HEAVY- METAL TOLERANCE IN GENUS *SILENE* – PLAY METALOTHIONEINS A CRUCIAL ROLE?

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ABSTRACT

Phenomenon of heavy metal tolerance was described in genus *Silene* for the first time. Up to now there is not enough evidence to explain physiological or genetic mechanisms that enable survival of plant genera, as well as genus *Silene*, in metalliferous sites like copper contaminated areas. On the basis of new investigation in other tolerant species it is proposed metallothioneins (MTs) are key compounds of copper tolerant homeostatic network. MTs are small metal binding proteins encoded in genome a lot of organisms. We discovered two new *MT3* genes in *Silene vulgaris* genome.

Key words: metallothioneins, *Silene vulgaris*, heavy metal tolerance

Acknowledgments: This work was supported by the IGA FA MENDELU No. TP 1/2010.

INTRODUCTION

Plant adaptation to soils with heavy metal contamination is a well-know ecological phenomenon. But only minority of plant species can grow on metalliferous soils. Red campion (*S. dioica* L.) is used in geology as a bioindicator of high concentrations of copper (Martinka and Lux, 2006). Copper homeostatic consisted of several components for example copper reductases, copper transporters and metallothioniens (MTs). Recent genetic studies of heavy metal plant models have showed MTs are tightly involved in copper resistance in many plant species (van Hoof *et al.,* 2001). Metallothioneins are cysteine-rich, metal-binding cytoplasmic proteins (Cobbett and Goldsbrough, 2002). They play a significant role in maintaining intracellular metal homeostasis, eliminating metal toxification and protecting against intracellular oxidative damages (Zhou *et al*., 2006). The genes enconding MTs occur both in eukaryotes and prokaryotes. The similarity among *MT* genes within one species is often high (Hudspeth *et al*., 1996). Some findings suggest that gene amplification is one of the mechanisms that play an important role in evolution of these genes (van Hoof *et al.*, 2001). This work was preformed to isolate and characterize *MT* genes of *S. vulgaris* accessions and investigate their role the copper homeostasis.

MATERIAL AND METHOD

Seeds of tolerant *S. vulgaris* plant were collected from copper mines near Spania Dolina. Hydroponic culture was used for test of tolerance, where tolerant and non-tolerant plants were exposed to linearly increasing concentrations of Cu^{2+} in the test solution (200µM $Cu(NO_3)_2$) in defined light and temperature conditions. RNA was isolated using TRIzol reagent. Single-strand cDNA was prepared from 2µg of RNA. To amplify candidate *MT* genes from cDNA we used specific primers designed based on sequences from cDNA databases of several *Silene* species. After purification and sequencing candidate *MT* genes NCBI database was searched by BLAST program. From our data set we chose two new sequences belonging to *MT3* type. Radioactively labelled probe was constructed by random priming method and incorporation of [α-P^{32}]dATP and the *S. vulgaris* BAC library was screened. One BAC clone was finally selected and sequenced by 454 sequencing method. The expression of *MT3* genes was measured by qPCR.

RESULTS AND DISCUSSION

We measured a level of copper accumulation (Fig. 1). Surprisingly, copper accumulation is higher in copper sensitive accession. However this accession can not tolerate excess of copper in tissues and after achievement of limiting concentration plants died (Fig. 2). Copper tolerant accession accumulated lower copper in their tissues than non-tolerant plants. However level of accumulation is very high in both populations. The most interesting discovery is a presence of local duplication *MT3* genes in BAC clone (Fig. 3). We suppose the duplication plays a role in evolution of copper tolerance. We have characterized flanking regulatory region of the *MT3A* gene and we found insertion of a retrotransposon in its promoter sequence. This *S. vulgaris* locus can be very crucial for copper homeostasis, our hypothesis is supported by occurrence of other genes associated with copper metabolism and collinearity with *Arabidopsis thaliana* locus. RT - qPCR of *MT3A* gene in leaves indicates unstable level of expression under linearly increasing concentrations of copper cations in solutions. The expression pattern can be influenced by different activation of retrotransposon. Root expression increases in excess of copper. Root expression correlates with root accumulation but not copper resistance. We indicated unequal expression level in tolerant and sensitive accessions. Up to now the biggest difference in expression of the *MT3* gene in the resistant and non resistant accessions was found by Hassinen *et al.* (2009) in *T. caerulescens*. MT3 protein of *S. vulgaris* showed a high amino acid sequence identity with the MT3 protein of *Brassica juncea, Typha angustifolia, Tamarix androssowii and Arabidopsis thaliana*, particularly in the cysteine-rich N-terminal and C-terminal sectors (Fig. 5). A copper sensitive yeast strain transformed with *MT3A* gene was able to grow at 75 $Cu(NO_3)_2$, whereas untransformed mutant died.

