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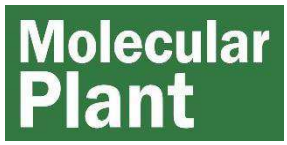
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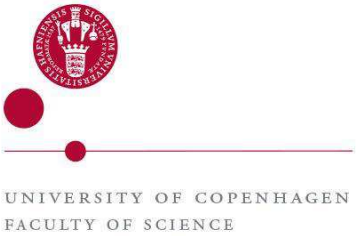
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Inducibility, tissue-specificity and product variation of three phytochelatin synthase homeologs from the cadmium-tolerant reed A. donax L.

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Phytochelatins (PC) are a family of Cys-rich oligopeptides constituting the main defence of plants against toxicity of heavy metals and metalloids like cadmium and arsenic. PCs are non-ribosomally synthesized from glutathione by the enzyme phytochelatin synthase (PCS). Dicotyledonous PCS have been characterized in detail, while much less information is available on monocotyledonous ones. In this study, we characterized three different *PCS* genes from giant reed (*Arundo donax* L.), a biomass/bionergy crop with remarkable tolerance to cadmium, to study the evolution of this trait in monocots. Phylogenetic reconstruction with PCS genes from fully sequenced monocotyledonous genomes indicated that the three *A. donax* PCS, *AdPCS1-3*, are most likely homeologs - resulting from lineage-specific whole-genome polyploidization. *AdPCS1-3* genes are tissue-specifically expressed, and *AdPCS1* is expressed about 5 times more than *AdPCS2* and *AdPCS3*. All three genes displayed cadmium-responsive expression in roots, and coded for functional PCSs, as once overexpressed in yeast they confer enhanced tolerance to cadmium stress. Overexpression of *AdPCS1-3* in *Arabidopsis thaliana* further confirmed the typical phenoytype associated to overexpression of functional PCS genes. Mass-spectral analyses detected statistically significant differences in the amount and spectral feature of the PCs synthesized, with AdPCS2 and AdPCS1 producing, respectively, the highest and lowest amount of total PCs in yeast cells. AdPCS1 synthesized the same amount of PC2, PC3 and PC4, while both AdPCS2 and AdPCS3 enzymes produced significantly higher amounts of PC2 and PC3 compared to PC4.
Taken together, these results indicate that the genetic bases of *A. donax* high capability to tolerate the presence of heavy metals is, at least in part, related to the high functional specialization of its *PCS* genes from a transcriptional as well as enzymatic point of view. Thus, transcriptional neofunctionalization and specialization seems to have played a major role in the evolution of Cd tolerance in *A. donax*.