論 文 の 内 容 の 要 旨

農学国際専攻
平成21年度博士課程　進学
氏　名　ウーゴ　マサヨシ　シモ
指導教員名 山川　隆

論文題目

Identification and characterization of new genes related to cadmium tolerance and accumulation in rice

(イネにおけるカドミウム耐性および蓄積に関連する新規遺伝子の同定とその性質)

1. Cadmium (Cd) pollutant and the molecular biology strategies to mitigate its toxic effects

Cadmium (Cd) is a heavy metal that has been widely released into the environment. Once released into the environment, Cd enters the biogeochemical cycle and tends to accumulate in soils and sediments, where it becomes available to rooted plants. Cd uptake is harmful to most plants; it can reduce plant growth, and even kill plants in extreme cases. Critical problems related to environmental exposure to Cd were reported in Japan in the early 20th century; people consuming contaminated food and water developed 'itai-itai disease', which caused renal abnormalities and weak bones. To suppress Cd pollution, most industrialized nations have set strict limits on Cd exposure, and in 2004 the Joint FAO/WHO Expert Committee on Food Additives, in association with the Codex Alimentarius Commission, established allowable limits for Cd levels in food; for rice, the limit was set at 0.4 mg/kg. The mechanisms that control Cd accumulation in the edible parts of plants are poorly understood. For the general population, the major route of Cd exposure is through food. Rice grains contaminated with Cd represent a major risk to the health of more than half of the world's population, which depends on rice as a basic staple. With the goal of improving food safety, the current study aimed to identify genes related to Cd tolerance and accumulation in rice.

2. Rice screening for Cd phenotype

Insertional mutagenesis is one of the most useful methods for analyzing gene function. When foreign DNA is inserted into a gene, it not only creates a mutation but also tags the affected gene, facilitating its isolation and characterization. In the present work, around 9500 seeds from 3993 independent knockout rice lines were screened for Cd tolerance tagged by the gene trap vector, pGA2707, resulting in selection of two tolerant lines (*lcd* and *mmm*) to MS medium containing Cd from this library and a third line (*pez2*) was obtained through screening of lines potentially related to Cd.

3. ***Low cadmium (LCD),* a novel gene related to cadmium tolerance and accumulation in rice** ([1] Shimo *et al., J. Exp. Bot. 2011*)

The *lcd* mutant showed tolerance to Cd on agar plates and in hydroponic culture during early plant development. Metal concentration measurements in hydroponically grown plants revealed significantly less Cd in the shoots of *lcd* plants compared with wild-type (WT) shoots. When cultured in the field in soil artificially contaminated with low levels of Cd, *lcd* showed no significant difference in the Cd content of its leaf blades; however, the Cd concentration in the grains was 55% lower in 2009 and 43% lower in 2010. There were no significant differences in plant dry weight or seed yield between *lcd* and WT plants. *LCD*, a novel gene, is not homologous to any other known gene. LCD localized to the cytoplasm and nucleus, and was expressed mainly in the vascular tissues in the roots and phloem companion cells in the leaves. These data indicate that *lcd* may be useful for understanding Cd transport mechanisms and is a promising candidate rice line for use in combating the threat of Cd to human health.

4. Characterizing a constitutively expressed Mn and Fe transporter in rice

([2] Ishimaru and Shimo *et al., Submitted*)

The *mmm* mutant showed tolerance to Cd on agar plates. The mutant showed an interesting low Mn and high Cd accumulation phenotype. Microarray analysis showed a very strong knockdown of the *CNP* gene, which was hypothesized as the causative gene for the altered metal accumulation phenotype. Transport activity assays revealed CNP as an Fe, Mn and Cd transporter. It localizes to the plasma membrane and is expressed mainly in the cortex and epidermis of the roots. *CNP* RNAi experiments confirmed the previous hypothesis showing a similar metal accumulation phenotype to *mmm* mutants.

Suppression of *CNP* led to accumulation of Cd in shoots. Anjana Dhan, a high biomass and high Cd accumulating variety had *CNP* suppressed by RNAi, showing even higher Cd accumulation in shoots. This line represents an important achievement for phytoremediation.

5. Rice phenolics efflux transporter 2 (PEZ2) plays an important role in solubilizing apoplasmic Fe ([3] Bashir and Shimo et al., *Soil Sci. Plant Nutrition. in press*)

Here the identification of a phenolics efflux transporter in rice is described named phenolics efflux zero 2 (*PEZ2*). In *pez2*, the amount of protocatechuic acid (PCA), caffeic acid (CA) and Fe in the xylem sap was dramatically reduced and Cd accumulation in seeds was higher, suggesting a link between xylem apoplasmic Fe solubility and Cd accumulation in grains. As reported by previously Cd translocation in rice is greatly affected by Fe status and its transport system. Despite the fact PEZ2 being a PCA transporter directly related to apoplasmic Fe solubility which directly affects Fe transport activity in rice; it can also affects the Cd distribuition inside the plant. In this scenario, reduction of PCA in the xylem would indirectly affects the Cd status in the plant by a cascade response related to Fe homeostasis. More studies are needed to elucidate Cd xylem transport in plants.

6. Conclusions and perspectives

Cd contamination poses a serious hazard to human health and uptake of Cd into plants and its translocation to the edible parts are the primary avenue through which Cd enters the food chain. In fact Cd is not an essential element for plants but due to its high solubility, and similar physical and chemical properties to other cations it is readily taken up by plants from soil and translocated to different parts of the plant. Although the mechanisms of Cd tolerance, accumulation and translocation in rice remain to be fully elucidated, these data taken together, shows the importance of the uptake (*CNP*) and transport systems (phloem, *LCD* and xylem, *PEZ2*) in rice for Cd toxicity. With the goal of combating the threat Cd represents to human health and using the knowledge acquired during this study, two important mutant lines were achieved. The *lcd* mutant, accumulating low amounts of Cd in grains for safer food production, and *CNPi*, accumulating high amounts of Cd in the upper parts of the plant for phytoremediation. The next steps toward safer food production would apply the Cd homeostasis knowledge available to combine the manipulation of different processes of Cd tolerance, uptake and translocation in order to breed new lines showing phenotypes even more distinct than the ones presented in this work.