

Contribution of *nas1* knockout in evolution of heavy metal hyperaccumulation trait in *Thlaspi caerulescens*

In recent years, researches on *Thlaspi caerulescens*, a model plant for heavy metal hyperaccumulation and hypertolerance, have been increased tremendously. They are aimed to understand the ecological and evolutionary aspects of metal hyperaccumulation and to explore applications of phytoremediation on metal-contaminated soils and applications on improving nutritional values of food. Nicotianamine (NA) involves widely in homeostasis of essential metals (Fe, Cu, Zn, Ni) in plants generally, as well as hyperaccumulation of non-essential toxic metals (Cd, As). Four NICOTIANAMINE SYNTHASE genes, *TcNAS1*, *TcNAS2*, *TcNAS3*, and *TcNAS4* responsible for producing NA, are identified in *T. caerulescens*. Their presences are necessary although redundant functionally. A mutated *TcNAS1* allele with a transposon insertion is found to be distributed in natural populations of accession La Calamine. In this study, we studied the effects of this mutated *TcNAS1* on their growth, heavy metal hyperaccumulation, and *TcNAS* expressions in various conditions to understand why this mutated allele is maintained in these populations. This knockout resulted in more susceptibility to Fe deficiency and high Ni/Cd supplies. It showed a partial *chloronerva*-like phenotype with more interveinal chlorosis, increased Fe uptake, and Fe concentration in leaves, especially in Fe deficiency. However, Cu deficiency was not observed in this mutant and it did not alter much the Mn homeostasis of this genotype. The lack of *TcNAS1* is proposed to cause a shortage of NA in leaf tissues and affected expressions of other *TcNAS* genes. Total functional transcripts levels of the other three *TcNAS* genes in the *nas1* mutant were higher than the wild type in Fe deficiency and excess Zn treatments. It was not different between the two phenotypes in Ni treatments and low Cd supply, but was lower in the mutant than the wild type in high Cd supply. The *nas1* mutant had higher capacity of Ni/Cd, but not Zn, hyperaccumulation. This is proposed to be an advantage of the mutant over the wild type to avoid insect herbivores, which suggests a balancing selection resulting in the maintenance of this mutated allele in the natural habitat. As a part of this thesis, an experiment to create a quadruple *nas* mutant in *T. caerulescens* was with RNA interference (RNAi) was performed. However, it needs more samples tested to conclude the silencing efficiency of our RNAi construct and the used protocol.

Characterization of three members of the Major Facilitator Superfamily (MFS) in *Phytophthora infestans*

Phytophthora infestans, an oomycetes, is the renowned causal agent of the late blight in potato. Field control of this pathogen is widely dependent on chemicals. Cyclic lipopeptides, including Massetolide A (Mass A), that have deleterious effects on *P. infestans* are promising for developing novel biocontrol strategies against late blight. Therefore, understanding the responses of *P. infestans* to Mass A treatment is necessary to obtain a more robust application. In this study, we partly unraveled the molecular basis of these responses by characterization of three *P. infestans* Major Facilitator Superfamily (MFS) proteins of which gene expression was responded to Mass A treatment. Our results showed that caf-MFS1, homologous to the yeast PHO84p, is a repressible high-affinity inorganic phosphate:H⁺ symporter that is localized in the plasma membrane. Caf-MFS1 may be involved in nutrient sensing in mycelia and specific early stage of sporangium germination. Caf-MFS2

is also localized in the mycelial plasma membrane but its function as a putative monocarboxylate/dicarboxylate:H⁺ symporter was not yet confirmed. Caf-MFS3 is predicted to be a oxalate:formate antiport that is localized in the inner membrane of the mitochondria and experimentally proved to be involved in sporulation. Silencing of *caf-MFSs* reduced vitality of *P. infestans* but seems to reduce its sensitivity to Mass A treatment. The function of proton flux regulation of the three *caf-MFSs* is proposed and may be involved in adjusting the acid-base equilibrium of *P. infestans* cells and the environment upon exposure to Mass A.