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IDENTIFICATION AND ANALYSIS OF GENES INVOLVED IN NICKEL TOLERANCE IN *NOCCAEA CAERULESCENS*

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Metal hyperaccumulator plants are able to accumulate extremely high concentrations of Heavy Metals (HMs) in shoots, in contrast with non-accumulator species. Among them, the european species *Noccaea caerulescens*, member of the Brassicaeae family, represents an interesting model, because it shows a great variability between different ecotypes in metal tolerance and accumulation, considering, for instance, Zinc (Zn), Nickel (Ni) and Cadmium (Cd), alone or in combination.

This work focused on the ecotype Monte Prinzera (MP, Italy) of *N. caerulescens*, autochthonous in a Natural Reserve in the Tosco-Emilian Appennins (Italy) characterized by serpentine soil; this particular ecotype is able to tolerate and accumulate Ni and Zn, a property that is shared with two other species of the same genus, i.e. *Noccaea goesingense* and *Noccaea japonica*.

Molecular mechanisms responsible for Ni hypertolerance and hyperaccumulation are still unknown, although different genes seem to be involved in these processes. Particularly, several studies suggested that metal transporters, also essential for metal homeostasis, have a fundamental role in HM tolerance and accumulation. Therefore, the expression of vacuolar transporters *MTP1* and *NRAMP4* and of plasma membrane transporter *ZNT1* was compared in *N. caerulescens* MP exposed to different Ni concentrations with those of *Thlaspi arvense* (non-accumulator) and *N. caerulescens* ecotype Ganges (GA, Zn/Cd hyperaccumulator). High expression of *MTP1* was observed in the presence of Ni excess in *N. caerulescens*, GA ecotype and MP ecotype in comparison to the control species *T. arvense,* suggesting a possible role of MTP1 in Ni hypertolerance. In both *N. caerulescens* ecotypes, two forms of MTP1 were found, differing for the length of the histidine-rich loop. Co-localization experiments in *Nicotiana tabacum* leaves confirmed that both *MTP1-long* and *MTP1-short* are localized in tonoplast. Complementation assays with yeast mutants showed that the two MTP1 forms may possibly have different metal specificity, and other tests are going to be done in order to confirm this results.

The vacuolar transporter *NRAMP4* and the plasma membrane transporter *ZNT1* have important roles in Fe and Zn homeostasis in plants, although several studies showed their possible involvement in Ni hyperaccumulation/hypertolerance. Upon Ni treatment, *ZNT1* was strongly induced in *N. caerulescens GA*; in the MP ecotype, *NRAMP4* showed an high and constitutive expression, whereas *ZNT1* was down-regulated in response to Ni treatment. These results point to a possible role of these transporters in Ni hypertolerance/hyperaccumulation. To study their involvement in Ni hyperaccumulation/hypertolerance, plants of *Arabidopsis thaliana* were transformed with constructs carrying CaMV35S*::ZNT1* and CaMV35S*::NRAMP4* and the overexpressing lines were crossed to obtain plants overexpressing both genes.