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Transgenic Indian Mustard Overexpressing Selenocysteine Lyase or Selenocysteine Methyltransferase Exhibit Enhanced Potential for Selenium Phytoremediation under Field Conditions

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Two new transgenic Indian mustard [*Brassica juncea* (L.) Czern.] lines were tested under field conditions for their ability to accumulate selenium (Se) from Se- and boron-contaminated saline sediment. The transgenic lines overexpress genes encoding the enzymes selenocysteine lyase (cpSL) and selenocysteine methyltransferase (SMT), respectively. In the first Spring planting, cpSL, SMT, and wildtype plants (WT) were compared, while SMT and WT were compared in a second, Fall planting. In the Spring planting, shoots of the cpSL transgenic plants accumulated 2-fold more Se ($p < 0.01$), had 1.8 times higher leaf Se concentrations ($p < 0.01$), and grew better on contaminated soil than WT. The SMT plants had a 1.7-fold higher leaf Se concentration than WT ($p < 0.05$). In the Fall planting, the SMT transgenic plants accumulated 1.6-fold more Se in their shoots than WT ($p < 0.01$) with Se concentrations being higher in both leaves and stems. These results conclusively demonstrate that cpSL and SMT transgenic lines have significantly greater Se phytoremediation potential than wildtype Indian mustard. Further, this study confirms the importance of field testing for evaluating future transgenic lines.

Introduction

Selenium (Se) contamination of irrigation drainage water represents one of the most serious problems confronting agriculture in Se-rich regions of the Western United States (e.g., California and Colorado) and other parts of the world with similar environmental and geological formations (*1*). With irrigation of soils in these regions, Se and other salts are leached into the subsurface drainage water, which may be channeled and stored in evaporation ponds. The bioaccumulation of Se in drainage water and its subsequent accumulation in surface waters represents a serious environmental hazard (as typified by the environmental disaster which occurred at the Kesterson Reservoir in the San Joaquin Valley in California in the 1980s) (*2*, *3*). An attempt was made to deal with the drainage water problem by channeling the Se- and salt-laden drainage water through the San Luis Drain (SLD), small sloughs, and the San Joaquin River toward the San Francisco Bay Delta (*4*). However, the final construction and use of the SLD was abandoned because of objections raised by environmentalists and by state and federal agencies. This, in turn, created a new problem of how to deal with the large quantities of Se-, salt-, and boron-contaminated sediment left in the drain after it was abandoned (*5*).

When compared with other methods of dealing with Se-contaminated sediments such as soil washing, excavation, and reburial of the contaminated sediment (*6*), phytoremediation offers a much less expensive alternative to managing Se in the drainage sediments. Plants can be used to remove Se from soil by absorbing and transporting it to above-ground tissues that are then harvested and disposed (*7*−*9*), as well as volatilization of Se by plants (*10*) and/or microbes (*11*), to the atmosphere. However, in drainage sediments, the high salt and boron (B) levels that are present along with the excessive Se create such harsh growing conditions that the uptake and volatilization of Se for many plant species is impaired (*7*, *9*, *12*, *13*). Even under less adverse growing conditions, the effective use of phytoremediation as a technology for the cleanup of Se is limited by the slow rate of the biological processes involved.

Because there is a need to develop plants that can remove Se from soil at much faster rates than are presently observed, we began a program of genetic engineering to increase the efficiency of Se phytoremediation. Our goal was to generate plants with enhanced abilities for Se uptake and volatilization (*14*). This research led to the first successful field trial using transgenic plants for phytoremediation (*12*). In this study, transgenic Indian mustard (*Brassica juncea* L. Czern) lines that were developed for accumulating Se and heavy metals (described in full by Pilon-Smits et al. (*15*) and Zhu et al. (*16*, *17*)) were tested in the field by growing them in poor-quality drainage sediment. The transgenic *B. juncea* lines, which overexpressed genes encoding the enzymes adenosine triphosphate sulfurylase (APS), γ-glutamyl-cysteine synthetase (ECS), and glutathione synthetase (GS), accumulated 4.3-, 2.8-, and 2.3-fold more Se in their leaves than wildtype plants, respectively ($p < 0.05$).

The research program on the genetic engineering of Indian mustard has been continued with the development of two new lines, selenocysteine lyase (cpSL, *18*) and selenocysteine methyltransferase (SMT, *19*), respectively (Figure 1). The cpSL transgenic plants were developed in order to diminish the toxic, nonspecific incorporation of selenocysteine into proteins. This is achieved by virtue of the fact that selenocysteine lyase breaks down selenocysteine into elemental Se and alanine (Figure 1). The mouse SL enzyme was chosen because of its reported high activity toward SeCys but negligible activity toward cysteine (*20*). Therefore, by introducing the mouse SL gene in the plant it was hoped to manipulate Se fluxes without interfering with S metabolism. The SMT transgenic plants (which overexpress a gene cloned from a Se hyperaccumulator, *Astragalus bisulcatus*) were developed to generate fast-growing Indian mustards with

