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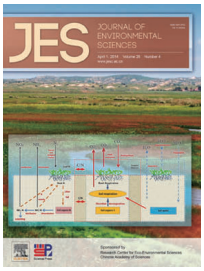

Arsenic dynamics in the rhizosphere and its sequestration on rice roots as affected by root oxidation

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ABSTRACT

A pot experiment was conducted to investigate the effects of root oxidation on arsenic (As) dynamics in the rhizosphere and As sequestration on rice roots. There were significant differences ($P < 0.05$) in pH values between rhizosphere and non-rhizosphere soils, with pH 5.68–6.16 in the rhizosphere and 6.30–6.37 in non-rhizosphere soils as well as differences in redox potentials ($P < 0.05$). Percentage arsenite was lower (4%–16%) in rhizosphere soil solutions from rice genotypes with higher radial oxygen loss (ROL) compared with genotypes with lower ROL ($P < 0.05$). Arsenic concentrations in iron plaque and rice straw were significantly negatively correlated ($R = -0.60$, $P < 0.05$). Genotypes with higher ROL (TD71 and Yinjingruanzhan) had significantly ($P < 0.001$) lower total As in rice grains (1.35 and 0.96 mg/kg, respectively) compared with genotypes with lower ROL (IAPAR9, 1.68 mg/kg; Nanyangzhan 2.24 mg/kg) in the As treatment, as well as lower inorganic As ($P < 0.05$). The present study showed that genotypes with higher ROL could oxidize more arsenite in rhizosphere soils, and induce more Fe plaque formation, which subsequently sequestered more As. This reduced As uptake in aboveground plant tissues and also reduced inorganic As accumulation in rice grains. The study has contributed to further understanding the mechanisms whereby ROL influences As uptake and accumulation in rice.

Introduction

Contamination of groundwater by arsenic has been frequently reported in the scientific literature (Stone, 2008; Zhu et al., 2008a, 2008b). In the arsenic-affected areas of Bangladesh, groundwater contains up to 2 mg As/L compared to the WHO recommended provisional limit of 0.01 mg As/L. The irrigated soils of Bangladesh generally contain 4–8 mg/kg As, while in areas where arsenic-contaminated water is used for irrigation, the soil As concentration can be as high as 83 mg/kg (Abedin et al., 2002). Rice grains collected in districts of As-contaminated soils in Bangladesh had concentrations that were 10-fold higher than the average concentration of 0.1 mg As/g (Meharg and Rahman, 2003). Rice is the staple diet of 3 billion people in the world, predominantly those in Asia. However, there is extensive As contamination of paddy soils around the world, as a result of irrigation using As-contaminated groundwater (Meharg, 2004; Liao et al., 2005) or mining activities around rice cultivation areas (Zhu et al., 2008a; Williams et al., 2009). The rice collected from mine-impacted regions in China were found to be highly enriched with As, reaching concentrations of up to 624 ng/g (Zhu et al., 2008b). For populations living on subsistence rice diets, As contamination in rice grain contributes greatly to their dietary As exposure. It has been reported that when drinking water levels of As

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are at the WHO's 10 mg/L limit, 0.05 mg/kg As in rice contributes to 60% of the dietary As exposure (Meharg et al., 2009; Williams et al., 2009). Inorganic arsenic species are of particular concern, as they are associated with various internal cancers and other health problems (IARC, 2004). Therefore, the food that sustains half of the world's population also increases a health risk (Stone, 2008). It is crucial that the physiology and genetics of rice uptake of As is better understood to counteract this widespread contamination of the food chain (Meharg, 2004).

