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## THE INVOLVEMENT OF DNA METHYLATION IN ENHANCING HEAVY METAL HYPERTOLERANCE

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Among abiotic stresses, excess heavy metals in the growth substrate is one of the most affecting arable lands, influencing plant physiology, reproduction and yield. Zinc (Zn) and cadmium (Cd) are two naturally occurring heavy metals, and their presence is often increased by anthropogenic activities such as agricultural practices and industrial processes. As sessile organisms, plants have evolved protective mechanisms to cope with high metal concentrations in soils that relies on expression of specific genes. Epigenetic modifications are also essential for maintenance of genome stability under genotoxic metal stress, and a role for heavy metals in inducing epigenetic modifications has been recently assessed.

In our research, we have analysed the effect of Cd and Zn on the nucleoid structure and DNA methylation, comparing the sensitive species *Arabidopsis thaliana* with species able to tolerate and accumulate metals, *Arabidopsis halleri* and *Noccaea caerulescens*. The populations I16 and PL22 of A.halleri were chosen, since they show contrasting Zn and Cd accumulation.With regard to N. caerulescens, the Ganges ecotype was selected, since itssuperior Cd accumulation and hyper-tolerance. We analysed compaction andrelaxation of leaf cell nucleoids, the CpG DNA methylation percentage andthe modulation of genes linked to epigenetic modifications in response toZn and Cd treatment.

The comet assay showed that Zn induced a mild reduction in the tail moment in *A. thaliana* and in both I16 and PL22. Cd treatment induced an increase in DNA migration in nuclei of *A. thaliana*, whereas no differences in DNA migration was observed for I16, and a significant increase in nucleoid condensation was found in PL22 Cd treated samples. This last population showed higher CpG DNA methylation upon Cd treatment than control conditions, and an upregulation of genes involved in symmetric methylation and histone deacetylation. On the other hand, compared with *A. thaliana*, Cd treatment induced a smaller increase in DNA migration in the Ganges ecotype, which showed higher CpG DNA methylation upon Cd treatment.

Our data suggest a possible role of epigenetic modifications in both *A. halleri* and *N. caerulescens* to face high Cd shoot concentrations while preserving genome integrity, limiting ROS production and contributing to Cd tolerance. The differences between *N. caerulescens* Ganges and *A. thaliana* regarding DNA damage and expression of genes coding for enzymes involved in DNA methylation support the hypothesis of different mechanisms to prevent the Cd-induced DNA damage which evolved in hyperaccumulator species. A deeper investigation is important to transfer the results obtained approaching hyperaccumulator species to agronomical important crops. The results obtained in PL22 and I16 reinforce the hypothesis that divergent strategies for metals detoxification evolved in different populations belonging to the same species.