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Technical Note

Sulfur-responsive promoter of sulfate transporter gene is potentially useful to detect and quantify selenate and chromate

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Abstract Plant-based assays for monitoring contaminated environments provide inexpensive and nontechnical means of environmental analysis. Here we report a model system for monitoring selenium and chromium, which are highly toxic heavy metals for living organisms. The major forms of selenium and chromium in nature are selenate and chromate. As toxic analogs of sulfate, they cause sulfur deficiency in plants by inhibiting the uptake of sulfate from the environment. We used a fusion gene construct consisting of a sulfur-responsive promoter region of the high-affinity sulfate transporter *SULTR1;2* from *Arabidopsis* and green fluorescent protein (GFP; $P_{SULTR1;2}$-*GFP*) to quantify the levels of selenate and chromate by GFP accumulation. The $P_{SULTR1;2}$-*GFP* transgenic *Arabidopsis* plants showed drastic increases in GFP with the addition of selenate or chromate to the medium. The increase in GFP was concentration-dependent relative to the amounts of contaminants in the medium, suggesting the potential of $P_{SULTR1;2}$-*GFP* plants as indicators in quantifying environmental selenate and chromate.

Key words: Chromate, environmental monitoring, selenate, sulfate transporter, SULTR1;2.

Heavy metals are highly toxic for living organisms when excess amounts exist in the environment (Salt et al. 1998; Kovalchuk et al. 2001). Soils and water contaminated with heavy metals cause major environmental and human health problems. During the last few decades, phytoremediation and plant-based assays for cleanup and monitoring of these contaminated environments have been developed (Salt et al. 1998; Kovalchuk et al. 2001; Krizek et al. 2003; Pilon-Smits 2005). These methods take advantage of the sedentary nature of plants, providing inexpensive and nontechnical means of environmental analysis and restoration (Kovalchuk et al. 2001; Krizek et al. 2003).

Among heavy metals, selenium and chromium function as toxic analogs of sulfur, and they exist in nature mainly as selenate (SeO_4^{2-}) and chromate (CrO_4^{2-}) anions at +6 oxidation states. Because of their structural similarities to sulfate (SO_4^{2-}), selenate and chromate are readily imported into plant cells through the activities of sulfate transporters. In fact, selenate competitively inhibits the influx of sulfate, and after incorporation into the cells, it is subsequently assimilated into seleno-cysteine and seleno-methionine by sharing the pathways of sulfur metabolism; ultimately, these selenium-containing amino acids are incorporated into proteins (Terry et al. 2000; Ellis and Salt 2003). Chromate also has an inhibitory effect on sulfate uptake, but is mainly reduced to +3 oxidation states, Cr^{3+} or Cr_2O_3, presumably by using reducing cofactors. Accordingly, excess amounts of selenate and chromate taken up from the environment can affect the cellular function of living organisms and sometimes have lethal effects. Indeed, several mutants lacking sulfate transporter genes have been isolated from yeast and plants by using the toxic effects of selenate and chromate as sulfate analogs (Smith et al. 1995a, b; Cherest et al. 1997; Shibagaki et al. 2002).

In *Arabidopsis*, two high-affinity sulfate transporters, SULTR1;1 and SULTR1;2, facilitate the initial uptake of sulfate into the roots (Takahashi et al. 2000; Vidmar et al. 2000; Shibagaki et al. 2002; Yoshimoto et al. 2002). These transporters are expressed in the epidermis and cortex of roots, and their transcripts can accumulate through sulfur depletion to maximize sulfate uptake for efficiently using limited amounts of sulfur in the soil environment (Takahashi et al. 2000; Vidmar et al. 2000; Shibagaki et al. 2002; Yoshimoto et al. 2002). The expression of these *SULTR*s depends on the promoter activities of their 5′-regions controlled by sulfur deficiency (Maruyama-Nakashita et al. 2004a, b, 2005). The presence of a specific sulfur-responsive *cis*-element in the *SULTR1;1* promoter region further elaborates the

Abbreviations: GFP, green fluorescent protein; GSH, reduced glutathione; SULTR, sulfate transporter.
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molecular mechanism of its regulation (Maruyama-Nakashita et al. 2005). In addition to sulfur limitation, selenate treatment induces the transcripts of *SULTR1;1* and *SULTR1;2* (Takahashi et al. 2000; Yoshimoto et al. 2002). Selenate treatment reduces the cysteine and reduced glutathione (GSH) contents in plants and induces the accumulation of sulfate transporters that facilitate the uptake and translocation of sulfate from roots to shoots (Takahashi et al. 2000; Yoshimoto et al. 2002). Selenate treatment appears to mimic sulfur-deficient stress by disturbing sulfur metabolism (Ellis and Salt 2003; Van Hoewyk et al. 2005). Alternatively, unknown mechanisms for detoxification of selenium-containing metabolites may promote the assimilatory sulfur metabolism, including transport and reduction processes.

