IDENTIFICATION OF CANDIDATE HEAVY-METAL RESISTANCE GENES IN GENUS SILENE

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ABSTRACT

Plants can survive on heavy metal contaminated areas. The phenomenon of heavy metal accumulation and resistance is described in many species of plants. First time was this peculiar trait observed in *S. dioica*, already in 1934. *S. dioica* is a typical plant growing on soils with copper enrichment. There are several known mechanisms that enable plant populations to survive in soils with high concentration of heavy metals. Metallothioneins (MTs) are small proteins with high cysteine residues content and play a key role in defensive reactions of many plant species. Recently, the role of metallothioneins has been investigated especially in the heavy metals detoxicant pathway. It was also shown that MTs are closely connected with the copper metabolism. *S. dioica* and *S. vulgaris* are growing in high copper concentrated sites. We suggested to isolate genes encoding metallothioneins and to determine their role in metabolism of copper. We used cDNA databases of several Silene species for the identification of these genes. By screening of the BAC library of *S. vulgaris* with the probe derived from MT3 gene we found positive clones that were subsequently sequenced. This way we characterized the first MT3 gene in genus Silene. Expression analysis indicates the importance of the MT3 gene in resistant species, since the expression of this gene is increasing in higher copper concentration in hydroponic cultures.

Key words: metallothioneins, heavy metal resistance and accumulation, copper, *Silene vulgaris*

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INTRODUCTION

Plant adaptation to soils with heavy metal enrichments is a well-know ecological phenomenon. But only minority of plant species can grow on metalliferous soils. Among such plants belongs genus Silene with some species growing on toxic metal contaminated areas. Red campion (*S. dioica* L.) is used in geology as a bioindicator of high concentrations of copper (Martinka and Lux, 2006). Metallothioneins (MTs) are known to be 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 an important role in maintaining intracellular metal homeostasis, eliminating metal toxification and protecting against intracellular oxidative damages (Zhou *et al*., 2006). Plants metallothioneins are classified into four types based on arrangement of cysteine residues. The genes enconding MTs occur both in eukaryotes and prokaryotes. The similarity between MT genes within one species is often very high (Hudspeth *et al*., 1996). Some findings suggest that gene amplification is one of the mechanisms that play a crucial role in evolution of these genes (van Hoof *et al.*, 2001). This work was preformed to isolate and characterize MT genes of some Silene species and investigate their role the copper resistance.

MATERIALS AND METHODS

Seeds of resistant plant of *S. vulgaris* and *S. dioica* were collected from copper mines near Spania Dolina. (Fig.1). Hydroponic culture was used for test of tolerance, where tolerant and non-tolerance plants were exposed to linearly increasing concentrations of copper in the test solution (0μM, 25μM, 100μM $CuNO_3$) in defined light and temperature conditions. RNA was isolated using TRIzol reagent. Single-strand cDNA was prepared from 2μg of RNA. To amplify candidate genes from cDNA we used specific primers designed based on sequences from cDNA databases of several *Silene* species. The primers (5' – GGTCATGGATGCAGGAGAGT – 3', 5' – TCAACACTTGTAGGCAGTGGA – 3') were used to amplify MT3 sequence using *S. dioica* cDNA as a template. After purification of a PCR product was constructed a radioactively labelled probe of MT3 gene by random priming method and incorporation of [α-P^{32}]dATP. This probe was used for screening of the BAC library of *S. vulgaris* and positive clones were chosen. These clones were tested on presence of MT3 gene by PCR reaction with MT3 specific primers and by Southern blot hybridization with the MT3 probe. One BAC clone was finally selected and sequenced by 454 sequencing method. The expression of MT3 genes was estimated by Northern blot hybridisation.

