Genetic and physiological approach to elucidation of Cd absorption mechanism by rice plants

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Abstract: This paper summarizes our recent research into the genetic and physiological mechanism (s) on Cd accumulation in rice. A large natural variation (40-fold difference) in grain Cd concentration was found in a diverse rice germplasm. Physiological research indicated that root-to shoot Cd translocation via the xylem is a major process determining the divergent difference in shoot and grain Cd accumulation in rice cultivars. Further, using a positron-emitting tracer imaging system (PETIS), we succeeded to visualize the difference in the real-time translocation of Cd among rice cultivars. For genetic analysis, Quantitative trait loci (QTL) mapping showed that a major and specific QTL for grain Cd concentration was detected on the short arm of chromosome 7. A conventional breeding enabled to produce the rice lines with lower grain Cd concentration than a popular rice cultivar. The results obtained by our group would useful for planning a breeding or selection strategy to exploit the new cultivars with low grain Cd trait.

Keywords: cadmium, genotypic variation, quantitative trait loci, rice, xylem loading

1. Introduction

There is widespread contamination of cadmium (Cd) in natural and agricultural environments through anthropogenic activities or geological sources. The contamination of Cd in rice is serious threat to the people living on rice in monsoon-Asia because rice among crops is the greatest sources of dietary intake of Cd. Therefore, it is urgently required to develop the technologies for reducing the Cd levels of rice grain. Based on the genotypic variation in Cd accumulation in rice (Arao and Ae, 2003), two promising strategies for reducing the Cd of rice grains have been proposed: phytoextraction of Cd by using high-Cd accumulating rice cultivar (Ishikawa et al., 2006; Murakami et al., 2007) and breeding of low-Cd accumulating one (Ishikawa et al., 2005). To lead the proposed strategies to a success, it is necessary to elucidate the mechanism(s) underlying the Cd transport in rice. This paper summarizes our work to elucidate genetic and physiological aspects underlying Cd accumulation in rice.

2. Genotypic variation in Cd concentration of grains in rice cultivars

Arao and Ae (2003) reported a large variation in grain Cd concentration was found in 49 rice cultivars cultivated on the Cd-polluted soil. On the basis of this information, we further screened using many cultivars to investigate the genetic diversity of Cd concentration in rice. The world rice core collection (WRC), consisting of 69 rice accessions which covers the genetic diversity of almost 32,000 accessions of cultivated rice, were obtained from the Genebank at the National Institute of Agro-biological Sciences (NIAS) in Japan (Kojima et al, 2005). The collection was cultivated in a paddy field with a naturally abundant level of Cd (0.1 M HCl extractable Cd; 0.21 mg kg-1) in soils under submerged conditions during the rice-growing season. The grain Cd concentration showed that almost all accessions were below 0.1 mg kg^{-1} because they were cultivated in unpolluted soil and also in un-aerobic submerged conditions in which available Cd in soils is quite low. Nevertheless, we found that two varieties, Jarjan and Anjana Dhan, showed high grain Cd concentration around 0.4 mg kg^{-1} which is the Codex limit. A maximum 40-fold difference in grain Cd concentration was found within the core collection (Fig. 1, Uraguchi et al., 2009). Further, these cultivars were used to dissect the physiological aspects related to Cd accumulation in rice.

3. Physiological process of Cd accumulation in rice cultivars

There are three transport processes most likely to mediate Cd accumulation into the shoots and, subsequently, into the seeds: 1) uptake by roots, 2) xylem-loading-mediated translocation to shoots, and 3) further translocation to seeds via the phloem (Clemens et al., 2002). Among them, a major factor determining the difference in Cd accumulation among rice cultivars were investigated. Seedlings (1-month-old) of 69 rice accessions from WRC and several additional cultivars were cultivated on the pots filled with the Cd-polluted paddy soil (0.1 M HCl extractable Cd; 1.8 mg kg-1). After culture for 1month under upland conditions, the xylem sap and shoots were collected from all genotypes. The root samples were also collected from 15 genotypes which showed significantly lower or higher Cd concentrations in xylem saps. A wide range of variation in Cd concentrations was observed, ranging from 6.96 to 54.4 mg kg^{-1} for shoot Cd, 56 to 630 μg L^{-1} for xylem Cd, and 78 to 402 mg kg^{-1} for root Cd concentration. The Cd concentrations in the xylem sap and shoots (r=0.78, P<0.01) and in the xylem sap and brown rice (r=0.30, P<0.05) were positively correlated, whereas those of roots and shoots of 15 selected genotypes were negatively correlated (r=-0.41, P=0.09) (Fig. 2, Uraguchi et al., 2009). These results suggest that not Cd uptake but root-to shoot Cd translocation via the xylem is a major process determining the divergent difference in shoot and grain Cd accumulation in rice cultivars.

