1.2 = *ACR3* is a key player in arsenic detoxification in bryophytes

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Bryophytes are the sister group to all tracheophytes and the second most numerous group of land plants. Therefore, elucidating in this plant lineage the molecular bases of traits shared with tracheophytes can provide important insights into trait evolution during the radiation of land plants.
Several bryophyte species display high levels of tolerance to arsenic (As), a highly toxic metalloid, but the molecular mechanisms underlying this trait have not been investigated until now. We recently demonstrated that the pathway of arsenic detoxification by vacuolar sequestration of phytochelatin::As conjugates does not play any significant role in As detoxification in the model bryophyte *Marchantia polymorpha* L. (1).
This finding leaves open the question of whether bryophytes rely on a mechanism of active As extrusion, as suggested by the presence in their genomes of genes homologs to *ACR3*, an arsenite transporter responsible for As hyperaccumulation in the fern *Pteris vittata* L.
We, thus, characterized the expression of the single *ACR3* gene present in *M. polymorpha*. The gene is expressed at relatively low levels in all *M. polymorpha* tissues. It is upregulated upon treatment of *M. polymorpha* gametophytes with As, suggesting that it may be involved in As detoxification. To functionally test this hypothesis, we overexpressed the full length CDS of Mp*ACR3* in *M. polymorpha* under the control of the strong constitutive promoter EF1α. Two transgenic lines with high transgene expression were selected from a total of 14 independent transformation events. Additionally, 2 knockout mutant lines corresponding to different target sites were obtained by CRISPR-Cas9 genome editing.
The overexpression lines consistently displayed strongly increased tolerance to both As(III) and As(V) addition to the growth media, while the knockout lines were hypersensitive to both As species compared to the Cam2 WT genotype based on plant fresh weight. Interestingly, the total As content per unit of dry weight in both overexpression lines did not significantly differ from that of Cam2, while both mutant lines accumulated much more As per unit of dry weight than the WT control.
Taken together, these results demonstrate that in bryophytes the *ACR3* gene plays a pivotal role in As detoxification and further suggest that the transporter may be localized to the plasma membrane where it extrudes As from *M. polymopha* cells. The presence of an efficient tolerance mechanism to As mediated by *ACR3* in bryophytes provides compelling evidence in support of the existence of the trait in the common ancestor of all land plants and that it was successively lost in the angiosperm lineage.

1) M. Li, M. Leso, M. Buti, E. Bellini, D. Bertoldi, A. Saba, R. Larcher, L. Sanità di Toppi, C. Varotto (2022) Journal of Hazardous Materials 440: 129844. https://doi.org/10.1016/j.jhazmat.2022.129844.