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Arsenic speciation in the phloem exudates of rice and its role in arsenic accumulation in rice grains☆

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ABSTRACT

Arsenic (As) speciation in the phloem sap of rice plants and its role in As accumulation in rice grains remain largely uncharacterized. In the present study, we tested As chemical species in the phloem exudates of rice treated with arsenate [As(V)], arsenite [As(III)], monomethylarsonic acid [MMA(V)], or dimethylarsinic acid [DMA(V)]. As(V) was the main species (58%) in the phloem exudates of As(V)-exposed rice, whereas As(III) predominated (69%) in As(III)-exposed rice. A large proportion of As(V) (41–45%) was observed in the phloem exudates when rice was treated with methylated As species. High concentrations of phytochelatins were detected in the phloem exudates when the As(V) treatment level was increased. The role of phloem transport was analyzed by applying a ± stem-girdling treatment to the rice plants, limiting phloem transport to the grain in rice pulsed with As(III), As(V), MMA(V), or DMA(V). The findings of the present study indicate that organic As is more mobile than inorganic As during phloem transport. Phloem transport accounted for 54% of As(III), 56% of As(V), 100% of MMA(V), and 89% of DMA(V) transport to the grain. The total As concentration and As(III) percentage in rice phloem and grain were significantly affected by increasing the phosphate concentration in the medium.

1. Introduction

The environmental fate and behavior of arsenic (As) have received increasing attention because of As pollution in Southeast Asia (Singh et al., 2015). Arsenic and its compounds are carcinogenic to humans (Chen et al., 2015) and may affect the quality of paddy rice (*Oryza sativa* L.) and its products, which are the staple food in Southeast and South Asia (Ma et al., 2016). Compared to other cereals such as barley, wheat, and maize, rice is more efficient in As uptake and accumulation (Wang et al., 2015; Williams et al., 2007; Zhao et al., 2009). An understanding of As accumulation, transport, and metabolism is thus needed to mitigate its contamination (Ye et al., 2010).

As is mainly found as arsenite [As(III)] and arsenate [As(V)] in paddy soil (Zheng et al., 2012). Methylated As compounds such as monomethylarsonic acid [MMA(V)], dimethylarsinic acid [DMA(V)], and trimethylarsine oxide (TMAO) occur as minor components in some soils (Huang and Matzner, 2006), but can reach high concentrations in plants. Several studies have demonstrated that oxic conditions in soils play an important role in As uptake and speciation in rice plants (Wu et al., 2017; Xu et al., 2008).

Arsenite is the predominant form of As found in the xylem of tomato, cucumber, rice, and Pteris *vittata* (Zhao et al., 2010). However, in other plant species such as *Brassica juncea*, wheat, and barley, arsenate account for a larger proportion (40–50%) of the total As in the xylem (Pickering et al., 2000; Su et al., 2010), whereas that of As(V) is significantly higher (55–83%) in the xylem exudate of castor bean (Ye et al., 2010). Arsenite forms complexes with thiols in the xylem, which decreases its mobility from the roots to the shoots (Zhao et al., 2010). Rice loads arsenite into the xylem more efficiently than barley or wheat due to the highly expressed Si pathway (Su et al., 2010).

Our current understanding of phloem transport of As, including As species and the transporters involved in phloem loading and unloading, is limited. Chemical speciation studies involving four different As species in castor bean (*Ricinus communis*) indicated that free inorganic As, mainly As(III), is transported via the phloem of castor bean exposed to either As(III) or As(V) (Ye et al., 2010). The methylated As species is

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more mobile than inorganic As (Ye et al., 2010). Moreover, phloem transport plays a significant role in As accumulation in rice (Carey et al., 2011, 2010; Zhao et al., 2012). Carey et al. (2010) reported that about 90% of As(III) was transported to rice grains via the phloem, whereas 55% of DMA(V) was transported when As(III) or DMA(V) were applied to excised rice panicles. Radiotracer studies showed that As had low mobility in rice plants and was transported to the grains primarily via the phloem (Zhao et al., 2012). In these two studies, the excised stems behaved differently from that of intact plants due to a loss of xylem tension. Furthermore, no studies on As speciation in the phloem sap and rice grains have been performed. In the present study, we investigated As speciation in the phloem exudates of rice and determined how phloem transport affects rice grain speciation.

2. Materials and methods

2.1. Plant culture

Rice seeds (*Oryza sativa* L. cv. Xinliangyou VI) were surface-sterilized (0.5% NaClO) for 10 min. The rice seeds were soaked overnight in deionized water after rinsing and then germinated in pure water.

After germination, the seedlings were cultured hydroponically with modified half-strength Hoagland nutrient solution and the composition of the pre-culture nutrient solution were as follows: 2 mM $Ca(NO_3)_2$, 3 mM KNO_3, 1 mM $MgSO_4{\cdot}7H_2O$, 100 μM Fe-EDTA, 2 mM MES, 0.55 μM $NaMoO_4{\cdot}2H_2O$, 9 μM $MnCl_2{\cdot}4H_2O$, 46 μM H_3BO_3, 0.35 μM $CuSO_4{\cdot}5H_2O$, 0.75 μM $ZnSO_4{\cdot}7H_2O$, and 500 μM $NH_4H_2PO_4$. The nutrient solution was replenished every 2 days. The growth conditions included a 15-h photoperiod with a light intensity of 350 μmol/m^2s, 32 °C:25 °C day:night temperatures, and 70% relative humidity.