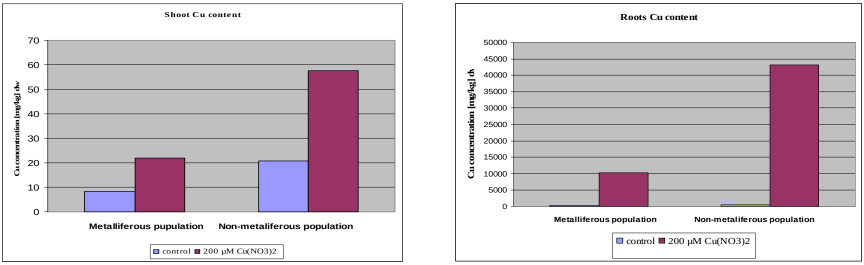

Fig. 1 Measurement of accumulation and tolerance

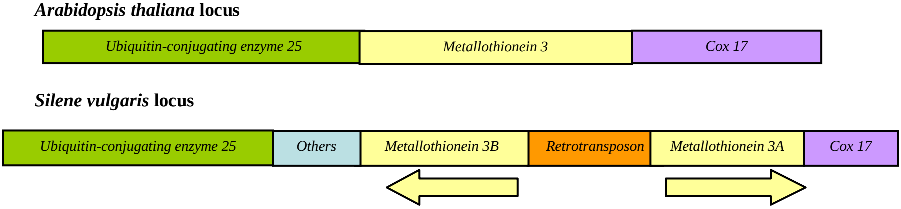

Fig. 2 The comparison of the S.vulgaris and A. thaliana locus containing MT3 genes

Fig. 3 Dead copper-sensitive plant

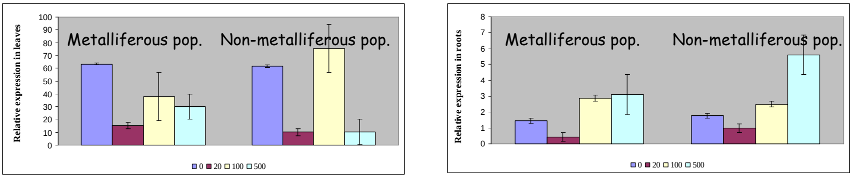

Fig. 4 Expression of MT3A gene in plants treated with different concentrations of $Cu(NO_3)_2$

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SVA/1-61  MSGMCANCDCSDKTNCV-KGNQYGINLIENYDV--DCQVMD----------AGE--CDSCKCGPSCACASCSCCSH
SVB/1-65  MSGMCANCDCSDKNNCV-KGAA------EYDDV--DALVVDSGVGMATAYYSGE--CDSCKCGPSCACASCSCCSH
BJ/1-67   MSDKCGSCDCADKTQCVKKGTSYTFDIVETQESYKEAMFMD--VGA-----EEN--GCQCKCGSTCSCVNCTCCPN
AT/1-69   MSSNCGSCDCADKTQCVKKGTSYTFDIVETQESYKEAMIMD--VGA-----EENNANCKCKCGSSCSCVNCTCCPN
TN/1-69   MSGKCGNCSCADKSQCVQKRNQYGFDLIETQTYAESTVVMDDPPTA-----AEN--GGQCKCGDRCACVNCTCGSH
TA/1-64   M-STCGNCDCADKSQCVKKGNSYGVEIIETEKSYHDGVF---EVAA-----AKN--EGNCKCGPSCACVDCHCG-N
          *355*+9*7*8**99**-*476311122*7554--676714--111-----437--455****56*8*87*4*916
          MSG+CGNCDCADKTQCVKKGNSYGFDIIETQ+SYKDA+VMD--VGA-----AEN--+++CKCGPSCACVNCTCCS+
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Fig. 5 Alignment of MT3 proteins of BJ=Brassica juncea, TA=Typha angustifolia, TN=Tamarix androssowii, SV=Silene vulgaris, AT=Arabidopsis thaliana

CONCLUSION

We isolated and characterised two *MT3* genes. These genes are duplicated locally in genomic locus of *S. vulgaris*. Deduced protein of MT3A showed a deletion of ten amino acids. Another feature of the MT3A in *S. vulgaris* is a presence of higher number of cysteine residues that are important parts of metal binding domains. It is likely this specific protein structure can enable better chelated function.

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