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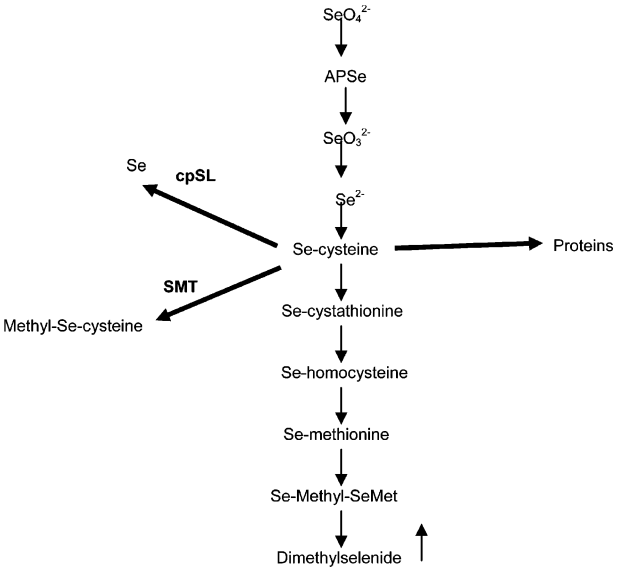

FIGURE 1. Simplified schematic overview of selenium assimilation in plants. The inorganic forms of selenium, selenate and selenite, are reduced to selenocysteine. Overexpression of SMT results in the methylation of selenocysteine. Overexpression of cpSL converts selenocysteine into elemental selenium and alanine. Other metabolic fates of selenocysteine are the production of selenomethionine and the eventual conversion to the substantially less toxic, volatile dimethylselenide or the highly toxic (nonspecific) incorporation of selenocysteine into proteins (not shown). Overexpression of cpSL and SMT were two strategies used to outcompete the latter, toxic process.

the ability to hyperaccumulate Se by methylating selenocysteine into nontoxic methylselenocysteine (Figure 1, *19*). In the present study, the cpSL and SMT transgenics were tested for the first time in the field to assess the potential ability of these newly obtained transgenic Indian mustard lines to phytoremediate highly Se-contaminated sediment under real environmental conditions.

Experimental Section

Field Characteristics. Drainage sediment was collected (Aug 30, 1999) at 0−25 cm depth from the San Luis Drain, Mendota, CA, and spread to a depth of 25 cm in a previously excavated field plot at the USDA-ARS research facility in Parlier, CA. Ten cm of good-quality soil (sandy loam; pH 7.2, EC 0.9 dS/m) was applied as a topsoil to enhance plant survival and to encourage biological activity. Under field conditions, the sediment soil is exposed to natural weathering and wetting/drying conditions, which influence speciation, mobility, and volatilization of Se. The topsoil and sediment were mechanically mixed by a tractor that was used for constructing twenty beds (33 m long and 1 m wide) on the field sediment plot. Adjacent to the sediment plot, a non-sediment field plot (control) of the same dimensions was constructed on a sandy loam soil. A surface drip irrigation system was installed that consisted of in-line turbulent flow emitters with a 4 L/h emitter discharge rate and an emitter spacing of 0.45 m for both the sediment and control field plots. All plants were surface-drip irrigated in part based upon weather data collected from a CIMIS weather station located less than 1 km away at the University of California Kearney Research Station, Parlier, CA, and from estimated crop water use data collected from plants grown on control plots. Additional water was periodically applied to the plants during the Spring planting because of the excessive salt precipitate forming on soil surface.

Plant Material. Indian mustard (*Brassica juncea*; Accession no. 173874) seeds were initially obtained from the North Central Regional Plant Introduction Station, Ames, IA, and propagated. The transgenic lines were obtained by transformation of this genotype with different DNA constructs. The (homozygous) transgenic lines were tested only under laboratory conditions and selected previously for high expression of the introduced gene. Transgenic *B. juncea* cpSL plants were obtained by overexpression of the mouse (*Mus musculus*) selenocysteine lyase (SL) cDNA fused to the transit sequence of ferredoxin from *Silene pratensis*, under the control of the constitutive CaMV 35S promoter, as described by Pilon et al. (*20*) and Garifullina et al. (*18*). Transgenic SMT plants were obtained as described by LeDuc et al. (*19*) by expression of the *Astragalus bisulcatus* SMT gene (encoding the enzyme selenocysteine methyltransferase), driven by the 35S CaMV promoter. For this field study, one representative line was chosen for each type of transgenic (SMT 3−11 and cpSL C3), as well as untransformed *B. juncea*.

