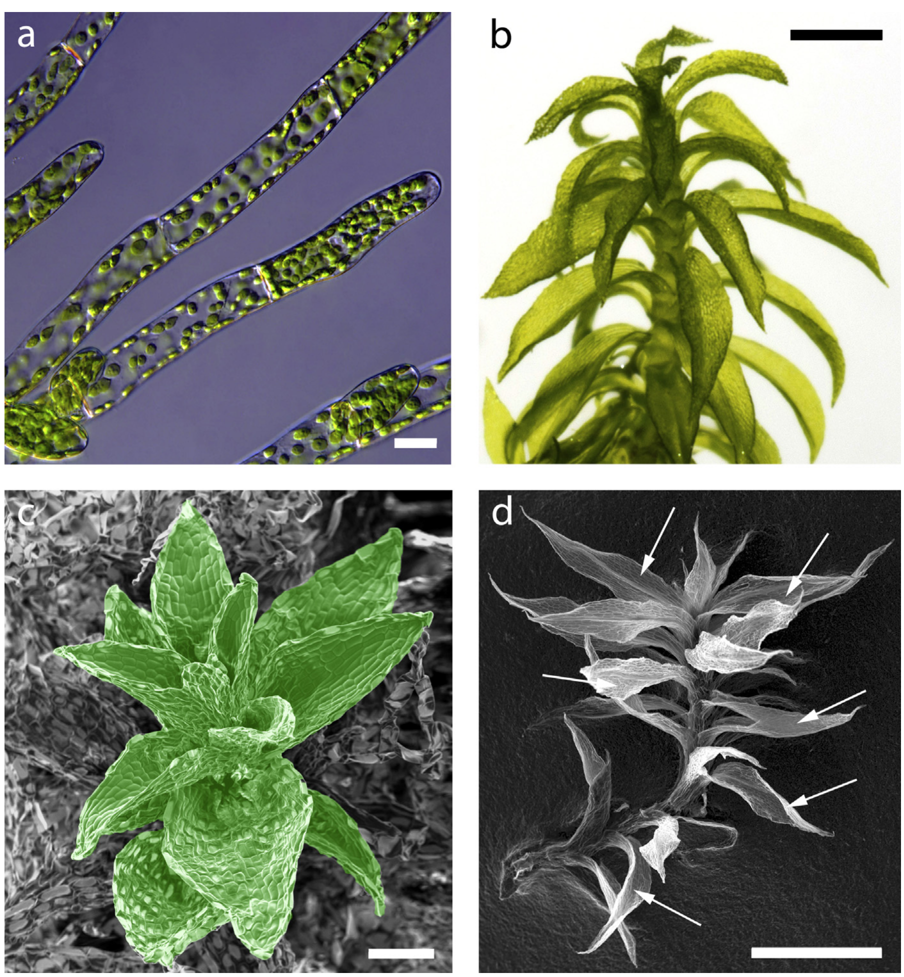

Fig. 1. (a) Differential interference contrast image of filamentous chloronema cells; (b) *P. patens* leafy gametophor; (c) colored SEM micrograph showing a young leafy gametophor (green); (d) example of SEM–EDX measurement sites on leaves (arrows); scale bar: $a = 25\ \mu M$; b,c and $d = 1$ cm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)