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ScienceDirect
Environmental Pollution 144 (2006) 70–76
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phytoremediation traits in Indian mustard

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Simultaneous overexpression of APS and SMT genes in Indian mustard greatly increases ability to accumulate selenate.

Abstract

A major goal of our selenium (Se) phytoremediation research is to use genetic engineering to develop fast-growing plants with an increased ability to tolerate, accumulate, and volatilize Se. To this end we incorporated a gene (encoding selenocysteine methyltransferase, SMT) from the Se hyperaccumulator, *Astragalus bisulcatus*, into Indian mustard (LeDuc, D.L., Tarun, A.S., Montes-Bayón, M., Meija, J., Malit, M.F., Wu, C.P., AbdelSamie, M., Chiang, C.-Y., Tagmount, A., deSouza, M., Neuhierl, B., Böck, A., Caruso, J., Terry, N., 2004. Overexpression of selenocysteine methyltransferase in Arabidopsis and Indian mustard increases selenium tolerance and accumulation Plant Physiol. 135, 377–383.). The resulting transgenic plants successfully enhanced Se phytoremediation in that the plants tolerated and accumulated Se from *selenite* significantly better than wild type. However, the advantage conferred by the SMT enzyme was much less when Se was supplied as *selenate*. In order to enhance the phytoremediation of selenate, we developed double transgenic plants that overexpressed the gene encoding ATP sulfurylase (APS) in addition to SMT, i.e., APS × SMT. The results showed that there was a substantial improvement in Se accumulation from selenate (4 to 9 times increase) in transgenic plants overexpressing both APS and SMT.

Keywords: Selenium; Indian mustard; Selenocysteine methyltransferase; ATP sulfurylase

1. Introduction

Although selenium (Se) is an essential micronutrient for humans and animals at very low doses, it is extremely toxic at higher doses (Wilber, 1983). In animals, fish, and wildlife, excess Se can cause birth defects, sterility and other disease symptoms, while in humans it can cause loss of hair, teeth, and nails, fatigue, and even death (Moxon, 1937; Eisler, 1985; Lemly and Smith, 1987; Sorenson, 1991). Living organisms become exposed to high Se concentrations through both natural and anthropogenic releases of Se to the environment. Selenium may be released naturally into soils formed from Se-bearing shales. This in turn can lead to the production of large quantities of Se-contaminated irrigation drainage water – one of the most serious agricultural problems in the western United States and other areas with similar environments and geological conditions (Presser and Ohlendorf, 1987). There are many examples of anthropogenic Se releases to the environment, including aqueous discharges from electric power plants, coal ash leachates, refinery effluents, and industrial wastewater (American Medical Association, 1989).

Abbreviations: SeCys, selenocysteine; SeMet, selenomethionine; SMT, selenocysteine methyltransferase; APS, ATP sulfurylase.

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doi:10.1016/j.envpol.2006.01.008

Because of the presence of excessive and potentially toxic levels of Se in the environment, it is important that we find ways of removing or detoxifying Se in Se-contaminated soil and water. Phytoremediation, using plants to remove, stabilize, or detoxify pollutants, is a promising technology to achieve this end (Terry et al., 2000). Because Se and sulfur are chemically similar, plants are able to extract Se from soils and water into their tissues, which can be harvested and removed. This process is referred to as *phytoextraction*.

Some unique species, called Se hyperaccumulators, are naturally able to accumulate high concentrations of Se (thousands of μg g^{-1} DW) in their tissues – a concentration even higher than the seleniferous soil upon which they thrive (Brown and Shrift, 1981). Although these hyperaccumulators are efficient Se extractors, their phytoremediation potential is often limited by their slow growth rate and low biomass (Cunningham et al., 1997). More effective Se phytoremediation has been achieved using fast-growing plant species with only moderate Se accumulation abilities, such as *Brassica juncea* (Indian mustard) (Bañuelos and Schrale, 1989; Bañuelos et al., 1997).

An important goal of our research has been to try to combine the fast-growing ability of Indian mustard with the superior Se accumulating ability of a Se hyperaccumulator. Recent field trials demonstrated that judicious genetic engineering is a viable means of enhancing the phytoremediation potential of Indian mustard (Bañuelos et al., 2005). In an earlier study, we introduced a gene from the slow-growing Se hyperaccumulator, *Astragalus bisulcatus*, into the fast-growing high biomass Indian mustard in order to create a much more efficient plant for Se phytoremediation (LeDuc et al., 2004). To this end, we overexpressed the gene encoding selenocysteine methyltransferase (SMT) because it is thought to confer Se tolerance to *Astragalus bisulcatus*. SMT specifically methylates selenocysteine (SeCys) to produce the non-protein amino acid, methylselenocysteine (MetSeCys); this is thought to reduce the intracellular concentrations of SeCys and selenomethionine (SeMet) (Neuhierl and Böck, 1996) thereby decreasing the chance of toxic misincorporation of these species into proteins.