The positive relationship between selenate treatment and the expression of *SULTR*s led us to construct a reporter system tracing *SULTR* expression for quantitative monitoring of selenate and chromate in the environment. We previously reported that transgenic plants expressing green fluorescent protein (GFP) under the control of a 2160-bp promoter region of *SULTR1;2*, i.e., $P_{SULTR1;2}$-*GFP* plants, accumulated GFP concomitantly with the increase in internal *SULTR1;2* mRNA levels (Maruyama-Nakashita et al. 2004b). Given this background, we sought to measure the external concentration of selenate and chromate through the fluorescence of GFP in $P_{SULTR1;2}$-*GFP* plants. In this study, we examined the effects of selenate and chromate on the GFP accumulation of $P_{SULTR1;2}$-*GFP* plants to develop a new system for detecting and quantifying the concentration of selenate and chromate in the environment.

T3 progenies of $P_{SULTR1;2}$-*GFP* transgenic plants were used for the experiment. $P_{SULTR1;2}$-*GFP* plants were vertically grown on agar medium (Hirai et al. 1995) at 22°C under a 16-h light/8-h dark cycle. When plants were germinated and grown on the media containing 1, 3, 10, 30, or 100 μM of selenate or chromate, plant growth was significantly inhibited by 10 μM or higher concentrations of selenate and chromate (data not shown). We then tested the effects of selenate and chromate using transfer experiments (Figures 1 and 2). Six-day-old plants were transferred to media containing 0, 1, 3, 10, 30, or 100 μM of selenate or chromate, and 2 days after the transfer, the expression of GFP in $P_{SULTR1;2}$-*GFP* plants was visualized using an image analyzer under 488 nm excitation (FluorImager 595; Molecular Dynamics, Sunnyvale, CA, USA). The GFP and auto-fluorescence of plants were detected using 530DF30 and 610RG filters, respectively. The relative intensities of GFP signals in plant roots were then quantified with ImageQuant Software (Molecular Dynamics).

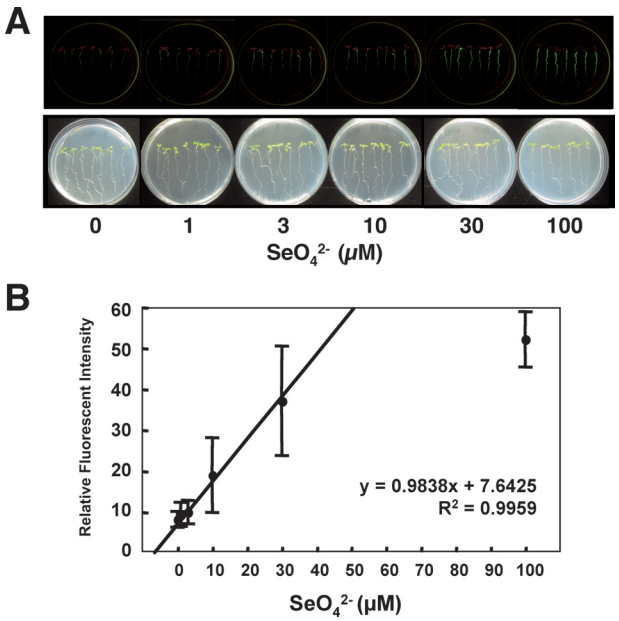

Figure 1. Induction of GFP accumulation in $P_{SULTR1;2}$-*GFP* plants by selenate application. $P_{SULTR1;2}$-*GFP* plants (n=7) were grown for 6 days on control agar medium, and then incubated for 2 days on the control medium or on a medium containing 1, 3, 10, 30, or 100 μM of selenate (Na_2SeO_4). The medium used for the experiment contained 1500 μM sulfate as a sulfur source. (A) The fluorescence of GFP in roots was visualized using FluorImager 595. (B) Relative fluorescent intensities quantified by ImageQuant software. Error bars indicate the standard deviation. The linear equation for the increase in fluorescence between 0 to 30 μM of selenate is indicated.