Plant Growth Conditions. Transgenic and wildtype plants were planted during two different growing seasons (Spring vs Fall) at two different locations on the sediment field plot during 2004 on the USDA-ARS Research Facility in Parlier, CA. For the Spring planting, the following lines were evaluated: untransformed *B. juncea* (WT) and *B. juncea* overexpressing selenocysteine methyltransferase (SMT) and chloroplastic selenocysteine lyase (cpSL). For the Fall planting, the SMT and WT lines were evaluated. The plants were initially grown in germination trays under controlled greenhouse conditions at 24 ± 2 °C with an average photosynthetic flux of approximately 450 μmol m^{-2} s^{-1}. The plantlets were lightly fertilized with a non-sulfur-containing ammonium nitrate fertilizer (15-15-15) and hardened by increasing the temperature to 32 ± 2 °C during both growing seasons and by reducing the amount of irrigation water. For the Spring and Fall plantings, 25 d old plants were ready to transplant from the greenhouse to the sediment and control field plots in May and in September 2004, respectively. Two beds were randomly selected from the sediment and control plots, respectively, for each growing season. Each bed was divided into two blocks. Within each block, four 2.3 × 1 m plots (Spring planting) and four 3.3 × 1 m plots (Fall planting) were randomly designated for the transgenic lines, the untransformed wild type Indian mustard, and the bare plot, respectively. Each planted bed in sediment and control field sites was surrounded by a 1 m high chicken wire fence to prevent entry by rabbits, birds, and other animals. Initial and final plant densities were between 30 and 35 plants (Spring planting) and 45 and 50 plants (Fall planting) per plot planted in double rows with 10−15 cm spacing between plants. The difference in the total number of plants planted was due to the fact that fewer transgenic seed were available for the Spring planting compared with the Fall planting. Prior to transplanting, a low rate of fertilizer was applied to the sediment soil (50 kg N ha^{-1}), and soil samples were taken in duplicate from each plot at depths of 0−25 and 25−50 cm for each growing season. Due to the anticipated short growing season, we did not expect to observe measurable differences in Se concentrations in the soil; hence, soil samples were collected only at pre-plant and not at harvest. Safer Insecticide Soap (containing 49% potassium salt of fatty acids as the active ingredient) was sprayed on all plants every 2−3 days to reduce insect infestation of aphids and spider mites for both growing seasons. Climatic conditions are presented as averages for each growing season as follows: Spring, temperature of 29 °C, solar radiation of 650 Lux, and zero precipitation; Fall, temperature of 23 °C, solar radiation of 310 Lux, and 51 mm precipitation.

Collection of Volatile Se. During each respective growing season, measurements of volatile Se production were taken 4 days a week from each line for at least 6 weeks. The chambers used for volatile Se collection were made of 6.6

mm thick Plexiglas and had dimensions of 0.71 m long, 0.71 m wide, and 0.76 m high, as described in detail by Lin et al. (*21*). Each chamber enclosed an area of 0.5 m^2 and had an internal volume of 0.38 m^3. Volatile Se was trapped in a series of three 500 mL gas-washing bottles (Corning 31760C) containing 200 mL of alkaline peroxide trap solution (6% H_2O_2 and ~0.05 M NaOH). The gas-washing bottles were connected to the outlet port of each chamber and to each other with Teflon tubing. The volatile Se produced inside each chamber was captured by pulling air out of the chamber through the trap solution with a $^1/_3$-horsepower vacuum pump. Volatile Se was sampled for 24 h continuously for each measurement, after which the solutions from the three gas-washing bottles were collected and taken to the lab for Se analysis (*22*). Volatilization of Se never exceeded 25 μg Se m^{-2} d^{-1} during both growing seasons. As there were no significant differences between plant lines, individual data points are not included for either growing season.

Plant Harvesting. Prior to harvest, plants were monitored daily and debudded manually to prevent flowering as much as possible. As mandated by the USDA-Animal and Plant Health Inspection Service (USDA-APHIS), all plants were harvested when 25% of any transgenic line flowered, which was 45 days after transplanting for the Spring planting and 73 days for the Fall planting. Warmer weather and higher soil salinity and B levels likely contributed to an earlier flowering during the Spring planting. At harvest, plant population density was counted in two 1 m^2 sections randomly selected from each plot. Plants were harvested by removing stems and leaves (shoots) and separating them from the roots 2 cm above the soil surface. Upon harvesting the roots, we observed that most of the root distribution was located in the 0−25 cm depth with some finer roots located in the 25−50 cm depths. Harvested plant material was washed with deionized water, oven-dried at 50 °C for 7 days, weighed, and ground in a stainless steel Wiley Mill equipped with a 1-mm screen. A low drying temperature of 50 °C was selected to reduce any potential loss of Se through volatilization during sample dehydration.

