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**Title**

Accumulation and transport mechanisms of arsenic in rice

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## Introduction

Arsenic (As) is a metalloid toxic to plant and animal and carcinogenic to human. Rice is the staple food crop for regions of the world like Bangladesh, West Bengal, China, Taiwan and Thailand which suffer from elevated As in soils and groundwater (Abedin *et al.*, 2002a). In Bangladesh, As-contaminated ground water is used not only as drinking water by tens of millions of people but also for rice cultivation, particularly during the dry season which has led to an arsenic build-up in the soil of paddy fields and a 10-fold elevation of As conc. in rice grain and straw (Meharg and Rahman, 2003). Elevated As accumulation in rice has the potential to become a new disaster for the population in Southeast Asia. Knowing how As is taken up by rice plants is important for understanding the accumulation and metabolism of As by rice, and is the starting point for developing strategies either breeding or molecular engineering for reducing arsenic uptake by rice..

In soils, the most abundant As species is arsenate [As(V)] (Brown *et al.*, 1999). The toxicity of arsenate is derived from its close chemical similarity to phosphate (Pi); this mimicry enables As(V) to alter Pi metabolism (Fitz and Wenzel, 2002). Indeed, the similarity between these anions makes plants to take up As(V) through the high affinity Pi transport system (Meharg and Macnair, 1990). Since this transport system is induced by Pi starvation, As(V) uptake is highly dependent upon the amount of Pi available in the soil. *Arabidopsis thaliana* mutants exhibiting As(V) tolerance harbor null alleles coding for the high affinity Pi transporters PHT1;1 or PHT1;4 (Shin *et al.*, 2004), indicating that these transporters play a major role in As(V) uptake. Although it has been demonstrated that phosphate transporters are important for arsenate uptake in *Arabidopsis*, still it is not known if it is also the case in rice. So it is worthwhile to examine the possible involvement of rice phosphate transporters in arsenate uptake, translocation and tolerance.

## Materials and Methods

### Plant materials

Rice plants (*Oryza sativa*) were grown in pots in a temperature controlled (30/25°C day/night) greenhouse under natural light. The plants were irrigated with various concentrations of As-contaminated water from shallow tube well, deep tube well, pond and river. The rice crop was harvested, and the samples of grain and straw were collected for subsequent analyses.

### Arsenic determination

Rice grain and straw samples were digested by nitric acid and the As content was measured by atomic absorption spectrophotometer. For digestion, 0.5 g grain/straw sample was transferred into a dry clean digestion vessel. Five mL of HNO<sub>3</sub> was added to it and it was allowed to stand overnight in a fume hood. On the following day, the vessels were placed on a heating block and heating was continued for 2-4 hours as the temperature is raised slowly to 120 °C. When brown fumes appeared, the step was repeated by adding conc. HNO<sub>3</sub> until no brown fumes give off. After cooling, 3 ml of 30% H<sub>2</sub>O<sub>2</sub> was added and the vessel was heated at 120 °C until the effervescence is minimal. Finally, the As content in the digest was determined by using atomic absorption spectrophotometer.

### Phylogenetic analysis

The predicted full-length amino acid sequence of rice phosphate transporters (OsPTs) was found in aramemnon database (<http://aramemnon.botanik.uni-koeln.de/>). Phylogenetic analysis was performed with the ClustalW program in DDBJ (<http://www.ddbj.nig.ac.jp/E-mail/clustulw-j.html>) and phylogenetic trees were generated with the application software, Tree Viwer.

### Results and Discussion

Arsenic accumulation in rice was monitored by measuring the content of total As in rice grain and straw. Rice plants were irrigated with different water sources such as Shallow tube well (STW), Deep tube well (DTW), pond and river. It may be mentioned here that the As content in STW, DTW, pond and river water was found to be 182.2, 70.79, 4.8 and 8.06  $\text{mg L}^{-1}$ , respectively. The data on As content of rice grain and straw have been presented in Figure 1. The results demonstrate that the As content of rice grain and straw was the highest when rice plants were irrigated with badly As-contaminated STW water. On the contrary, the As concentration in both grain and straw of rice decreased when the plants were supplied water from the lowest As containing pond water (Fig. 1). Similar results were obtained when rice plants were supplied with soil- and water-added arsenate (data not shown). So it is clear from the results that the As accumulation in rice grain and straw vary with the concentration of arsenic in soil or in irrigation water.

