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Effect of arsenic on spatial pattern of radial oxygen loss and iron plaque formation in rice

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Abstract: The effects of different arsenic (As) treatments on spatial pattern of radial oxygen loss (ROL), iron (Fe) plaque formation and As accumulation in rice were investigated using three rice genotypes, planted under greenhouse conditions. Arsenic was applied to soil at 50 and 100 mg/kg, with untreated soil used as a control having an average As concentration of 8.5 mg/kg. It was demonstrated that the ratio of ROL in root tips to that at the root base slightly decreased with increasing As concentration, suggesting that the spatial ROL patterns in these groups may be shifted from the "tight" barrier towards the "partial" barrier form. Furthermore, increasing As concentration led to a increase in Fe plaque formation on root surfaces. In addition, root As concentrations of genotypes in 50 and 100 mg/kg As treatments were significantly higher than that of control treatment (P<0.05). Grain As concentration of genotype Nanyangzhan (with lower ROL) was significantly higher (P<0.05) than that of genotype CNT87059-3 with higher ROL.

Key words: arsenic; iron plaque; rice; spatial pattern of ROL

1 Introduction

Rice (*Oryza sativa* L.) is the staple food in Asia, particularly China, but recently it has caused great attention due to its high arsenic (As) concentration [1−3]. Rice grown under anoxic soil environment where more toxic and labile species (arsenite) dominates the rhizosphere has 10 times higher arsenic accumulation over other cereal crops [4,5]. Furthermore, As-enriched rice grain and straw have been widely used as domestic bird and cattle feed and subsequently the metalloid has been introduced into the food chain [6,7].

Paddy rice belongs to the wetland plants which generally grow in waterlogged soil, resulting in oxygen (O_2) deficiency [8−10]. In order to cope with anoxic conditions, wetland plants develope large volumes of root aerenchyma which is spongy tissue with large air spaces providing low resistance for the exchange of O_2 or other gases between tissues above water and submerged tissues [8,11]. The process of oxygen diffusion from aerenchyma to the rhizosphere occurs in the roots of wetland plants and is termed radial oxygen loss (ROL) [8,12], which establishes an oxic rhizosphere around the root tip [9,13]. On the other hand, to prevent excessive oxygen loss from basal zones and to enhance longitudinal oxygen diffusion towards the root tip, a barrier to ROL was induced in the basal root zones of plants grown in anoxic condition [8]. "Tight" barrier has low radial permeability to oxygen in the root base of plants grown in anoxic condition while "partial" barrier has high radial permeability to oxygen when grown in oxic condition [8]. In particular, the "tight" barrier to ROL provides a higher oxygen release rate at the root tip to detoxify phytotoxins which consequently ensure root elongation; this is considered as the most tolerant form to flooding and contaminants [9,14,15].

Due to the fact that there are five main As species found in rice plants, arsenate (As(V)), arsenite (As(III)), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and trimethylarsine (TMA) [4,16,17]. The ROL process in the rhizosphere is likely to influence the As species and as a result affect the tolerance and uptake of As in rice [12,18]. Furthermore, it has been demonstrated

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that ROL contributes to As tolerance in rice plants and rice genotypes with high ROL accumulate less As in shoots than in genotypes with low ROL [12,18,19].

The oxygenation of plants root by ROL and microbial activities may oxidize dissolved Fe(II) and subsequently resulted in the formation of iron plaque on the surface of rice roots [20−22]. In rhizosphere soil, iron plaque has an effect on the bioavailability of As and its subsequent uptake by the rice roots [20,23,24], and iron plaque did not intercept As entry into rice roots directly but rather acted as "barrier" for As uptake and accumulation in rice plants [21,22]. More recently, PAN et al [24] discovered that genotypes with higher ROL may possibly oxidize more arsenite in rhizosphere soils, inducing additional Fe plaque formation and subsequently sequestering more As on rice root surfaces. Iron plaque formation on roots was characterized as amorphous or crystalline mineralogy and was constituted of lepidocrocite (γ-FeOOH), goethite (α-FeOOH) and ferrihydrite ($Fe(OH)_3 \cdot nH_2O$ [21,25,26]. Previous studies have suggested that Fe plaque plays an important role in the adsorption of As on rice root surfaces, which sequester As and prevent its translocation to shoots [27,28].