Elemental Analysis. Pre-plant sediment samples were collected and composited from within each plot for each respective depth, dried at 50 °C for 7 days, and ground to pass an 850 μm sieve. Due to sulfur's chemical similarity with Se and the potential toxicity of B, Na, and Cl to normal plant growth, these ions were also determined in plant and soil samples (as described below). Water-soluble fractions of soil Se, B, S, Na, and electrical conductivity (EC) were determined from a sediment/soil water extract of approximately 1:1. The water extractable fraction of Se was likely immediately available for plant uptake. Total Se (the total reservoir of Se present in sediment) was determined in a 500 mg ground sediment sample after wet acid digestion with nitric acid, hydrogen peroxide, and hydrochloric acid (*23*). Other forms of Se, e.g., selenite, organic Se, or elemental Se, were not specifically identified; however, they are included as part of the total Se identified. Plant samples (leaves, stems, and roots) were acid digested with nitric acid, hydrogen peroxide, and hydrochloric acid as described by Banuelos and Akohoue (*24*). Selenium concentrations in plant and soil, including trapped volatile Se, were analyzed by an atomic absorption spectrometer (Thermo Jarrell Ash, Smith Hieftje 1000, Franklin, MA) with an automatic vapor accessory (AVA 880). B, S, and Na were analyzed using an inductively coupled plasma spectrometer (Perkin-Elmer Plasma 2000 Emission spectrometer, Norwalk, CT). External quality control standards for soils, soil extract, and plant tissue samples were obtained from The National Institute of Standards and Technology (NIST). They included wheat flour (SRM 1567; Se content of 1.1 ± 0.2 $\mu g\ g^{-1}$ DM, 94% recovery) and internal soil standards (sediment collected from Kesterson Reservoir, CA) with a total Se content of 7.5 and 25 mg kg^{-1}, 94% recovery, respectively. Soil and plant samples were also acidified with nitric acid and extracted with 2% acetic acid, respectively, and analyzed for Cl by potentiometric titration with silver nitrate. The model 160 conductivity/salinity meter measured soil EC in the soil extract. The software package JMP-IN from the SAS Institute (Cary, NC, *25*) was used to analyze the data by means of analysis of variance and least mean comparisons between the wild type Indian mustard and transgenic lines.

Results

Selenium Accumulation and Volatilization. Selenium accumulation (i.e., total Se per shoot) was significantly enhanced in the shoots of the cpSL and SMT transgenic plants, compared to WT. In the Spring planting, the cpSL transgenic line accumulated 2-fold more Se in their shoots than WT (Figure 2a bottom); this was accompanied by 1.8- and 1.4-fold increases in the Se concentrations of cpSL leaves and stems, respectively (Figure 2a top). The SMT transgenic line in the Spring trial also had significantly higher leaf Se concentrations (1.7-fold more). Although the SMT plants accumulated 1.6-fold more shoot Se than WT, the variation was large, and the increase was not statistically significant ($p > 0.05$). For this reason, we decided to test the SMT transgenic line again in the Fall field trial. The results from the Fall planting showed conclusively that the SMT transgenics performed better than WT; they accumulated 1.6-fold more Se in their shoots (Figure 2b, bottom) and had 1.8-fold higher Se concentrations in leaves and stems (Figure 2b, top). Concentrations and accumulation of Se did not significantly differ in the roots between the transgenic lines and the WT (except SMT in the fall) for either growing season (Figure 2a, top and bottom).

Rates of Se volatilization were generally quite low (<25 $\mu g/m^2/day$) and did not differ significantly among the plant lines for either of the two growing seasons; consequently, the data are not presented here. However, biological volatilization of Se via both plant and microorganism activity would likely be more significant under more favorable conditions in the upper sediment profile, such as more organic matter or soil moisture.

Growth and Tolerance. The Indian mustard plant lines generally grew at substantially lower rates on the Se-, boron-, and salt-contaminated sediment-soil when compared to their growth on control soils for both Spring and Fall plantings (Figure 3). For all lines, most of the root biomass was observed to be between 0 and 25 cm, although some root growth was observed between 25 and 50 cm. In the Spring planting, the cpSL transgenic line attained the greatest shoot biomass among all lines on sediment-soil (Figure 3). Plants of the cpSL line grown on sediment-soil attained a total aboveground shoot biomass that was 71% of that of cpSL plants grown on control soil (Figure 2). In comparison, WT plants achieved only 59% of control shoot biomass when grown on sediment-contaminated soil ($p < 0.01$; Figure 3). SMT plants grew significantly better on control soil than either WT or cpSL plants; however, the relative growth of SMT plants on contaminated soil was 51% of that on control soil ($p < 0.01$).

In the Fall trial, all plant lines grew much better than in the Spring trial ($p < 0.01$; Figure 3), probably because of more favorable weather conditions, such as cooler temperatures and increased precipitation. In the Fall planting, there were no significant differences in growth between WT and SMT plants on either control or sediment-contaminated soil (Figure 3): WT plants achieved 85% of control shoot biomass when grown on sediment-soil, while the SMT line achieved 76% (Figure 3).