2.2. As exposure and plant treatment

A set of experiments was conducted using rice plants at 10 d post-anthesis (DPA). In the first experiment, rice plants were exposed to As in the form of As(III) ($NaAsO_2$), As(V) (Na_2HAsO_4), MMA(V), or DMA(V) at a concentration of 10 μM. Plants were exposed to 10 μM As(V) in the second experiment with either a low (10 μM) or high concentration (200 μM) of phosphate. Both treatments were replicated in three 1-L pots and maintained for nine days, and the As treatment pots were changed every two days. Each pot had at least three similarly sized, healthy looking panicles. One was used in collecting the phloem exudates, whereas the other two panicles were utilized as a stem-girdled panicle and non-stem-girdled panicle. Phloem exudates were collected from plants that were exposed to 10 μM and 100 μM As(V) in the third experiment for phytochelatin (PC) analysis. Each treatment was replicated in three 1-L pots and maintained for nine days, with feeding pots changed every two days. For all experiments, the phloem exudates, the rice grains of the stem-girdled panicle, and the non-stem-girdled panicles of the rice plants were collected after nine days of As treatment, when these plants were nearly at full ripeness. The stem-girdling treatments were immediately carried out at the beginning of rice plants being exposed to As. Stem-girdled rice was exposed to a 30 s jet of steam in a 1 cm area of the rice stem between 1 cm and 2 cm below the panicle head. This process destroys phloem cells and prevents further phloem transport into the rice grains while the xylem vessels remain functional (Carey et al., 2010; Geng and Zhu, 2006; Martin, 1982; Wu et al., 2016). The grains were separated from its straw and dehusked. Phloem exudates were collected according to the method of King and Zeevaart (1974). Briefly, rice stems were cut with a sharp blade at about 1 cm above the roots. Then, the petioles were recut under 20 mM EDTA and transported to a fresh 20 mM EDTA solution (pH 7) for 4 h extraction at once. A strip of aluminum foil was applied to the panicles to limit evaporation, and the plants were kept in a dark room during the extraction. Rubidium (Rb) and strontium (Sr) were added to the treatment nutrient solutions as markers for phloem and xylem transport, respectively, at a final concentration of 1 mM (Carey et al., 2010).

2.3. Chemical analyses

For determination of As speciation in brown rice, 0.2 g of each powdered sample was placed in a microwave vessel and extracted with 1% HNO_3 in a microwave digester (Zhu et al., 2008). For quality assurance, we added a certified reference material (NIST 1568a rice flour) and included blanks. High-performance liquid chromatography inductively coupled with plasma-mass spectrometry (HPLC-ICP-MS; 7500a Agilent Technologies, Palo Alto, CA, USA) was used for As speciation. The procedure was performed as described previously (Ye et al., 2010). Chromatographic columns consisted of a Hamilton PRP-X100 10-μm anion-exchange column (240 × 4.1 mm) and a Hamilton precolumn (11.2 mm, 12–20 mm). The mobile phase consisted of 6.67 mM ammonium dihydrogen phosphate and ammonium nitrate, adjusted to pH 6.2 by using ammonia. As speciation of the samples was identified by comparing the retention times to that of the standards.

Total non-protein thiol (TNP-SH) compounds and glutathione (GSH) were detected using an Mlbio reagent kit. PC concentrations were calculated as PC-SH levels by subtracting the amount of GSH from the amount of total non-protein SH compounds (Bankaji et al., 2015).

2.4. Data analysis

The experimental data were analyzed using ANOVA with Windows-based SPSS 19.0.

3. Results

3.1. As speciation in phloem exudates of rice

As speciation in phloem exudates significantly differed among the four treatments. As(III) and As(V) were detected in the phloem exudates in both As(III) and As(V) treatments (Fig. 1). The major species of As in the phloem exudates was As(III), which was 69% of the total As in the As(III) treatment. However, when plants were treated with As(V), As(V) was the predominant species in phloem exudates, which accounted for 58% of the total As, and the remainder was As(III). No methylated As species were detected in either treatment.

The main As speciation was MMA(V) (55%) or DMA(V) (59%) when rice was treated with MMA(V) or DMA(V), respectively. A considerable proportion of As(V) was found in phloem exudates, representing 45% and 41% of the total As in MMA(V) and DMA(V) treatments, respectively.

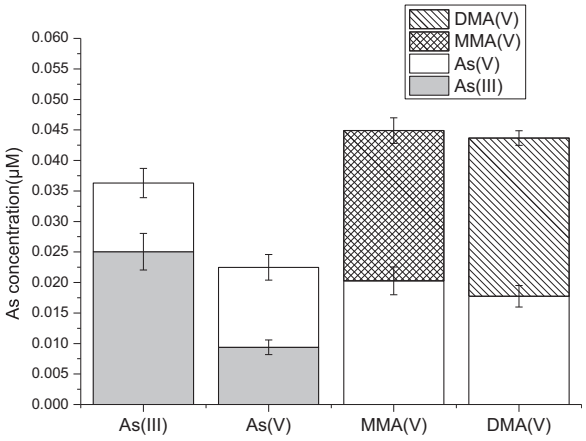

Fig. 1. As speciation in phloem exudates of rice exposed to 10 μM different As species for 9 d. Error bars represent ± SE of three replicates.