INTRACELLULAR RESPONSE TO CADMIUM IN THE MOSS *LEPTODICTYUM RIPARIUM*

Amira El Shall[1*], Erika Bellini[1,2*], Viviana Maresca[3*], Luca Paoli[1], Laura Bruno[2], Sergio Sorbo[4], Adriana Basile[3], Luigi Sanità di Toppi[1]
*equally contributed

[1]Department of Biology, University of Pisa, Italy (a.elshall@studenti.unipi.it – erikabellini1990@gmail.com - luca.paoli@unipi.it – debora.fontanini@unipi.it – luigi.sanita@unipi.it); [2]Department of Biology, University of Rome "Tor Vergata", Italy (erikabellini1990@gmail.com – laura.bruno@uniroma2.it); [3]Department of Biology, University of Naples "Federico II", Italy (viviana.maresca@unina.it - adriana.basile@unina.it); [4]CeSMA, Microscopy Section, University of Naples "Federico II", Italy (sersorbo@unina.it)

Heavy metals pose a problem for many plants that grow in contaminated environments, due to their toxicity effects. One of the most toxic metals is cadmium (Cd), a non-essential element, particularly harmful to most living organisms. Phytochelatin synthase (PCS) is a γ-glutamylcysteine transpeptidase (Grill et al., 1985) that, starting from glutathione (GSH), is able to synthesize phytochelatins (PCn), thiol peptides having a general structure γ-(glutamate-cysteine)$_n$-glycine, with n commonly included between 2 and 5. By virtue of their thiol groups, PCn can bind Cd and other heavy metals, sequestering them in the vacuole and thus reducing their toxic effects for the plant cell. The presence of widespread and constitutive PCSs has been found in a number of early plants, including bryophytes (Degola et al., 2014; Petraglia et al., 2014); amongst mosses, only *Sphagnum palustre* showed to possess a PCS with a molecular mass of about 36 kDa, able to synthesize PCn up to the oligomer PC_4 (Petraglia et al., 2014). By contrast, no PCS is expressed by the "model-moss" *Physcomitrella patens*. In the present work, we functionally characterized for the first time the PCS of *Leptodictyum riparium*, a cosmopolitan moss that appears to be capable of accumulating a wide amount of heavy metals compared to other plants. Indeed, HPLC-ESI-MS-MS assays of *L. riparium* exposed to 36 or 360 μM Cd allowed to detect the presence of PCn up to PC4, other than GSH and γ-glutamylcysteine. Moreover, TEM observation and Comet assay showed ultrastructural alterations and damage to DNA, proportional to the supplied Cd concentrations. Likewise, the increase in Cd concentration led to a progressively risen ROS level, with activation of antioxidant- (catalase, superoxide dismutase) and detoxifying- (glutathione transferase) enzymes. Thus, also in *L. riparium*, PCn and other detoxifying intracellular mechanisms come simultaneously into play to counteract Cd toxicity.

References:

Grill E., Winnacker E.-L., and Zenk M.H. 1985. Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Science, 230, 574 – 576.

Degola F., De Benedictis M., Petraglia A., Massimi A., Fattorini L., Sorbo S., Basile A., and Sanità di Toppi L. 2014. A Cd/Fe/Zn-Responsive phytochelatin synthase is constitutively present in the ancient liverwort *Lunularia cruciata* (L.) Dumort. Plant Cell Physiol, 55(11), 1884–1891.

Petraglia A., De Benedictis M., Degola F., Pastore G., Calcagno M., Ruotolo R., Mengoni A., and Sanità di Toppi L. 2014. The capability to synthesize phytochelatins and the presence of constitutive and functional phytochelatin synthases are ancestral (plesiomorphic) characters for basal land plants. Journal of Experimental Botany, 65(4), 1153–1163.