Accumulation of Potentially Toxic Ions with Respect to Soil Characteristics. Pre-plant concentrations of total and

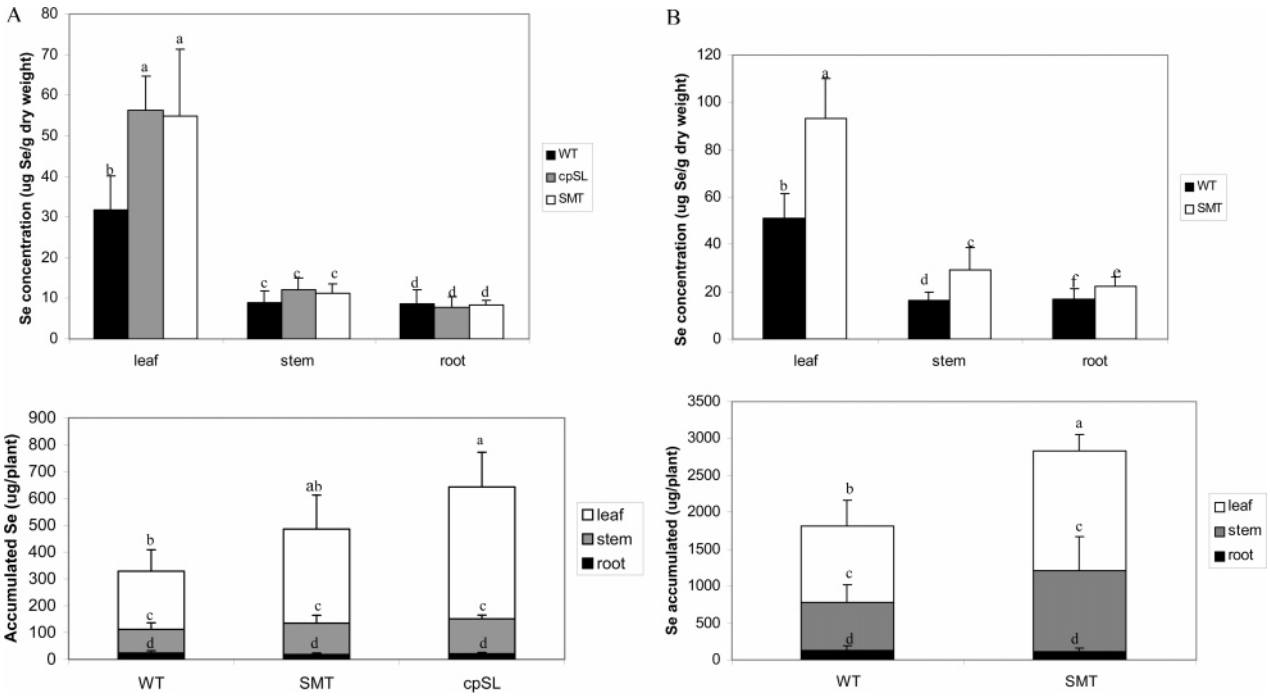

FIGURE 2. (A) Se concentrations (top panel) and amount of Se accumulated (bottom panel) in leaves, stems, and roots for WT, SMT, and cpSL plants in the Spring planting. Values represent the means and standard deviation from four blocks. Each block consisted of a sample composed of a minimum of 30 plants. Bars with the same letter are not significantly different at the $p < 0.05$ level between plant lines for that tissue. In the bottom graph, the letters above the bars refer to both leaf Se accumulation and total shoot Se accumulation. (B) Se concentrations (top panel) and amount of Se accumulated (bottom panel) in leaves, stems, and roots for WT and SMT plants in the Fall planting. Values represent the means and standard deviation from four blocks. Each block consisted of a sample composed of a minimum of 30 plants. Bars with the same letter are not significantly different at the $p < 0.05$ level between plant lines for that tissue. In the bottom graph, the letters above the bars refer to both leaf Se accumulation and total shoot Se accumulation.

extractable Se, EC values, and extractable B concentrations were generally greater at the 25−50 cm depth compared to 0−25 cm (Table 1). Overall, the plots used for the Spring planting were more saline (to the extent that white salt precipitates formed on the soil surface) and contained greater concentrations of water soluble B than those of the Fall planting (see Table 1). Among the potentially toxic ions tested, concentrations of leaf and stem B and accumulated leaf B were greatest in the Spring trial, and greatest for accumulated stem B in the Fall trial. Concentrations of Na and S in the leaves and stem were generally similar between both trials, while leaf Cl was greater in the Spring trial. Accumulated totals of Na, S, and Cl in both leaves and stem were, however, greatest in the Fall trial (Table 2).