Further research was conducted by using a positron-emitting tracer imaging system (PETIS) to analyze the real-time translocation of Cd in 6 selected rice cultivars (low Cd cultivars 'Nipponbare', 'Koshihikari' and 'Sasanishiki'; high Cd cultivars 'Jarjan', 'Anjana Dhan', and 'Cho-ko-koku') with different Cd accumulation in aerial parts including grains. Seedlings (2-3 weeks-old) of each rice cultivar cultured in plastic syringes were fixed on an acrylic board and placed between a pair of PETIS detectors in a chamber. PETIS analysis was started by adding purified ^{107}Cd (half-life 6.5h) with 0.1μM Cd as a carrier to $CaCl_2$ solution. Time-series images of the ^{107}Cd distribution were monitored simultaneously in 6 rice cultivars. Using PETIS, we succeeded to visualize the difference in the real-time translocation of Cd among 6 rice cultivars (Fig. 3). The serial images revealed that ^{107}Cd first appeared at the basal portion, irrespective of rice cultivars. The strengths of signals not only at the basal portion but also at the upper portion of the shoots were greater in high Cd cultivars than in low Cd ones during a 36 h-exposure of Cd. Thus, the root-to-shoot Cd translocation is the most important physiological process controlling Cd accumulation in rice.

4. QTL (quantitative trait loci) analysis for grain Cd concentration in rice

Quantitative trait loci (QTL) mapping is powerful tools for understanding the genetic control underlying complex traits that are important in agriculture (Yamamoto et al. 2009). Here, QTL analysis was performed to identify the gene loci for grain Cd concentration in rice. A mapping population consisting of 85 back-cross inbred lines (BILs) derived from the cross between 'Sasanishiki' (a japonica cultivar) and 'Habataki' (an indica cultivar) was obtained from Rice Genome Resource Center (RGRC: http://www.rgrc.dna.affrc.go.jp/index.html) in Japan. This population is often used to analyze the QTLs for several important agronomic traits such as grain ripening and panicle architecture (Nagata et al., 2002, Ando et al., 2008). For phenotypic evaluation in grain Cd levels, the BILs population and their parental cultivars were cultivated on a paddy field that was contaminated with Cd after being irrigated using with river water originating from an abandoned mine area (0.1 M HCl extractable Cd; 2.8mg kg-1). A large difference in grain Cd concentration was observed in parental cultivars; it was approximately 4-times higher in 'Habataki' (2.82 mg kg^{-1}) than in 'Sasanishiki' (0.65 mg kg^{-1}). The grain Cd concentrations in the BILs ranged from 0.38 to 2.31 mg kg^{-1}, and segregated mostly between 'Sasanishiki' and 'Habataki'. Based on Linkage map data and all data set of the phenotypes and genotypes in BILs, QTL analysis was performed by composite interval mapping using the software QTL cartographer version 2.5. When putative QTLs were estimated based on a threshold of logarithm of odds (LOD)>3.0, three QTLs for grain Cd concentration was mapped on chromosomes 2, 7, and 12, and designated tentatively as *qGCd2*, *qGCd7*, and *qGCd12*, respectively (Fig. 4). The 'Habataki' alleles at these QTLs were responsible for increasing grain Cd concentration. Among the QTLs found, a major QTL (*qGCd7*) was detected on chromosome 7 between genetic markers C383 and G1068; this QTL showed the LOD value of 13.6 and accounted for 35.5 % of total phenotypic variance (Table 1). The genetic effect of *qGCd7* was verified by using advanced backcross progenies such as chromosome segment substitution lines (CSSLs). This QTL was not genetically related to any QTLs for essential heavy metals (Cu, Fe, Mn, and Zn) concentration and those for agronomic traits such as heading dates and grain yield, suggesting being the specific QTL to Cd.