Arsenic chemistry in the rhizosphere is complex and is controlled by several factors (Fitz and Wenzel, 2002). Under paddy field conditions, inorganic As is inter-converted between the reduced inorganic species arsenite (As(III)) and the oxidized species arsenate (As(V)) (Marin, 1993). Soil microbes can also methylate inorganic As to produce monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMA) (Turpeinen et al., 1999). In roots, oxygen transported within the root aerenchyma is consumed by adjacent tissue cells, or diffused towards the root apex or the rhizosphere; the transfer of oxygen from aerenchyma to the rhizosphere is termed radial oxygen loss (ROL) (Colmer, 2003a, 2003b). ROL can oxidize rhizosphere soil elements (e.g. Fe^{2+} to Fe^{3+}) and cause precipitation of toxic metals in rhizosphere soil and on root surfaces (Otte et al., 1989; Smolders and Roelofs, 1996), subsequently altering rhizosphere metal mobility. Caetano and Vale (2002) reported that iron-rich concretions are frequently found around plant roots in the Tagus estuary where radial delivery of O_2 takes place. Furthermore, the oxidizing ability of the plant roots is considered the most important biotic factor controlling iron plaque formation (Mendellsohn et al., 1995). Rice plants develop aerenchyma to transfer O_2 from the aerial parts of the plant to the roots, resulting in the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), and the precipitation of Fe oxides or hydroxides (Fe-plaque) on the root surfaces (Chen et al., 1980). Iron plaque can sequester metals on wetland plant roots (Hansel et al., 2001; Blute et al., 2004) and prevent translocation of As from roots to shoots (Liu et al., 2004a, 2004b). Therefore rhizosphere interactions play a key role in controlling As bioavailability to crop plants (Hinsinger, 2001; Fitz and Wenzel, 2002).

A previous study noted that there was a significant correlation between root aeration (ROL) and As tolerance and accumulation in rice (Mei et al., 2009; Wu et al., 2011). However, the mechanism involved in terms of effects of ROL on As tolerance and accumulation is unclear, and may due to its effects on the rhizosphere. This study focuses on root oxidation and the effect it has on As dynamics in the rhizosphere and subsequent As sequestration on rice roots. In this investigation, we studied (1) the effects of root oxidation on As dynamics in soil solutions; and (2) the effects of root oxidation on As sequestration by rice roots, and uptake and translocation in rice plants.

1 Materials and methods

1.1 Hydroponic investigation

Genotypes IAPAR, Nanyangzhan, TD71 and Yinjingruanzhan were chosen for this study with porosities of 19%, 12.4%, 31.1% and 26.3% and ROL's of 14.7, 5.3, 27.1 and 22.1 μmol O_2/(g dry weight · day) respectively (Wu et al., 2011). All seeds were sterilized in 30% H_2O_2 for 15 min, then washed with deionized water. The seeds were germinated in petri dishes each containing a moist filter paper in a controlled chamber (28°C and relative humidity 70%). After 1 week, uniform seedlings were transplanted to 2-L plastic vessels (four plants per vessel) with deoxygenated nutrient solution containing 0.1% (*W*/*V*) agar ('stagnant' treatment/solution, which more closely resembled the waterlogged soil than either semi-stagnant or N_2-flushed solution, because dilute agar prevents convective movements in solution) (Wiengweera et al., 1997). The nutrient solution contained 40 mg N/L as NH_4NO_3, 10 mg P/L as $NaH_2PO_4{\cdot}H_2O$, 40 mg K/L as K_2SO_4, 40 mg Ca/L as $CaCl_2{\cdot}2H_2O$, 40 mg Mg/L as $MgSO_4{\cdot}7H_2O$, and traces of Mn, B, Zn, Cu, and Fe (Yoshida et al., 1976). Solution pH was maintained at 5.8 with KOH. The nutrient solutions were renewed every five days.

All the vessels were arranged randomly in a greenhouse (25°C during the day and 20°C during the night, relative humidity 70%. Natural light was supplemented with sodium light (1200 Lux), providing a photoperiod of 12 hr light/12 hr dark per day. Measurement of ROL was determined at stem elongation and flowering stages using the titanium(III) citrate buffer method (Kludze et al., 1994). The method was described in more detail in our previous study (Wu et al., 2011).

1.2 Pot investigation

1.2.1 Plant culture

The same four genotypes used in the hydroponic experiment were used in the pot investigation. Rice seeds of the genotypes were germinated as previously mentioned and grown in Yoshida Nutrient solution for two weeks. Soils were collected in a paddy field on the campus of South China Agricultural University (sandy clay, pH 6.43, low As concentration 8.6 mg/kg, particle size distribution: clay 33%, silt 36% and sand 31%). The soils were air dried and sieved through a 2 mm sieve. A rhizobag system with two compartments was established using 3.5 L plastic pots (150 mm diameter × 200 mm height). These consisted of a central compartment in which the plants roots were grown as rhizosphere conditions and an outside compartment that served as the bulk soil. The compartments were separated using 30 μm nylon mesh cloths mounted on cylindrical plastic frames (60 mm diameter × 200 mm height). There were 500 g soil in the central compartment and 2500 g in

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