Discussion

The results of the present work are important because they provide evidence, for only the second time ever, that it is possible to genetically engineer Indian mustard to enhance its ability for Se phytoremediation under field conditions. As with the three transgenic lines of the earlier work (*12*), the two new transgenic lines, cpSL and SMT, performed significantly better than wild type. When the plants were grown in the Se-contaminated sediment-soil, shoots of the cpSL and SMT transgenics accumulated 2-fold more Se in the Spring planting and 1.6-fold more in the Fall planting than wild type, respectively. In addition, the cpSL plants appeared to be more tolerant of the toxic sediment-soil than the other lines or wild type; this is based on the fact that the cpSL plants, despite having higher Se concentrations, exhibited a 1.2-fold increase in biomass compared to WT (Figure 2). However, despite the fact that SMT transgenic lines have previously been shown to volatilize substantially more Se than WT in laboratory experiments (*19*), we were not able

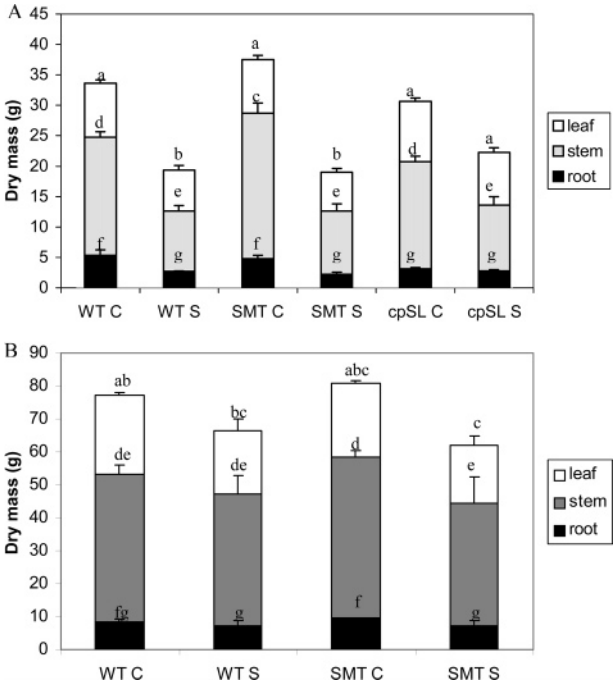

FIGURE 3. Plant dry weight. Dry weights of leaves, stems, and roots of plants grown on clean soil (C) and on contaminated sediment-soil (S). Values represent the means and standard deviations from four blocks. Each block consisted of a composite sample composed of a minimum of 30 plant samples. Note the difference in scales in A (Spring planting) versus B (Fall planting). Bars with the same letter are not significantly different at the $p < 0.05$ level between plant lines for that planting and plant part.

TABLE 1. Mean Values for Total and Extractable Se and Other Chemical Parameters from 0 to 50 cm Depth in Sediment and Control Plots Prior to Spring and Fall Plantings

treatment	total Se (μg g^{-1})	water-extractable concentrations (μg mL^{-1}) Se	S	Na	B	Cl	EC (mS cm^{-1})	pH
Spring Trial								
0−25 cm								
control soil	0.14 ± 0.02[b]	0.030 ± 0.008	330 ± 20	127 ± 20	1.00 ± 0.02	96 ± 10	0.75 ± 0.06	7.10 ± 0.08
bare soil	7.40 ± 2.78	3.332 ± 2.062	1180 ± 200	1422 ± 438	13.40 ± 2.60	709 ± 332	8.10 ± 1.99	8.18 ± 0.08
WT[a]	6.14 ± 2.67	4.105 ± 2.499	2200 ± 650	4100 ± 2757	19.08 ± 1.70	1949 ± 1636	12.97 ± 3.68	8.54 ± 0.25
cpSL[a]	6.54 ± 3.97	3.060 ± 1.982	1800 ± 220	2564 ± 712	16.03 ± 5.45	982 ± 540	10.00 ± 2.09	8.25 ± 0.32
SMT[a]	10.58 ± 5.69	5.840 ± 2.790	2890 ± 890	4546 ± 3904	27.50 ± 2.07	1861 ± 679	16.78 ± 6.07	8.42 ± 0.28
25−50 cm								
control soil	0.23 ± 0.03	0.028 ± 0.005	470 ± 20	172 ± 7	1.0 ± 0.02	229 ± 9	0.85 ± 0.06	7.23 ± 0.05
bare soil	10.04 ± 2.45	3.730 ± 1.308	1550 ± 260	2377 ± 595	12.55 ± 0.93	1445 ± 342	12.52 ± 2.45	8.22 ± 0.10
WT[a]	9.00 ± 3.11	6.077 ± 1.900	2680 ± 570	4549 ± 1685	20.32 ± 3.24	2820 ± 1662	17.99 ± 6.08	8.48 ± 0.06
cpSL[a]	8.18 ± 4.80	4.483 ± 2.807	2800 ± 580	3929 ± 3010	12.57 ± 2.54	1689 ± 1302	10.68 ± 1.59	8.15 ± 0.29
SMT[a]	9.93 ± 5.11	5.870 ± 2.193	2440 ± 1550	6613 ± 2942	18.10 ± 4.81	4113 ± 2783	13.49 ± 5.46	8.17 ± 0.28
Fall Trial								
0−25 cm								
control soil	0.12 ± 0.01	0.023 ± 0.005	260 ± 10	94 ± 5	1.00 ± 0.01	85 ± 7	0.60 ± 0.08	7.15 ± 0.06
bare soil	4.93 ± 1.84	1.900 ± 0.408	910 ± 190	1007 ± 366	5.52 ± 2.29	492 ± 168	5.71 ± 1.14	8.04 ± 0.03
WT[a]	4.53 ± 1.13	2.775 ± 1.763	990 ± 270	1426 ± 713	6.40 ± 1.99	937 ± 530	7.45 ± 2.65	8.05 ± 0.02
SMT[a]	6.05 ± 0.37	3.600 ± 0.887	1040 ± 220	1642 ± 748	6.80 ± 1.12	1087 ± 671	8.49 ± 3.03	8.03 ± 0.05
25−50 cm								
control soil	0.26 ± 0.03	0.028 ± 0.005	420 ± 20	161 ± 11	1.26 ± 0.50	194 ± 5	0.90 ± 0.08	7.15 ± 0.06
bare soil	4.30 ± 1.38	2.800 ± 0.762	980 ± 130	1340 ± 306	7.20 ± 1.69	729 ± 196	7.02 ± 1.10	8.01 ± 0.02
WT[a]	5.45 ± 2.15	3.750 ± 1.763	1200 ± 280	1839 ± 762	8.68 ± 2.80	1058 ± 523	8.93 ± 2.57	8.04 ± 0.07
SMT[a]	6.68 ± 1.80	4.100 ± 1.236	1180 ± 240	1868 ± 671	8.43 ± 1.13	1100 ± 600	9.15 ± 2.65	8.02 ± 0.04

