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Molecular mechanisms for reducing inorganic arsenic level in rice grains

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Abstract

Dietary exposure to arsenic (As) has become a serious issue because it may pose a health risk. In particular, rice is a major source of inorganic As (the more toxic form) for a large part of the world's population. The greater assimilation of As by rice than by other crops is mainly attributed to two reasons: the high arsenite bioavailability in reductive paddy soil and the high ability to transport arsenite through silicon transporters. The molecular mechanisms relating to As uptake and transport from soil to rice grains have been increasingly explored. Here, we report that two genes, *OsABCC1* and *OsPCS1*, are essential to control inorganic As in rice grains. The *OsABCC1* encodes an ABC transporter located in the vacuolar membrane and its transporter can trap inorganic As by sequestering in As-phytochelatin (PC) complexes in vacuoles of rice nodes. PCs are synthesized from glutathione by phytochelatin synthase (OsPCS1) and PC synthesis is induced by the contact of PCSs with harzardous metals such as As and cadmium. The transgenic rice lines over-expressing *OsPCS1* showed a significantly lower grain As level than the wild-type rice. Therefore, modifying the *OsPCS1* expression would be an approach to breeding rice cultivars with low inorganic As in the grains.

Keywords: food safety, inorganic arsenic, phytochelatin, rice, transporter

1. INTORDDUCTION

Arsenic (As) is a metalloid that is widely dispersed in the environment, and it is a primary concern in the public health field because it is a carcinogen. Arable soils inevitably contain naturally derived As, and crops grown on these fields assimilate As from the soil. The As levels in crops vary depending on the species, growing conditions, and As availability in soils. Paddy rice is a plant of particular interest because it accumulates As at higher concentrations in the grains than other crops [1]. Arsenite has high toxicity in living organisms and it is present as a major As species in paddy soils under aerobic conditions [2]. Rice can efficiently absorb arsenite via silicon transporters [3]. Rice is a major contributor to inorganic As exposure for Asian populations that consume rice as a staple food. For example, in Japan, rice and rice cakes contributed 97% of the estimated daily inorganic As intake from cereals, occupying the greatest proportion (62%) of the total daily inorganic As intake [4]. Therefore, we urgently need to establish practicable techniques for reducing inorganic As in rice to diminish the risk it poses to human health.

Understanding how inorganic As is taken up by rice roots and subsequently transported to rice grains is necessary for reducing As concentrations in rice. In this study, we found two rice mutants showing significantly higher grain As concentrations than the wild type (WT) plants. Using such mutants, we identified two genes by which inorganic As levels in rice grains are controlled. In this symposium, we

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discuss the molecular mechanisms for controlling As level in rice and propose how to breed a low-As rice.

2. MATERIALS AND METHODS

Rice cultivar 'Koshihikari' (*Oryza sativa* L.) was mutagenized with carbon ions [5] and M_2 progeny were used for screening of the mutants that differed from grain As levels in WT plants. The M_2 plants (approximately 3,000) and WT were grown in a paddy soil under continuous flooded conditions, the As concentrations of grains harvested from all plants were measured, and we selected wo mutants with high As traits (*has1* and *has2*). In addition, the shoots of WT and two mutants were divided into several parts and As concentration in each part was analysed. The mutant genes of *has1* and *has2* was identified by the methods of SNP linkage mapping and whole-genome resequencing.

In *has2* mutant, we tested a functional complementation using transgenic plants. Full-length ORF of a target gene (*OsPCS1*) was linked to the 5' flanking region, a native OsPCS1 promoter or CaMV 35S promoter and nopaline synthase terminator, and then inserted into the pKS221 gateway entry vector. The DNA constructs were transferred into a binary vector and transgenic rice plants were generated by *Agrobacterium*-mediated transformation. T_1 plants transformed into *has2* were cultivated in water culture for evaluating As or Cd phenotypes.

3. RESULTS AND DISCUSSION

Quantitative analysis of As showed that *has1* and *has2* had approximately five-times higher inorganic As concentrations in grains than the WT. (Table 1). On the other hand, we found that As concentrations were substantially lower in node I of *has1* and *has2* than in that of WT. As concentrations in flag leaves were almost equivalent between *has2* and WT, but was lower in *has1* than the WT. These results suggest that *has1* and *has2* have defects in trapping As in the node I with different mutations.

The genetic analysis revealed *has1* had a large mutation of *OsABCC1* which encodes an ABC transporter, a known player in sequestering As into vacuoles [6]. The *has2* had a three-base deletion in the *OsPCS1* (*Os05g0415200*) which encodes phytochelatin synthases and replaced Thr118 and Phe119 with an Ile residue. Complementation test showed that grain As concentration in *has2* were decreased to the WT level by the introduction of wild-type *OsPCS1* (data not shown), indicating that As phenotype in *has2* is due to the loss of function of *OsPCS1*. Grains harvested from transgenic lines overexpressing *OsPCS1* in *has2* under the control of the CaMV 35S promoter showed substantially lower As levels than those in WT grains (Fig. 1a). On the other hand, grain Cd concentrations in transgenic lines overexpressing *OsPCS1* were almost equivalent to those in the WT (Fig. 1b). These results suggest that *OsPCS1* regulates As but not Cd in rice grains [7].

Our results revealed that *OsPCS1* is crucial for reducing As levels in rice grains. Modification of *OsPCS1* expression would be an approach to breeding rice cultivars with low As levels in grains, and thereby to decrease human As exposure via food chain.

Table 1 As concentration of grain, node I and flag leaf in wild type (WT) and two mutants grown in a paddy field.

	Grain		Node I	Flag leaf
	Total	iAs		
	mg kg^{-1}			
WT	0.25	0.20	9.13	4.24
has1	1.26	1.01	0.64	1.44
has2	1.17	0.95	0.55	3.71

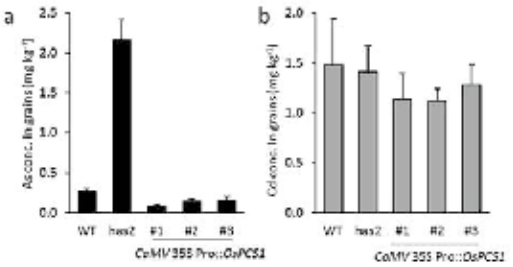

Fig.1 As and Cd concentrations of grains in WT, *has2*, and transgenic lines (T_1) highly expressing *OsPCS1*. The plants were hydroponically treated with 0.5mg L^{-1} of As or Cd.

4. ACKNOWLEDGEMENTS

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