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ARTICLE ADDENDUM

Plants prioritize phytochelatin synthesis during cadmium exposure even under reduced sulfate uptake caused by the disruption of *SULTR1;2*

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ABSTRACT

Glutathione and phytochelatins are sulfur containing compounds playing an important role in cadmium (Cd) detoxification. We examined the Cd-induced changes in the percentage of sulfur containing compounds to total sulfur in wild-type and *sulfate transporter 1;2* knockout mutant, *sel1–10*. Cd treatment increased the proportion of sulfate and thiols in the total sulfur content. Among the thiols analyzed, the proportion of cysteine and glutathione were decreased by the Cd treatment and that of the phytochelatins were increased. Although the total sulfur content in *sel1–10* was decreased compared with that in wild-type, the percentages of individual thiol in the total thiol content were similarly maintained between *sel1–10* and wild-type, suggesting that plants tightly controlled the balance of each thiol under Cd treatment.

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Cadmium (Cd) is a harmful element for plants, and so plants have developed detoxification mechanisms for conditions of Cd exposure.[1,2,3,4,5,6] Under heavy metal stress, plants synthesize low molecular weight sulfur-containing compounds such as glutathione (GSH) and phytochelatins (PCs).[6,7] PCs are the compounds synthesized from GSH with the structure of $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, which are named depending on the numbers of γ-Glu-Cys residues, such as PC2 for $(\gamma\text{-Glu-Cys})_2\text{-Gly}$, PC3 for $(\gamma\text{-Glu-Cys})_3\text{-Gly}$ and PC4 for $(\gamma\text{-Glu-Cys})_4\text{-Gly}$.[8] In plants like *A. thaliana* that do not accumulate high levels of Cd, detoxification mainly relies on the chelation by GSH and PCs, followed by sequestration of Cd-thiol complexes to the vacuoles.[1,9] Both GSH and PCs are synthesized from cysteine,[8,10] and cysteine is synthesized from sulfate through the sulfate assimilatory pathway.[11,12] SULTR1;2 is a sulfate transporter responsible for sulfate uptake from the roots in *Arabidopsis*.[13,14,15,16,17] We investigated the influence of Cd treatment on the sulfur usage and how sulfur availability contribute to Cd resistance in plants by using a T-DNA insertion mutant of *SULTR1;2, sel1–10*.[17,18]

In *sel1–10*, the growth inhibition under Cd exposure was slightly severe, and Cd level was significantly decreased compared with wild-type (WT). Sulfate uptake activity was increased in WT by Cd treatment, but not in *sel1–10*, indicating that the increased sulfate uptake activity by Cd treatment is due to the presence of SULTR1;2.

Cd treatment significantly increased sulfate, PCs, and the total sulfur content in the shoots; and PCs content in the roots, in both WT and *sel1–10*. The majority of the increased sulfur in shoots was sulfate, and the sulfate content in xylem sap was increased by Cd treatment, indicated that the root-to-shoot sulfate transport was accelerated by Cd treatment. Thus, uptake and transport of sulfate and synthesis of sulfur-containing compounds undergo demand-driven control in Cd treated plants.

The percentage of sulfate and thiols to total sulfur content was increased by Cd treatment

The percentage of sulfate and thiols (cysteine, GSH, PC2, PC3 and PC4) to total sulfur content was calculated (Fig. 1). The shoot and root tissues of WT and *sel1–10* seedlings grown for 10 d on MGRL agar medium containing 0, 20, 40 μM $CdCl_2$ were harvested and then extracted with 10 mM HCl. Using the plant extracts, sulfate contents were determined by ion chromatography, and cysteine, GSH and PCs contents were analyzed by HPLC-fluorescent detection system after labeling of thiol bases by monobromobimane.[18,19] The percentage of sulfate, thiols, and other sulfur containing compounds in total sulfur were calculated on a dry weight basis using the data previously reported.[18] Total sulfur content in *sel1–10* was lower than that in the WT for both shoots and roots in all conditions. Treatment with 20 and 40 μM $CdCl_2$ increased total sulfur content in the shoots of both WT and *sel1–10*. Under the control conditions, 54.8% and 16.2% of total sulfur were attributed to sulfate in the shoots and roots of WT, respectively; and 33.4% and 21.3% in those of *sel1–10*, respectively. Upon Cd treatment, the sulfate content was increased by more than 85% and 40% in the shoots of WT and *sel1–10*, respectively, whereas the sulfate content in the roots was

