OsRCS3 functions as a cytosolic *O*-acetylserine(thiol)lyase and regulates arsenic accumulation in rice

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ABSTRACT: Reduction of the levels of arsenic in rice shoot is important challenge for agriculture. OsRCS3 is identified as an *O*-acetylserine(thiol)lyase in present study. *OsRCS3* is able to complement an *Escherichia coli* cys-auxotroph mutant. OsRCS3 is localized to the cytoplasm and the expression of *OsRCS3* in roots was up-regulated by arsenic and cadmium treatment. In the presence of As(V), *OsRCS3* mutants showed significantly lower levels of Cys, GSH, PC₂ in roots compared with wild-type. Knockout of *OsRCS3* reduced As accumulation in the roots but increased As accumulation in shoots. We conclude that *OsRCS3* is the cytosolic *O*-acetylserine(thiol)lyase that plays an important role in non-protein thiol synthesis and in restricting As accumulation in rice shoots.

1 INTRODUCTION

Complexation of arsenite by thiols is an important mechanism of arsenic (As) detoxification in non-hyperaccumulating plants. Plants could decrease the translocation of As from roots to shoots by increasing the synthesis of a range of thiol-containing compounds in roots (Liu *et al.*, 2010). Cysteine biosynthesis is a central metabolic pathway to incorporate inorganic sulfur into organic molecules. In plants, cysteine is synthesized from *O*-acetyl-L-serine and sulfide by *O*-acetylserine(thiol)lyase (OAS-TL). OAS-TL activity is present in the cytosol, plastids and mitochondria (Hell *et al.*, 2002). In the present study, we investigated the As accumulation of rice (*Oryza sativa*) T-DNA insertion mutants for *OsRCS3*.

2 METHODS/EXPERIMENTAL

2.1 Functional complementation of *OsRCS3* in *Escherichia coli*

The full-length coding sequence of *OsRCS3* (*Os03g0747800*) was cloned into the prokaryotic expression vector pET-29a. The cysteine auxotroph *E. coli* strain NK3 transformed with the vector or pET-29a were cultured at 37°C for 2-d.

2.2 RNA extraction and transcriptional analysis by qRT-PCR

Four-week-old rice seedlings (cv. *Nipponbare* (NPB)) grown in hydroponics were exposed to 10 µM of As(III/V) or cadmium for 24 h. Total RNA were extracted from roots. Rice *Actin* and *HistoneH3* were used as reference genes.

2.3 Subcellular localization of *OsRCS3*

The *OsRCS3* full coding cDNA was cloned into pSAT6-EGFP-C1 vector between *HindIII* and *BamHI*. Then 35S::eGFP::*OsRCS3* fragment was subcloned into the vector pRCS2-ocs-nptII with *PI-PspI*. The protoplast was isolated from the tobacco leaves transformed with the two-expression vector and observed using a confocal laser scanning microscope (Zeiss).

2.4 Analysis of total As content and non-protein thiol levels

Four-week-old *OsRCS3* mutants (Tos17) and wild-type plants were exposed to 2 µM As(V) for 3 days. Phosphate was withheld in the As(V) experiments to facilitate As(V) uptake. Total As and non-protein thiol content were analyzed, as described previously (Wang *et al.*, 2018).

3 RESULTS AND DISCUSSION

3.1 *OsRCS3* functions as *O*-acetylserine(thiol)lyase

To test if *OsRCS3* is able to synthesize cysteine, we expressed the gene in the cysteine synthase-deficient *E. coli* strain NK3. The mutant strain transformed empty vector was unable to grow on M9 medium without cysteine. While heterologous expression of *OsRCS3* restored the growth of the *E. coli* strain NK3 on the medium.

3.2 *OsRCS3* was localized in the cytoplasm

To determine the subcellular localization of the *OsRCS3*, we isolated protoplasts from the transgenic tobacco leaves expressing *OsRCS3* under the control of cauliflower mosaic virus 35S promoter. Analysis

Figure 1. Subcellular localization of OsRCS3 in cytoplasm.

of OsRCS3-GFP-expressing protoplast by confocal microscopy showed that the green fluorescence was confined to cytoplasm. Thus, we confirmed that OsRCS3 is a cytosolic *O*-acetylserine(thiol)lyase (Fig. 1).

3.3 Arsenic and cadmium-inducible expression of *OsRCS3*

The transcription level of the *OAS-TL* genes was examined by qRT-PCR from 4-week-old roots treated with or without arsenic (III/V) or cadmium for 24 h. A high level of expression was observed for *OsRCS3* and *OsRCS1* (*Os12g0625000*). In contrast, the expression of other *OAS-TL* genes was low. Expression of *OsRCS3* increased after As(III/V) and cadmium treatment.

3.4 Cytosolic *OsRCS3* predominantly contributes to the cellular *OAS-TL* activity in root

To investigate the contribution of *OsRCS3* to the cellular *OAS-TL* activity, the *OAS-TL* activities in the roots and shoots of the *OsRCS3* mutants were determined using crude protein extracts of the mutants and wild-type. We obtained three independent knockout lines of *OsRCS3* mutants from the Rice *Tos17* insertion mutant database. Analysis using RT-PCR showed that the expressions of *OsRCS3* were abolished in these mutants.

In the root, significant reductions in the *OAS-TL* activity were observed in three *OsRCS3* mutants, NG6001, NG5746, and ND9034. The *OAS-TL* activity in shoot displayed lower levels than that in root and showed no difference between *OsRCS3* and wild-type. These results indicated that cytosolic *OsRCS3* showed predominant *OAS-TL* activity in the root (Fig. 2).

3.5 The total As and thiol levels in *OsRCS3* mutants

To determine the contribution of *OsRCS3* to cysteine synthesis and subsequent GSH and PCs synthesis in vivo, thiol levels and total As of plants grown in hydroponics treated with 2 μ M As(V) for 3-d were measured in the shoots and roots of *OsRCS3* mutants and compared with those of the wild type. The *OsRCS3* mutants showed significant reduction both in the total As content and Cys, GSH, PC₂ levels in the roots (Fig. 3). In the shoots, total As content and PC₂ level were significantly increased in the mutants.

Figure 2. Expression of *OsRCS3* (A) and *OAS-TL* activity (B) in *OsRCS3* mutants and wild-type in hydroponics without arsenic.

Figure 3. Total As content (A) and thiol levels (B) in roots of *OsRCS3* mutants and wild-type in hydroponics with 2 μ M As(V) for 3 days.

4 CONCLUSIONS

The results indicate that *OsRCS3* serves as a cytosolic *O*-acetylserine(thiol)lyase that plays an important role in non-protein thiol synthesis and in restricting As accumulation in rice shoots.

ACKNOWLEDGEMENTS

We thank Professor Jian Feng Ma for the provision of *OsRCS3* mutants. This work was supported by the Natural Science Foundation of China (31520103914).

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