Fig.1 S. dioica (left side) S. vulgaris (right side) growing on Cu contaminated area in Slovakia



RESULTS AND DISCUSSION

The part of the sequence of the BAC clone of *S. vulgaris* showed a high amino acid sequence identity with the MT3 genes in *A. thaliana*, *T. caerulescens and O. sativa*, particularly in the cysteine-rich N-terminal and C-terminal sectors (Fig. 2). The MT3 gene of *S. vulgaris* includes four Cys-residues in the N-terminal domain. We have characterized flanking regulatory region of the MT3 gene and found that the major difference between *A. thaliana* and *S. vulgaris* is in a presence of a retrotransposon in its promotor sequence. Surprisingly except this retrotransposon the region containing several important genes is completely collinear with *A. thaliana* genome sequence (Fig. 3). Using Northern blot hybridization was revealed the differential expression in tolerant and non-tolerant species as well as in Cu treated and non-treated Silene plants (up to now the biggest difference in expression of the MT3 gene in the tolerant and non tolerant accessions was found by Hassinen *et al*. (2009) in *T. caerulescens*). Most of the type MT3 genes characterized in other plants are expressed primarily in fruit tissues, such as papaya, banana, kiwifruit. Guo *et al*. (2003) show that *Arabidopsis* MT3 gene is expressed more in leaves and it cannot be detected in seeds. This finding can suggest that this gene is not only involved in heavy metals detoxification, but in different developmental processes too. We revealed the expression of this gene in both roots and leaves.

Fig.2 Alignment of S. vulgaris, T. caerulescens, A. thaliana and O. sativa MT3 amino acid sequences.

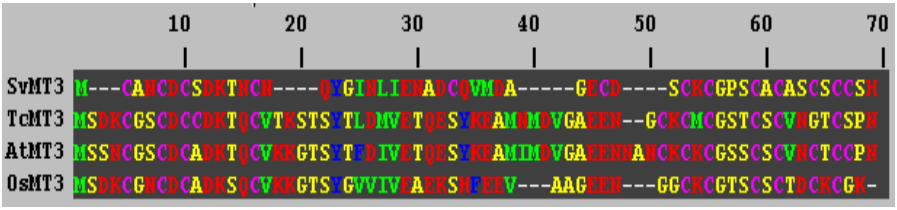
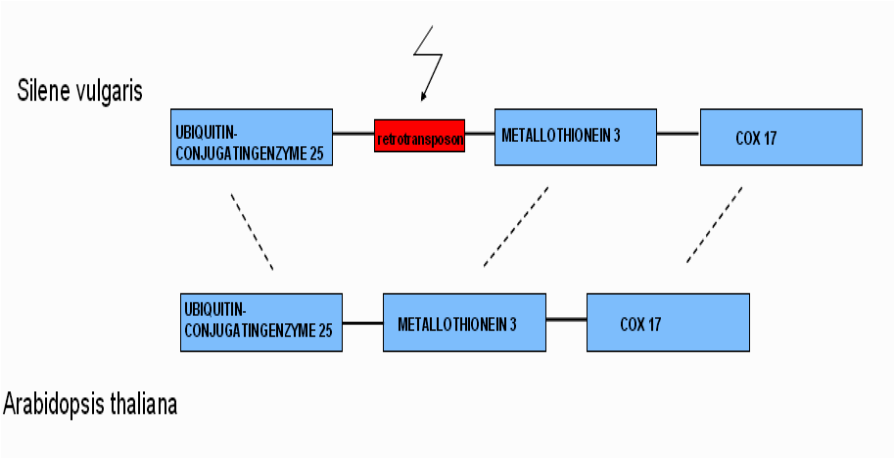


Fig.3 The comparison of the S.vulgaris and A. thaliana locus containing MT3 gene. Both loci are completely collinear except an intergenic region with the inserted retroelement in S. vulgaris.



CONCLUSION

We have isolated the MT 3 gene from heavy metal resistant plant *S. vulgaris*. We have sequenced the BAC clone containing the MT3 gene and found out that the major difference between *A. thaliana* the collinear region is a presence of a retrotransposon in the promotor of the MT3 gene in *S. vulgaris*. Another important feature of the MT3 gene in *S. vulgaris* is a presence of higher number of cysteine residues that are important parts of metal binding domains. Our aim is to answer following questions: Is the MT3 gene a key player in the metabolism of copper in Silene species? Can the retrotransposon influence expression of MT3 gene in stress conditions? Is the structure of isolated MT3 gene crucial for affinity of the MT3 protein to heavy metals?

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