5. Development of rice varieties with low grain Cd trait

The QTLs results demonstrated that grain Cd concentration in rice is genetically controlled, indicating that it would be possible to breed new varieties with low grain Cd concentration. To begin with, we tried to produce the varieties with low Cd trait by means of a conventional breeding. Based on the information of grain Cd concentration in diverse rice cultivars, 'LAC23', a tropical japonica cultivar, was selected as one parental line. This cultivar was less than half as low grain Cd concentration as popular *japonica* rice cultivars such as 'Koshihikari' (Arao and Ae, 2003). While, 'Fukuhibiki' a japonica cultivar, was selected as another parental line because this cultivar possesses good agronomic traits such as a high grain yield, short culm length, and so on. The F_3 progeny (total 126 lines) produced by a cross between 'LAC23' and 'Fukuhibiki' were cultivated on the Cd paddy filed and the continuous variation in their grain Cd concentrations was observed. Finally, five promising lines (named tentatively as Ukei1118, Ukei1119, Ukei1120, Ukei1121, and Ukei1122) with low grain Cd trait were selected from F_3 to F_5 progenies (Fig. 5). Their grain Cd concentrations were approximately 50 % lower than those of 'Fukuhibiki' and 'Hitomebore', a popular rice cultivar in Japan. However, the concentrations of essential metals (Cu, Fe, Mn, and Zn) in their grains were almost similar levels to those of 'Fukuhibiki' and 'Hitomebore'. Although other traits related on grain yield and tasty must be further improved in promising lines, it was proven to be feasible to breed the new varieties with low grain Cd concentration in rice.

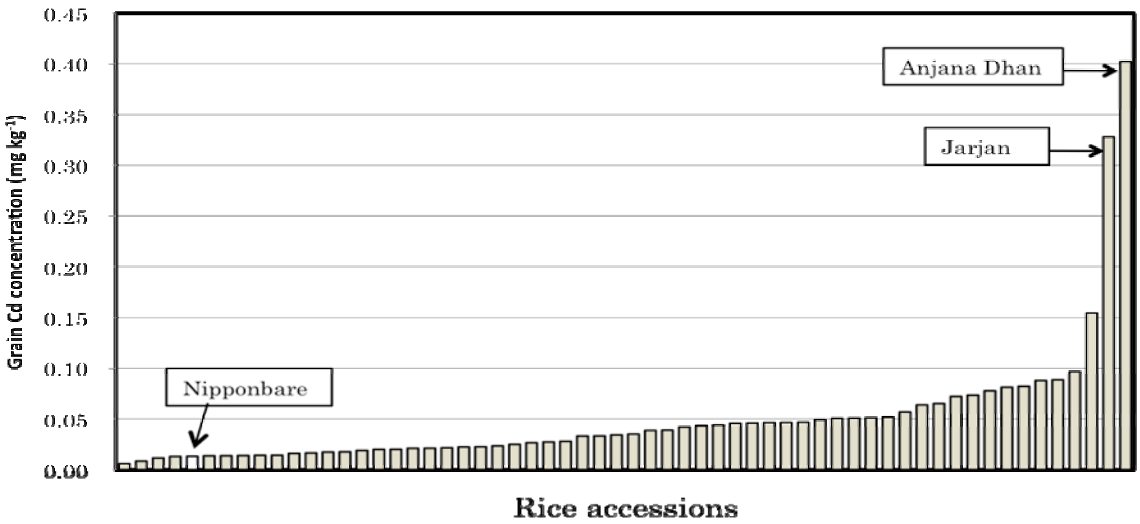

Fig. 1 Grain Cd concentration in diverse rice germplasm. The orders of rice accessions were arranged in lines according to their grain Cd concentrations.

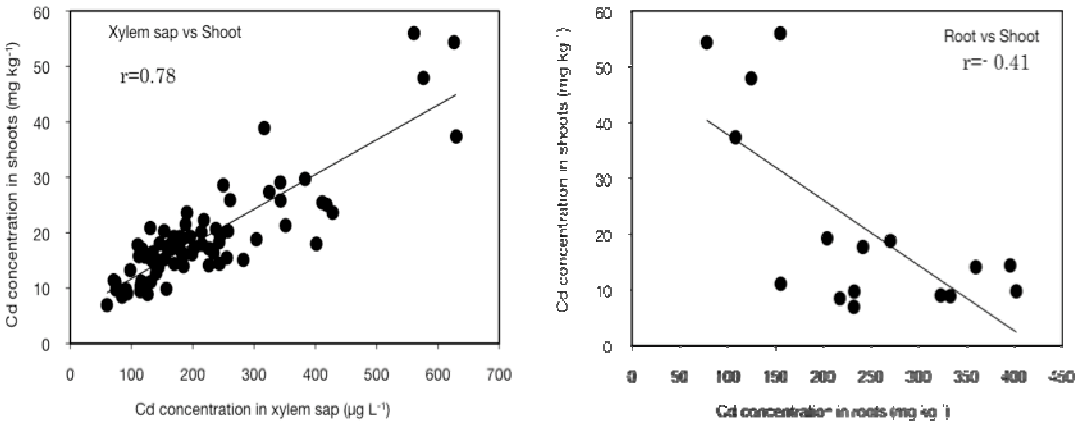

Fig. 2 Relationship between Cd concentration in shoots and that of xylem sap or between Cd concentration in shoots and that of roots in diverse rice germplasms.