The Indian mustard SMT plants did indeed have an increased ability to tolerate, accumulate, and volatilize Se, particularly when the transgenic plants were supplied with *selenite*. The advantage conferred by the SMT enzyme was much less when the transgenic plants were grown in the presence of selenate. The use of SMT transgenic plants to remove Se from seleniferous soils is of little value in soils which predominately contain selenate. Plants take up selenate actively, accumulating 10- to 20-fold higher Se concentrations than with selenite (Ulrich and Shrift, 1968). The conversion of these huge amounts of selenate to selenite is slowed substantially by rate-limiting amounts of ATP sulfurylase (APS), which catalyzes selenate reduction to organic forms of Se (Pilon-Smits et al., 1999a,b). Thus, plants overexpressing SMT were restricted in their ability to convert selenate to MetSeCys because of their limited capacity for selenate reduction.

In an earlier study, we overcame the rate-limiting step imposed by APS in Indian mustard by overexpressing the gene encoding ATP sulfurylase from *Arabidopsis thaliana* (Pilon-Smits et al., 1999a,b). The resulting APS transgenic plants were able to rapidly reduce selenate to selenite, thereby generating organic forms of Se. The APS transgenics exhibited increased tolerance to selenate and an increased ability to accumulate Se (probably as the non-toxic amino acid, methylselenocysteine, LeDuc et al., unpublished).

When Indian mustard plants take up selenite, they rapidly metabolize it to SeCys, which can then be converted to Met SeCys by SMT (Asher et al., 1967; Arvy, 1993; Terry et al., 2000). This conversion of toxic selenite to non-toxic methylselenocysteine explains why the transgenic plants overexpressing SMT had a superior ability to tolerate, accumulate, and volatilize Se when they were supplied with selenite (LeDuc et al., 2004). On the other hand, when the SMT transgenic plants took up selenate, they were less able to convert it to MetSeCys because of the rate-limiting step associated with the reduction of selenate to selenite (de Souza et al., 1998). In the present work we tested the hypothesis that Indian mustard plants overexpressing *both APS and SMT* should be able to carry out Se phytoremediation more efficiently, i.e., by using the overexpressed APS to increase selenate uptake and reduction, and then to use the overexpressed SMT to detoxify the resulting larger pool of SeCys. By this means, the double transgenic plants, APS × SMT, should be able to accumulate Se better than wild type plants or plants overexpressing either SMT or APS alone.

2. Materials and methods

2.1. Enzymes and chemicals

DNA modifying enzymes were from New England Biolabs (Beverly, MA) and Promega (Madison, WI). Acrylamide and SDS-PAGE gel reagents were from Bio-Rad (Hercules, CA). All other chemicals were from Sigma (St. Louis) and at least reagent grade.

2.2. Molecular characterization

APS × SMT double homozygous transgenic plants were identified among the kanamycin-resistant lines by PCR using primers directed against the APS and SMT genes. The APS primer sequences were: 5′-AAAGCACG TATCGGCGAGTC-3′and 5′-CCAGCGTAATGCATAGGTGA-3′. The SMT primer sequences were 5′-GGTCGTTCTGCAACAGCCACC-3′ and 5′-CCCTGCTTAGGAGAAGTGTTG-3′. APS and SMT transcript levels in Indian mustard plants were compared using RT-PCR. mRNA was converted to cDNA using the SUPERSCRIPT II, RNaseH Reverse Transcriptase kit (Gibco-BRL, Cleveland). SMT and APS cDNA was amplified using primers directed towards SMT and APS, respectively. For immunoblot analysis, Indian mustard protein was isolated by grinding 1 g of seedling tissue in liquid nitrogen, homogenizing in 2 ml of extraction buffer (25 mM Tricine pH 7.5, 1 mm EDTA, 10 mM ß-mercaptoethanol, 1% PVPP, 1 μM leupeptin, 1 μM pepstatin, 200 μg PMSF), and precipitating with ammonium sulfate. Protein concentrations were estimated using Bradford reagent (Biorad), with a BSA standard. 10 μg of protein from each sample was separated on a 12% SDS-PAGE gel, transferred onto PVDF membrane (Pharmacia), and hybridized with anti-SMT polyclonal antibody (Wang et al., 1999). Blots were probed with anti-AP secondary antibodies and detected using a colorometric method.

2.3. Selenium tolerance

To determine Se tolerance and accumulation in Indian mustard seedlings, seeds were sterilized by rinsing in 96% ethanol for 30 s, in 0.65% hypochlorite solution for 30 min, and in sterile deionized water for 5 × 10 min, on a rocking