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Roles of rhizospheric organic acids and microorganisms in mercury accumulation and translocation to different winter wheat cultivars

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ABSTRACT

Elevated mercury concentrations in wheat grains pose a potential health risk to human. In this study, we selected two low-mercury-accumulating wheat cultivars (Nongda-3163, Gaocheng-8901) and two high-mercury-accumulating wheat cultivars (Jimai-21, Taishan-21) to investigate the role of rhizospheric organic acids and microorganisms in mercury accumulation and translocation to different wheat plants. The wheat grew in greenhouse with three levels of mercury treatment: control (no added mercury), low treatment (1 mg kg^{-1} total mercury) and high treatment (5 mg kg^{-1} total mercury). Under low mercury treatment condition, Jimai-21 and Taishan 21 had significantly higher mercury concentrations in roots and secreted higher amounts of oxalic acid and citric acid in the rhizosphere soil than Nongda-3163 and Gaocheng-8901. Meanwhile, abundances of gram positive bacteria, gram negative bacteria and fungi in the rhizosphere soil of Jimai-21 were significantly higher than those of Nongda-3163 and Gaocheng-8901, while abundance of protozoa in the rhizosphere soil of Jimai-21 was significantly lower than those of Gaocheng-8901. These facts may lead to higher mercury concentrations in grains of Jimai-21 and Taishan-21 than those of Nongda-3163 and Gaocheng-8901. In the current study, the oxalic acid had the largest contribution on mercury accumulation and translocation (p = .002), followed by citric acid (p = .002) and protozoa (p = .038) under low mercury treatment condition. Under high mercury treatment condition, Jimai-21 and Taishan-21 had significantly higher translocation factors from roots to leaves, glumes and grains than Nongda-3163 and Gaocheng-8901, and the significant contribution sequentially were citric acid (p = .002), fungi (p = .02) and gram positive bacteria (p = .018). Improving the beneficial relationship among rhizospheric organic acids, microorganisms and wheat cultivars was a realizable strategy to ensure wheat safety for mercury.

1. Introduction

Mercury is a contaminant of global concern owing to its persistent and bioaccumulative nature and neurotoxicity to humans (Wang et al., 2016). Mercury ranks third in the Priority List of Hazardous Substances (www.atsdr.cdc.gov/SPL/index.html). Anthropogenic activities have led to severe mercury contamination in soil (Clarkson, 2002; Du et al., 2005). The Ministry of Environmental Protection of China (2014) has concluded that approximately 1.6% of soil samples is contaminated by mercury in China. This has posed a latent threat to human health through food chains. Wheat is an important staple agricultural crop worldwide with cultivated area of 2.18×10^8 ha (Wang et al., 2016). Past studies have well documented that the mercury concentrations in wheat grains exceed the national edible health standard (20 μg kg^{-1}) in some areas (Chen et al., 2012). Previous studies have also revealed that mercury accumulation and translocation differ obviously among wheat cultivars (Xie et al., 2014; Zhang et al., 2011; Zhao et al., 2013). Thus, selecting low-mercury-accumulating wheat cultivars can be an effective way to ensure lower mercury concentration in grains (Hang et al., 2016). However, it remains unclear why some wheat cultivars accumulate more mercury than other cultivars.

Roots are crucial for the accumulation and translocation of heavy metals in plants. The plant can change root secretions upon heavy metal stress (Bais et al., 2006). The exudation from the root, particularly for the low molecular weight organic acids, affects the uptake of heavy metals from soil and their transfer to shoots or leaves through the xylem tissues (DalCorso et al., 2013; Liński et al., 1998; Mendoza-Cózatl et al., 2011; Shen et al., 2005). On the one hand, organic acids can immobilize

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heavy metals by precipitation reactions (Sasaki et al., 2004; Yang et al., 2000, 2006); on the other hand, organic acids can also enhance the mobility and bioavailability of heavy metals by complexation (Wang et al., 2015; Zeng et al., 2008). Organic acids affect the accumulation and translocation of heavy metals differently depending on the plant species and metal species (Ma et al., 1997; Zhu et al., 2011). Wang et al. (2015) revealed that low-mercury-accumulating rice cultivars secreted smaller amounts of organic acids than high-mercury-accumulating rice. Compared with rice cultivars, little is reported about wheat cultivars.

In addition, oxalic acid and citric acid are the most common and important low molecular weight organic acids secreted by plants (Fox, 1995; Jones et al., 2001). These acids are known to play an important role in heavy metal translocation from roots to shoots through the xylem and in vacuolar sequestration (Davies et al., 2001; García-Gonzalo et al., 2016; Rauser, 1999). Thus, the current study focused on the role of oxalic acid and citric acid in the mercury accumulation and translocation in wheat.

The interactions between plants and microorganisms are important processes affecting the translocation of heavy metals from root to aboveground parts (García-Gonzalo et al., 2016). On the one hand, the microorganisms can immobilize the heavy metals to reduce metal toxicity by producing chelators and adsorbing metal ions directly; on the other hand, they can enhance the heavy metals translocation from root and accumulation in the aboveground parts of the plant by secreting organic acids, producing biosurfactants and changing the soil pH (Abou-Shanab et al., 2008; Bakker et al., 2013). The phospholipid fatty acid analysis (PLFA) is a rapid and reliable method that has been widely used for studying microbial communities in soil (Francisco et al., 2016; Yu et al., 2009). Although the plant-microorganism interactions have been a topic of active research, the rhizospheric microorganisms vary among plants species, genotype levels and surrounded environments (Costa et al., 2006; Micallef et al., 2009; Smalla et al., 2001). Thus far, few studies have attempted to elucidate the role of rhizospheric microorganisms in mercury accumulation and translocation among wheat cultivars.