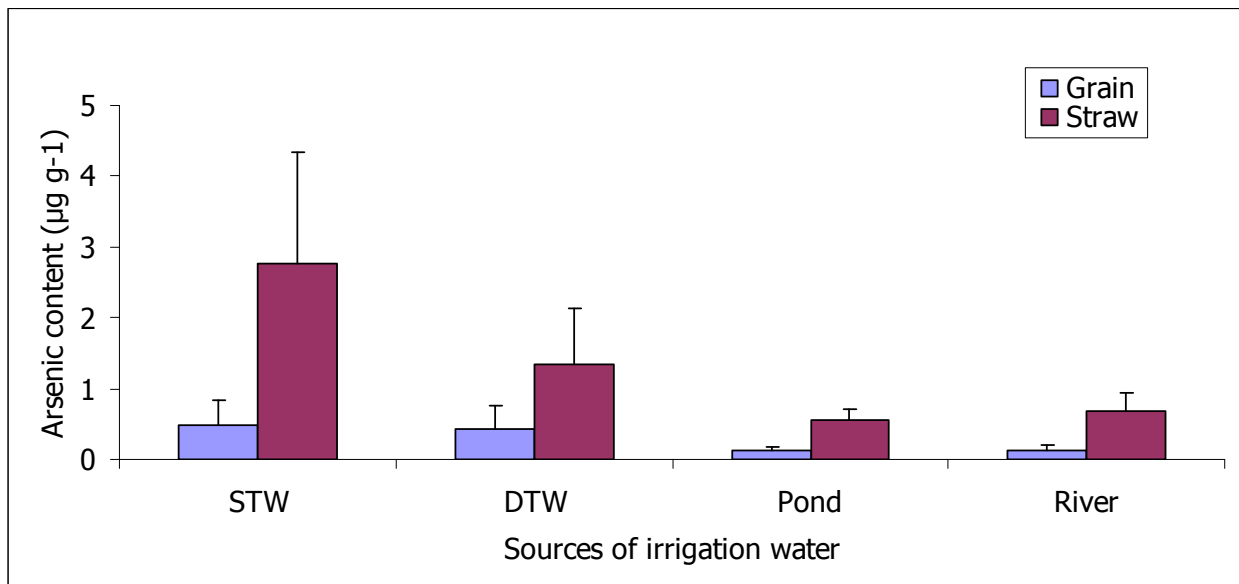


Figure 1. Arsenic accumulation in rice grain and straw. The data are the means of three replications. The bar indicates standard error.

Now it is important to investigate how the As is transported to rice plants for minimizing the As uptake by plants. Very recently, the arsenite transporter in plants has been reported (Ma *et al.*, 2008; Kamiya *et al.*, 2009). So our focus is on the transport mechanisms of arsenate in rice. There are some reports in Arabidopsis that arsenate is taken by plants through phosphate transporters. However, it is not well characterized in rice plants. Altogether there are 13 putative phosphate transporters in rice (OsPTS). We tried to find out the relationship among the OsPTS by

phylogenetic analyses of OsPT family proteins. The dendrogram indicates relative evolutionary distance among the OsPT-related family proteins (Fig. 2). We see from the phylogenetic tree that there are three groups of OsPTs having close relationship among each group. We are in progress to investigate the involvement of some phosphate transporters in arsenate transport in rice.

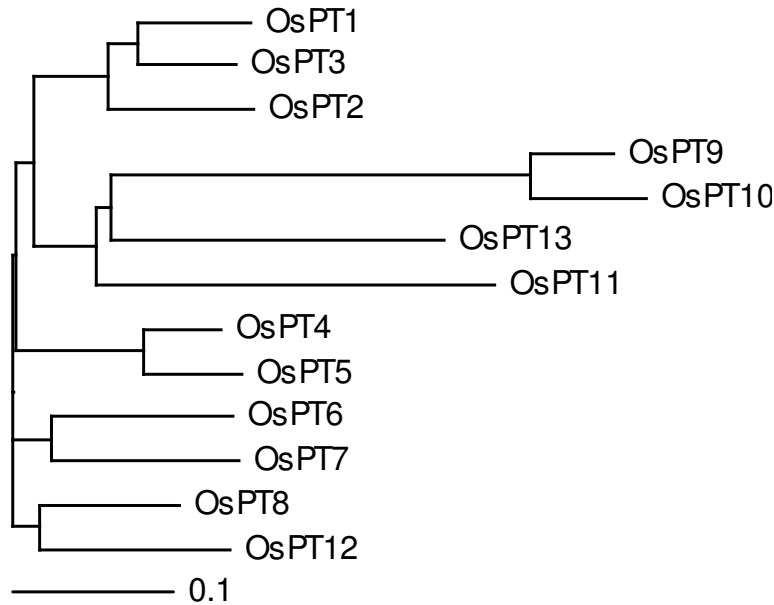


Figure 2. Phylogenetic analysis of rice phosphate transporter (OsPT) paralogs. The bar indicates the genetic distance for 0.1 amino acid substitution/site. The parenthesis include the gene code of OsPTs: OsPT1 (Os03g05620); OsPT2 (Os03g05640); OsPT3 (Os10g30770); OsPT4 (Os04g10750); OsPT5 (Os04g10690); OsPT6 (Os08g45000); OsPT7 (Os03g04360); OsPT8 (Os10g30790); OsPT9 (Os06g21920); OsPT10 (Os06g21950); OsPT11 (Os01g46860); OsPT12 (Os03g05610); OsPT13 (Os04g10800).

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