It has been recognized that Fe plaque and ROL are related to As tolerance and uptake in rice [10,19,20]. Nevertheless, previous studies have mainly focused independently on either the effects of Fe plaque [27−29] or ROL [20]. In addition, most of the earlier studies have focused on the relationship of As, root anatomy and Fe plaque conducted in solution cultures [12,28,29], which is substantially different from the rhizosphere under natural environmental conditions [30,31]. It has been reported that heavy metals induced the alteration of root anatomical structure and decreased root porosity and caused the decrease of ROL [14,20,32,33]. And WU et al [34] found that the As treatments significantly affected total ROL. However, there is little study regarding the effects of As on spatial pattern of ROL in rice.

Therefore, we hypothesize that As concentrations in As-contaminated paddy soils may induce the change of ROL along rice roots and affect the iron plaque formation. This hypothesis was tested in a series of pot experiments designed to investigate: 1) the effects of different As treatments (low, medium and high) on Fe plaque formation and the spatial pattern of ROL in the rice rhizosphere, and 2) the effects of ROL on As concentration in rice plants.

2 Experimental

2.1 Plant culture

Three rice genotypes including Nanyangzhan, Yuxiangyouzhan, CNT87059-3 were selected for this study with the rates of ROL as follows: "Nanyangzhan" 5.3 μmol O_2/(g·d); "Yuxiangyouzhan" 17.5 μmol O_2 /(g·d); "CNT87059-3" 7.0 μmol O_2/(g·d) [19]. Rice seeds were germinated on moist filter paper and subsequently grown in the Yoshida nutrient solution [10,34,35]. After 30 d, the rice seedlings were transplanted into rhizosphere bags, which were filled with acid-washed quartz sand used to mitigate any damage to rice roots when ROL was determined as previous study [19]. The rhizosphere bag was placed in a PVC pot filled with 1.5 kg soil (sandy clay, pH 6.5 and mean As concentration of 8.5 mg/kg, collected from a paddy field located on campus at Hunan Agricultural University, China). Arsenic was applied as an arsenate solution ($Na_2HAsO_4 \cdot 7H_2O$) to the soils at three treatment concentrations (control, 50 and 100 mg/kg) as the previous study [19]. Pots were randomly arranged in a greenhouse with the same conditions as previous studies [10,19,20]. All plants were grown under waterlogged conditions for 120 d until maturity.

2.2 Iron plaque analysis with scanning electron microscopy

A LEO 1530 field emission scanning electron microscope (Leo/Zeiss 1530, Germany) with energy dispersive X-ray (EDX) from OXFORD was used to investigate elemental distribution of Fe plaque formation. After harvest, some roots were oven dried, cut into 2 cm section and pressed flat for the detection of iron plaque on root side. An accelerating voltage of 15 kV was employed.

2.3 Measurement of ROL spatial patterns

Two rice genotypes (Nanyangzhan and Yuxiang-youzhan) were selected for this investigation due to their respective low and high rates of ROL, identified in a previous study [19]. The ROL spatial patterns were measured using root-sleeving O_2 electrodes [9], of which further details are described in a previous study [10]. Briefly, the intact selected lateral root was carefully passed through a cylindrical O_2 electrode (internal diameter of 2.25 mm, height of 5.0 mm), fitted with a guide to keep the root near the center of the electrode [9]. Plants were left in the cylinder for at least 2 h prior to the first ROL measurement. The flux of ROL from the root to the electrode was then taken along each root with the center of the electrode positioned at the root tip and 2, 4, 8, 12 and 15 cm from the tip. After measurements were made, the root diameter at each position was determined using a Vernier microscope (150 mm Arc Headed Digital Caliper, UK). All measurements of ROL were carried out at 25 °C and light intensity was fixed at approximately 120 μmol/(m^2·s). Three roots from each