The aim was to elucidate the role of rhizospheric organic acids and microorganisms in mercury accumulation and translocation among different winter wheat cultivars. Understanding their complex relationships can help explain why some wheat cultivars are less mercury accumulation in the grains and, thus, ensure food safety associated with wheat and wheat products.

2. Materials and methods

2.1. Wheat cultivars

The four wheat cultivars we selected, Nongda-3163, Gaocheng-8901, Jimai-21, Taishan-21, differed significantly in mercury tolerance and mercury concentrations in their grains, based on our previous study (Liu et al., 2017). Nongda-3163 and Gaocheng-8901 were low mercury accumulation in grains while Jimai-21 and Taishan-21 were high mercury accumulation. When the soil contained 1 mg kg^{-1} total mercury, the mercury concentrations in the grains were 14.2 and 16.7 μg kg^{-1} for the two low-mercury-accumulating cultivars and 25.8 and 25.7 μg kg^{-1}, respectively, for the two high-mercury-accumulating cultivars. When the total mercury concentration was 5 mg kg^{-1} in soil, the mercury concentrations in grains were also lower in Nongda-3163 and Gaocheng-8901 than in Jimai-21 and Taishan-21. At the same time, Nongda-3163 and Gaocheng-8901 were mercury tolerant, while Jimai-21 and Taishan-21 were relatively mercury sensitive based on the comprehensive membership value in our previous study (Liu et al., 2017). Selecting these wheat cultivars allowed us to study different patterns of mercury distribution in wheat tissues and the role of rhizospheric organic acids and microorganisms in mercury accumulation and translocation.

2.2. Greenhouse experiment and mercury treatment

In the current study, we designed a randomized block with four wheat cultivars and three mercury treatments. Three mercury treatments (control, 1 and 5 mg kg^{-1} total mercury) were designed based on the environmental quality standard (grade II) in China and the nationwide soil pollution investigation communique (The Ministry of Environmental Protection, 2014). Triplicate experiments were conducted for each combination of a wheat cultivar and a mercury treatment level. The untreated experimental soil had a pH of 7.6 (1:2.5 soil/water), EC of 0.40 mS cm^{-1} (1:2.5 soil/water), organic matter of 10.75 g kg^{-1} (Walkleye-Black method), cation exchange capacity of 14.07 cmol kg^{-1} (ammonium acetate replacement method), soil particle size with 12.8% sand, 79.7% silt, 7.50% clay (micropipette method) and total mercury of 0.08 mg kg^{-1}. The air-dried soil (900 g for each pot) was adequately blended with mercury chloride solution and base fertilizers ($CO(NH_2)_2$ and KH_2PO_4). The blended soil contained 150 mg kg^{-1} N, 150 mg kg^{-1} P, and 189 mg kg^{-1} K. The plastic pots were 11.9 cm long, 11.9 cm wide and 12.5 cm tall. After being vernalized for a month, the wheat seeds were sown on October 24, 2015 and the plants were harvested six months later. The growth conditions of wheat seedlings were as follows: natural light with a day/night temperature of 25 °C/15 °C, and soil moisture for 70% of the field holding capacity.

2.3. Determination of mercury concentration in wheat tissues

We divided the wheat into roots, stems, leaves, glumes and grains. The roots were rinsed thoroughly, first with tap-water, then with deionized water in an ultrasonic cleaner (SB-5200 DTD, Ningbo Scientz Biotechnology Co., Ltd., China) to remove the external adsorbed mercury (Millán et al., 2013; Sierra et al., 2017). Upon harvesting, the wheat tissues were placed in an oven at 105 °C for 15 min to deactivate the enzymes and then oven dried at 55 °C for 48 h. The wheat tissues were ground into powder with a food processor (AQ-180E, Cixi Nail Electrical Appliance Co., Ltd., China). Mercury concentrations in wheat tissues were determined according to the national standard methods of China (GB 5009.17-2014) with slight modifications. Briefly, wheat sample (0.2000 g) was acid-digested with HNO_3-H_2O_2 (5:3; v/v) in a thermostatic water bath at 80 °C for 10 h. The samples were analyzed for mercury by atomic fluorescence spectrometry (AFS-930, Beijing Titan Instrument Co., China, detection limit for mercury = 0.003 mg L^{-1}). The certified wheat reference material (GBW10011 [GSB-2]) was used for quality control and the mercury concentrations we determined were within the allowable error range. A standard mercury solution was used as the calibration check standard and was run after analyzing twenty wheat samples. The analyses also included two reagent blanks and three duplicates.

2.4. Extraction and measurement of organic acids in rhizosphere soil

2.4.1. Extraction of organic acids

The rhizospheric soil was collected by shaking the root gently and then brushing off the adhered soil (Chen et al., 2001). The soil samples were stored at −80 °C. Fresh soil (1.0000 g) was extracted in a 100 mL centrifuge tube containing 25 mL sodium hydroxide solution (0.01 M) by ultrasonication for 20 min. After centrifugation at 4000 rpm for 10 min, the supernatant was transferred to a volumetric flask (100 mL). This extraction procedure was repeated two more times. Then, 100 mL flask was filled with deionized water to the calibration mark. Finally, the solution was filtered through a 0.45 μm membrane. At the same time, the soil moisture content was also determined by the oven drying method at 105 °C for 8 h.

2.4.2. Preparation of standard solution

Sodium oxalate (GR, Sinopharm, China) and sodium citrate (AR,