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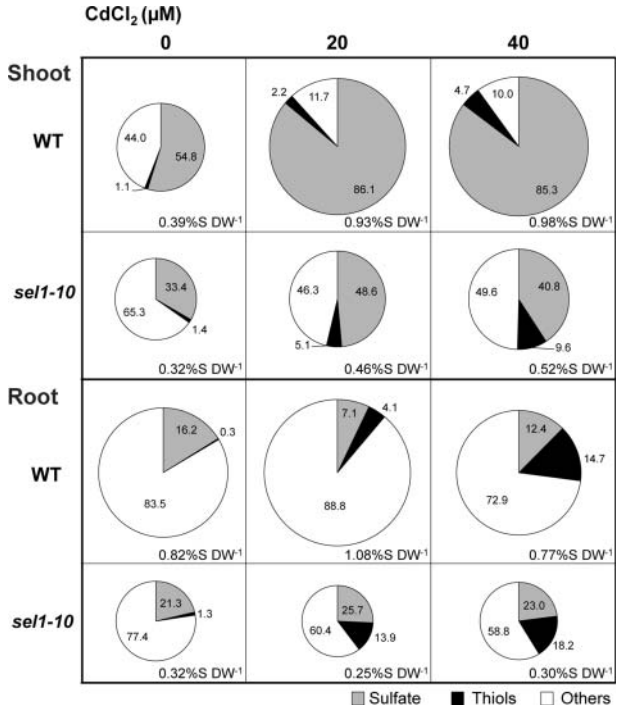

Figure 1. Effects of Cd treatment on the composition of sulfur containing compounds in WT and *sel1–10*. The percentage of sulfate (gray), thiols (black), and other sulfur containing compounds (white) in total sulfur were calculated. Thiols mean the sum of cysteine, GSH, PC2, PC3 and PC4. The numbers on the corners indicate total sulfur content per dry weight.

decreased in WT and not significantly influenced in *sel1–10*. Sulfate was the major chemical form of sulfur in Cd-treated shoots.[18] This Cd-induced increase of sulfate is due to the increased uptake and the root-to-shoot transport.

The contents and percentages of thiols were also increased by Cd treatment in the shoots and roots of both WT and the mutant (Fig. 1). In *sel1–10*, the proportion of thiols was higher than that of WT in control condition, and the induction rate of thiol contents by Cd treatment was higher in *sel1–10* than in WT. The increased percentage of thiols to the total sulfur content was dose dependent, and was particularly pronounced in the roots.

The percentage of individual thiol content was similar between WT and *sel1–10* plants treated with or without Cd

Although the contents and percentages of thiols were different between WT and *sel1–10*, percentages of cysteine, GSH, PC2, PC3, and PC4 in the total thiol content were quite similar between WT and *sel1–10* under all conditions (Fig. 2). Concentrations of cysteine, GSH, PC2, PC3 and PC4 were calculated to the percentage in sum of the thiol contents. In the control conditions, GSH occupied about 92% in shoots, and around 65% in roots of both WT and *sel1–10*. Cysteine occupied second biggest percentage in shoots and roots of WT and *sel1–10*, about 7% and 32%, respectively. Upon Cd treatment, percentage of GSH and cysteine in shoots and roots were decreased, whereas those of PCs were increased. When plants were treated with 20 and 40 μM $CdCl_2$, the percentages of GSH and cysteine were decreased in both plant lines, although their contents were not decreased.[18] The contents and percentage of PCs were

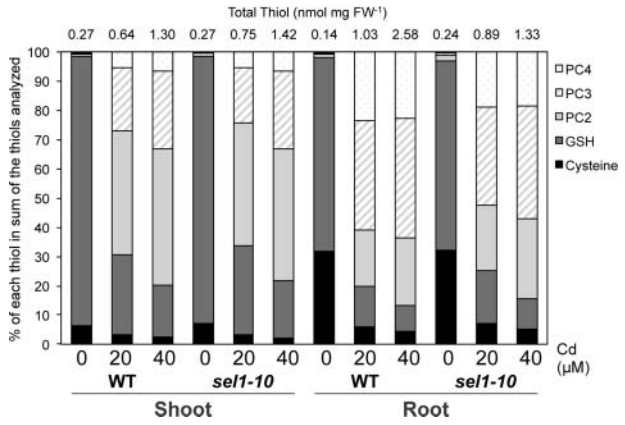

Figure 2. Percentage of each thiol content in the sum of them in WT and *sel1–10* plants treated with Cd. Concentrations of cysteine (black), GSH (gray), PC2 (light gray), PC3 (gray stripe), PC4 (gray dotted) were calculated to the percentage in sum of the thiol contents analyzed which are indicated on the top of each column (nmol mg FW^{-1}).

greatly increased under Cd treatment in both WT and *sel1–10*; in shoots, percentage of PC2 was 42% and 46%, that of PC3 was 20% and 26%, and that of PC4 was 5.5% and 6.6%, respectively, when treated with 20 and 40 μM $CdCl_2$. In roots, percentage of PC2 was 20% and 25%, that of PC3 was 35% and 40%, and that of PC4 was 20% and 19%, in both plant lines treated with 20 and 40 mM CdCl2, respectively.

Even when the sulfur metabolism sifts to PC synthesis, a mechanism to maintain the proportion of thiols seems to exist in both shoots and roots (Fig. 2). These results suggest that GSH and PC synthesis is controlled not only by metabolite levels but also by the ratios of precursors for GSH synthesis, cysteine, and γ-Glu-Cys, and those for PCs, γ-Glu-Cys and GSH. PC2 is also a substrate for PC3 synthesis, and PC3 is that for PC4 synthesis.[8] The balance between substrates and products should be important with respect to the thiol synthesis. To further explore this hypothesis, we need to know the thiol levels in each cellular compartment, and especially in the cytosol.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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