ROOT-APPLIED GLUTATHIONE INHIBITS CADMIUM TRANSLOCATION FROM ROOTS TO SHOOTS SELECTIVELY IN OIL SEED RAPE PLANTS

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Introduction

Cadmium (Cd) is categorized into toxic heavy metal. Cd accumulation in agricultural crops causes serious health problems for us. These days, the maximum acceptable Cd accumulation levels in various crops were determined. It is necessary to elucidate molecular mechanisms of Cd translocation from roots to shoots in order to control Cd accumulation in agricultural crops. However, these mechanisms are not fully understood so far. Glutathione (GSH) is one of thiol-containing peptides, related to various aspects of plant metabolisms. In our previous work, we have found GSH concentration in the phloem sap, collected from oilseed rape plants, responded to Cd treatment (Nakamura et al., 2005). These experimental results suggest that GSH in the sieve tubes increased in response to the request from sink tissues (roots or unexpanded leaves). Therefore, we applied GSH to oilseed rape plants site-specifically. Cd content and Cd long-distance transport in these treated plants were investigated. Additionally, effects of GSH concentration in root zone and GSH and Cd treatment period on Cd behaviors is investigated. We also tried to visualize and analyze root-to-shoot translocation of Cd by using a positron-emitting tracer imaging system (PETIS).

Methods

Oilseed rape plants (var. Nourin No. 16) were grown in a growth chamber under controlled growth conditions, according to the methods of Nakamura et al., 2013. In each experiment, plants were exposed to 10μM Cd ($CdCl_2$). First, effects of GSH, applied to plants site-specifically, on Cd behaviors were investigated. After two days of Cd exposure, plants were harvested and Cd contents in their shoots and roots were determined by using an ICP atomic emission spectrometer. After Cd treatment, xylem sap and phloem sap were collected according to the methods of Nakamura et al., (phloem sap; 2005, xylem sap; 2008). Cd concentrations in these saps were determined by using an atomic absorption photometer. Second, effects of GSH concentration in root zone and GSH and Cd treatment period on Cd behaviors were tested. Third, effects of sulfur containing compounds on Cd behaviors were tested. Obtained data from these experiments were analyzed by Student's t-test. PETIS experiments were started by adding purified ^{107}Cd in nutrient solutions after setting plants in the chamber according to the methods of Fujimaki et al., 2010 with a small modification. Two week old seedlings were used for these PETIS experiments. Imaging data obtained from PETIS experiments were analyzed in detail to investigate effects of GSH on Cd behaviors in plants.

Results

Cd contents in shoots of control plants and plants treated with GSH to their leaves for two days were approximately 0.4 μmol/gDW (dry weight). GSH, applied to leaves, had no effects on Cd behaviors in oilseed rape plants. In contrast, Cd content was about 0.1μmol/gDW in plants exposed to GSH in their root

zone. Root-applied GSH inhibited Cd translocation from roots to shoots significantly. Glutathione, applied to roots, showed no inhibitory effects on Fe, Mn and Zn translocation from roots to shoots. Glutathione, oxidized form, (GSSG) have different effects on Cd behaviors when GSSG was applied to nutrient solutions. Inhibitory effects of Cd translocation from roots to shoots decreased when GSH in root zone were reduced. These inhibitory effects were still seen in plants exposed to 0.1 mM GSH. However, these effects were completely lost when plants were exposed to 0.01 mM GSH. These inhibitory effects were dependent on the Cd and GSH treatment period. Our experimental results revealed that root-applied dithiothreitol (DTT), just as GSH, also had inhibitory effects on Cd translocation from roots to shoots. We succeeded in visualizing Cd behaviors in oilseed rape plants by using a PETIS. Inhibition of root-to-shoot translocation of Cd was visualized clearly. Imaging data also revealed that Cd efflux from root cells was activated by GSH.

Conclusion

Our study demonstrated root-applied GSH inhibits Cd translocation from roots to shoots by activating Cd efflux from root cells. GSH concentration in roots zone have a significant impact on Cd behaviors in oilseed rape plants. GSH, exceeding a certain concentration in the root zone, is required to trigger these inhibitory effects in oilseed rape plants. GSH and DTT have different inhibitory effects on Cd translocation. Further researches enable us to establish the best application conditions of GSH in root zone for suppressing Cd accumulation in agricultural crops.

References

Fujimaki S.; Suzui N.; Ishioka N.S.; Kawachi N.; Ito S.; Chino M.; Nakamura S. (2010) Tracing cadmium from culture to spikelet: non-invasive imaging and quantitative characterization of absorption, transport and accumulation of cadmium in an intact rice plant. *Plant Physiol. 152*, 1796-1806.

Nakamura S.; Maruyama K.; Watanabe A.; Hattori H.; Chino M. (2005) Response of glutathione in the sieve tube of Brassica napus L. to cadmium treatment. In: Saito K, De Kok LJ, Stulen I, Hawkesford MJ, Schnug E, eds. Sulfur transport and assimilation in plants in the Post Genomic Era.: Leiden, NL: Backhuys Publishers, 229-232.

Nakamura S.; Akiyama C.; Sasaki T.; Hattori H.; Chino M. (2008) Effect of cadmium on the chemical composition of xylem exudate from oilseed rape plants (*Brassica napus* L.). *Soil Sci. Plant Nutr. 54*, 118-127.

Nakamura S.; Suzui N.; Nagasaka T.; Komatsu F.; Ishioka N.S.; Ito-Tanabata S.; Kawachi N.; Rai H.; Hattori H.; Chino M.; Fujimaki S. (2013) Application of glutathione to roots selectively inhibits cadmium transport from roots to shoots in oilseed rape. *J. Exp. Bot. 64*, 1073-1081