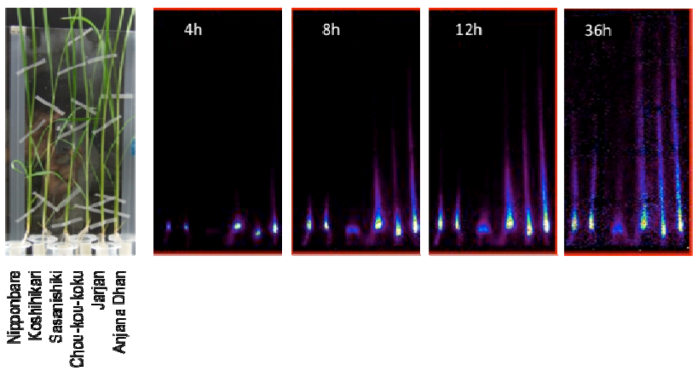

Fig. 3 serial images of shoot ^{107}Cd accumulation obtained from PETIS.

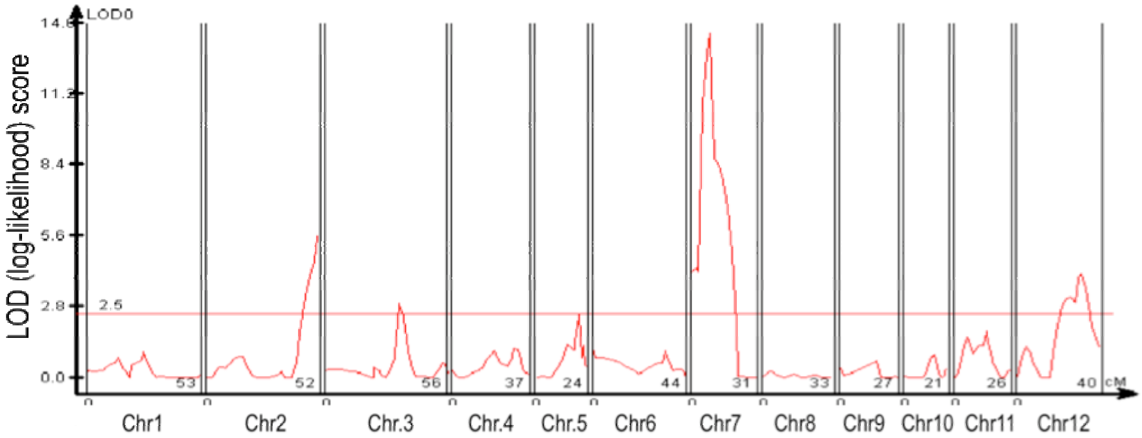

Fig. 4 Log-likelihood score for grain Cd concentration of BILs derived from 'Sasanishiki' and 'Habataki'.

Table 1. Quantitative trait loci (QTLs) for grain Cd concentration in the BIL population

Trait	Chromosome	QTL	Marker interval	LOD	Variance explained (%)	Additive effect	Positive allele
Grain Cd	2	*qGCd2*	R2511-C1470	5.64	12	-0.17	Habataki
	7	*qGCd7*	C383-G1068	13.64	35	-0.31	Habataki
	12	*qGCd12*	C1069-G1406	4.08	8	0.14	Sasanishiki

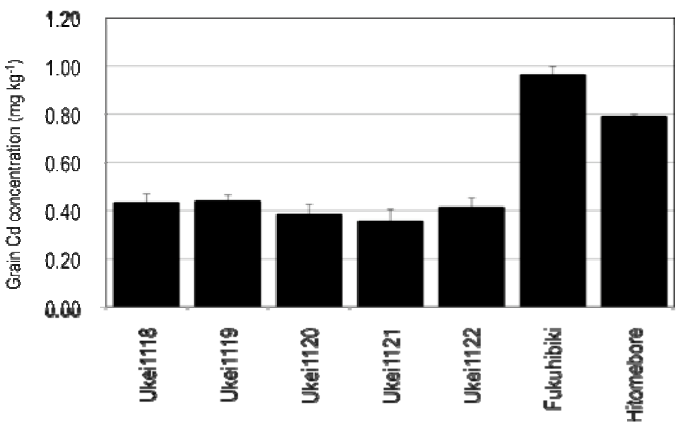

Fig. 5 Development of the rice lines with low grain Cd trait. Five promising lines (Ukei1118-Ukei1122) with low grain Cd trait were selected from F_3 to F_5 progenies produced from a cross between 'LAC23' and 'Fukuhibiki'. 'Hitomebore' is one of popular cultivars in Japan.

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