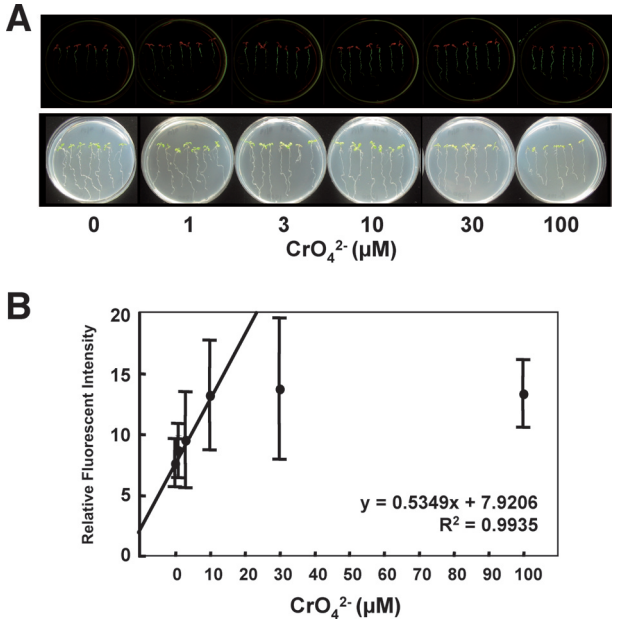

Figure 2. Induction of GFP accumulation in $P_{SULTR1;2}$-*GFP* plants by chromate application. GFP accumulation in $P_{SULTR1;2}$-*GFP* plants (n=7) was detected using FluorImager. Experiments and measurements of GFP fluorescence were performed as described in Figure 1, except that the 2-day exposure to toxic ions occurred on a medium containing 1, 3, 10, 30, or 100 μM of chromate (Na_2CrO_4). (A) Fluorescence of GFP in roots. (B) Quantification of relative fluorescent intensities. Error bars indicate the standard deviation. The linear equation for the increase in fluorescence between 0 to 10 μM of chromate is indicated.

As expected from the increase in *SULTR1;2* mRNA contents (Yoshimoto et al. 2002), selenate treatment caused a drastic increase of GFP in $P_{SULTR1;2}$-*GFP* plants (Figure 1). A significant accumulation of GFP was observed at 10 μM or higher concentrations of selenate (Figure 1A). GFP accumulated linearly relative to selenate concentrations between 1 and 30 μM, which enabled us to estimate the external concentration of selenate (Figure 1B). An increase in GFP was observed with exposure of plants to chromate (Figure 2A). However, the induction of GFP peaked at 10 μM (Figure 2B). Presumably, chromate affects plant viability more severely than selenate, causing a distortion of GFP reporter gene expression in $P_{SULTR1;2}$-*GFP* plants at higher concentrations.

In this study, we experimentally demonstrated the capability of $P_{SULTR1;2}$-*GFP* plants to detect environmental concentrations of selenate and chromate. In particular, a concentration-dependent increase of GFP levels in $P_{SULTR1;2}$-*GFP* plants by selenate suggests their potential for direct use as indicator plants in quantifying selenate and chromate. For practical use, this promoter-reporter system cannot distinguish between selenate and chromate; however detection of either of these heavy metals is useful since both are highly toxic for living organisms. In addition, since this promoter is responsive also to sulfur deficiency and its activity is rather restricted to roots, transfer of laboratory-grown plants to potentially contaminated soil or water supplemented with sufficient amount of sulfate would become necessary in practice. At this stage, the sensitivity of $P_{SULTR1;2}$-GFP plants for selenate and chromate detection is not sufficient to meet with the environmental limitations of selenium and chromium. According to the standards in Japan, the limits are 0.01 mg l^{-1} for Se and 0.05 mg l^{-1} for Cr, respectively, which are equivalent to 0.13 μM of selenate and 0.96 μM of chromate. Both are still less than the detection limit of the current version of $P_{SULTR1;2}$-*GFP* plants (Figures 1 and 2) for experimental use. However, by taking this as a model of a plant-based Se and Cr monitoring system, more sensitive indicators can be designed through arranging the promoter regions and reporter proteins.

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