4.8 = Divergent patterns of metal/metalloid responsiveness and detoxification of two *Medicago truncatula* Gaertn. *phytochelatin synthase* genes

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Introduction

- Phytochelatins (PCn) are low molecular weight oligopeptides synthesized non-ribosomally by PCS from glutathione (GSH). They chelate toxic divalent metal cations and trivalent arsenic in the cytoplasm and mediate their sequestration in the vacuole; *PCS* genes are present in one or two constitutively expressed copies in most diploid angiosperm genomes studied so far.
- In this study, responsiveness to different essential and non-essential divalent metallic cations and to arsenic of the two PCS copies (MtPCS1 and MtPCS2) from the model legume *Medicago truncatula* Gaertn. were compared both *in vitro* and *in vivo.*

Result 1

- Enzymatic activities of both PCS copies in *M. truncatula* are modulated by heavy metals (HMs).
- Cd is the strongest activator (**Fig. 1**).

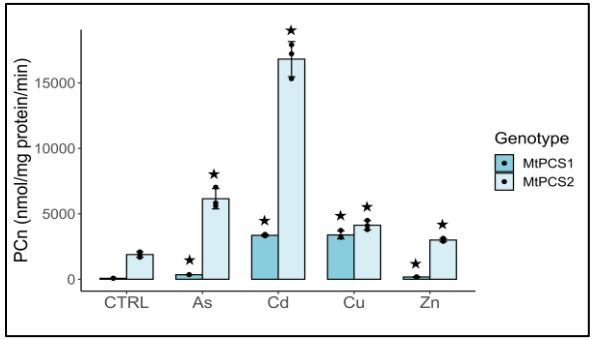

Figure 1*: In vitro* analyses of PCn productions by recombinant proteins MtPCS1 and MtPCS2 activated by different heavy metals. Black asterisks indicate amount higher than in the respective control.

Result 2

- Zn and to lesser extent Cd are the HMs causing the largest variation of the GSH pool in the shoots.
- Cd has the largest effect on the induction of PCn production in the shoots (**Fig. 2**).
- Cd tends to increase and As to decrease the GSH pool in the roots.
- Cd and to a lesser extent As have the largest effect on the induction of PCn production in the roots (**Fig. 3**).

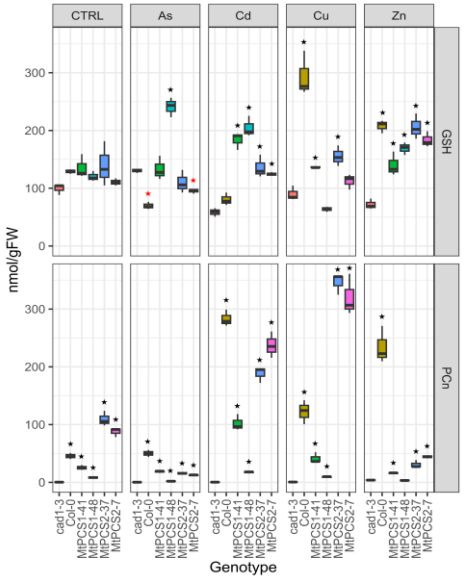

Figure 2: Shoot GSH and PCn levels in response to different heavy metal treatments of Arabidopsis transgenic plants complemented with *PCS* genes from *M. truncatula.*

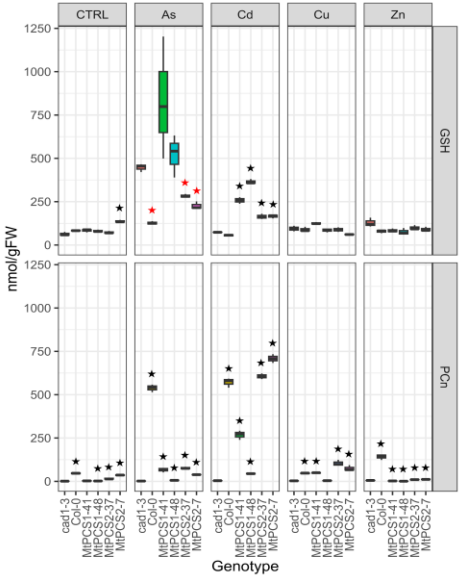

Figure 3: Root GSH and PCn levels in response to different heavy metal treatments of Arabidopsis transgenic plants complemented with *PCS* genes from *M. truncatula.*

Conclusions

- Several lines of evidence suggest a significant sub-functionalization of MtPCS1 compared to MtPCS2, as reported for all *PCS* genes functionally characterized until now.
- Cd is the strongest inducer of both MtPCS copies activity both *in vitro* and *in vivo*, suggesting that detoxification of this highly toxic metal is an important function of PCS in extant land plants.

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