[a] WT, wild-type Indian mustard; cpSL, chloroplastic selenocysteine lyase overexpressing Indian mustard; SMT, selenocysteine methyltransferase overexpressing Indian mustard; bare soil (unplanted). [b] Values represent the means and standard deviations from four blocks.

TABLE 2. Concentrations and Accumulated Totals of Total S, Na, B, and Chloride (Cl^-) in Shoot Tissue of Wild Type, CpSL, and SMT Indian Mustard Plants Grown in Contaminated Sediment-Soil

	Leaf Tissue Concentrations				Leaf Tissue Accumulated Totals			
		mg/g			mg			
Plant Line	B μg/g	Na	S	Cl	B	Na	S	Cl
				Spring				
cpSL[a]	741 ± 124[ab]	16 ± 3 [efg]	21.2 ± 2.8 [h]	28.0 ± 2.7 [l]	6.4 ± 1.26 [n]	139 ± 28 [s]	184 ± 42 [v]	241 ± 32 [y]
SMT[a]	854 ± 248 [ac]	17 ± 8 [e]	20.3 ± 6 [h]	33.1 ± 3.4 [kl]	5.39 ± 1.54 [n]	104 ± 41 [s]	127 ± 28 [vw]	208 ± 6 [y]
WT[a]	868 ± 115 [a]	9 ± 2 [g]	18.2 ± 2.3 [hi]	26.1 ± 3.5 [l]	5.69 ± 1.20 [n]	60 ± 19 [s]	123 ± 29 [vw]	175 ± 29 [y]
				Fall				
SMT[a]	133 ± 22 [b]	14 ± 2 [fg]	21.0 ± 1.2 [h]	39.6 ± 4.8 [k]	2.33 ± 0.3 [o]	239 ± 39 [r]	373 ± 53 [tu]	708 ± 151 [x]
WT[a]	121 ± 25 [b]	13 ± 3 [efg]	21.5 ± 1.6 [h]	36.7 ± 3.7 [k]	2.43 ± 0.9 [o]	251 ± 52 [r]	424 ± 100 [t]	708 ± 83 [x]

	Stem Tissue Concentrations				Stem Tissue Accumulated Totals			
		mg/g			mg			
	B μg/g	Na	S	Cl	B	Na	S	Cl
				Spring				
cpSL[a]	36 ± 6 [bc]	6 ± 2 [f]	5.9 ± 1.1 [ij]	12.8 ± 2.4 [lm]	0.39 ± 0.08 q	69 ± 5 [s]	64 ± 16 [v]	137 ± 23 [z]
SMT[a]	41 ± 11 [b]	12 ± 3 [e]	8.1 ± 1.6 [h]	21.0 ± 4.7 [k]	0.43 ± 0.14 q	124 ± 38 [s]	85 ± 23 [v]	222 ± 65 [z]
WT[a]	55 ± 19 [a]	7 ± 5 [f]	6.7 ± 0.2 [i]	14.8 ± 0.8 [lm]	0.54 ± 0.18 q	71 ± 50 [s]	70 ± 7 [v]	147 ± 20 [z]
				Fall				
SMT[a]	20 ± 1 [d]	1 ± 1 [ef]	5.6 ± 0.8 [ij]	17.1 ± 1.9 [k]	0.75 ± 0.15 [op]	353 ± 100 [r]	207 ± 59 [tu]	635 ± 173 [x]
WT[a]	22 ± 2 [d]	7 ± 2 [f]	5.1 ± 0.3 [j]	12.9 ± 2 [lm]	0.86 ± 0.1 [no]	273 ± 38 [r]	203 ± 290 [u]	505 ± 70 [xy]

[a] WT, wild-type Indian mustard; cpSL, chloroplastic selenocysteine lyase overexpressing Indian mustard; SMT, selenocysteine methyltransferase overexpressing Indian mustard. [b] Values represent the means and standard deviations from four blocks. Sample size consisted of a composite sample from 30 plants for each respective block. [c] Means followed by the same letter are not significantly different at the $p < 0.05$ level within each column.

to detect any differences in volatilization rate among all plant lines in the field.

Bearing in mind that the main objective of Se phytoremediation is to reduce Se levels in Se-contaminated soil/sediment, we thought it would be useful to develop estimates of the time required for the different plant lines to "clean up" soluble Se from the major root zone (i.e., 0−25 cm depth). For the Spring trial, we calculated that a volume of soil 1 m^2 in area and 0−25 cm deep (total soil dry weight of 208 kg m^{-2}) with a mean Se concentration of 4 mg Se L^{-1} (from Table 1) should contain 832 mg of available Se. The highest mass of Se accumulated in the aboveground plant material for the transgenic plants (i.e., Se mass = yield of 30 plants m^{-2} × shoot Se concentration) was 18.6 and 81.0 mg Se m^{-2} in the Spring and Fall, respectively. In contrast, the wild type Indian mustard accumulated 6.8 and 50 mg Se m^{-2} for the

Spring and Fall, respectively. Hence, over both the Spring and Fall growing seasons, the transgenics accumulated 99.6 mg Se m^{-2}, and the wild type Indian mustard accumulated 56.8 mg Se m^{-2}, respectively, in 1 year. Using these values, it would take the transgenic and wild type Indian mustard 8.4 and 14.6 years, respectively, to completely remove soluble Se from 0−25 cm contaminated soil, if there were no additional contributions of soluble Se from below 25 cm zone due to upward migration. These conservative approximations do not include potential losses of soluble Se occurring via biological volatilization, leaching, species transformation, and changes in plant Se accumulation.

The physiological mechanism for the superior performance of the cpSL and SMT transgenic lines may be related to the fact that both lines more effectively detoxify selenocysteine (SeCys) than wildtype. The cpSL line overexpressing mouse selenocysteine lyase in its chloroplasts and the SMT line overexpressing selenocysteine methyltransferase convert SeCys into elemental Se and methylSeCys, respectively. This prevents the misincorporation of SeCys (and eventually selenomethionine) into proteins, a process that is believed to cause Se toxicity (*26*). Earlier laboratory experiments also support the idea that the cpSL and SMT transgenic lines detoxify SeCys more efficiently than wildtype (*18, 19*). CpSL transgenic seedlings accumulated up to 50% higher Se levels than wild type. Even at a high level of supplied Se, the cpSL lines contained approximately 40% less Se in proteins than wild type, suggesting that nonspecific incorporation of SeCys into protein was reduced in these transgenic lines (*18*). SMT lines also demonstrated increased tolerance, while accumulating up to three times as much Se as wild type plants (*19*). The increased selenocysteine methyltransferase activity in SMT plants resulted in greater accumulation of the nontoxic MetSeCys, up to 10 times more than wild type (*19*).

All plants accumulated significantly more Se during the Fall planting than during the Spring planting (Figure 2). The greater Se accumulation in the Fall was associated with increases in both Se concentration and biomass. Leaves and stems from WT and SMT lines in the Fall planting had 60−80% higher Se concentrations than those in the Spring planting (Figures 2 and 3). The superior biomass accumulation in the Fall was almost certainly due in large part to a substantially longer duration of growth (10 weeks for Fall vs 6 weeks for Spring). However, it is also likely that plants grew better in the Fall because they were subjected to less environmental stress: for example, plants grown in the Spring were exposed to higher soil salinity levels at preplant and exhibited signs of B toxicity along the leaf margins, while no visible symptoms of B or salt toxicity were observed in the Fall trial plants. The higher concentrations of B in leaves and stems of all plant lines in the Spring versus Fall supports the view that B is likely to be the factor most responsible for lower yields in the Spring trial (Table 2).

In conclusion, the present field trial along with the earlier one (*12*) has resulted in the identification of five distinctly different transgenic lines of Indian mustard that are able to accumulate from ∼2- to 4.8-fold more Se than wild type (*12*). These findings are especially promising because the transgenic plants were in the ground for very limited growth periods, as mandated by USDA-APHIS requirements on the testing of transgenic plants in the field. It is also encouraging that the transgenic lines were able to perform well even under field conditions that are highly variable and quite harsh for normal biological activities. Thus, the results of both field trials support the view that the genetic engineering of Indian mustard has a significant potential for enhancing Se phytoremediation.

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