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1 Effect of arsenic on the spatial pattern of radial oxygen loss (ROL) and iron 2 plaque formation in rice

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13
14 **Abstract :** The effects of different arsenic (As) treatments on spatial pattern of radial oxygen
15 loss (ROL), iron (Fe) plaque formation and As accumulation in rice were investigated using three
16 rice genotypes, planted under greenhouse conditions. Arsenic was applied to soil at 50 and 100
17 mg/kg, with untreated soil used as a control having an average of 8.5 mg/kg As. It was demonstrated
18 that the ratio of ROL in root tips to that at the root base slightly decreased with increasing As
19 concentration, suggesting that the spatial ROL patterns in these groups may be shifted. Furthermore,
20 increasing As concentration led to a increase in Fe plaque formation on root surfaces. In addition,
21 root As concentrations of genotypes in 50 and 100 mg As kg⁻¹ treatments were significantly higher
22 than control treatment ($P < 0.05$). Grain As concentration from genotype Nanyangzhan (with lower
23 ROL) was significantly higher ($P < 0.05$) than that of genotype CNT85079 with higher ROL.

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

25 26 1 Introduction

27
28 Rice (*Oryza sativa* L.) is the staple food in Asia, particularly China, but recently it has caused
29 great attention due to its high Arsenic (As) concentrations [1-3]. Rice grown under anoxic soil
30 environment where more toxic and labile species (arsenite) dominates the rhizosphere, this makes
31 rice 10 times higher accumulator of arsenic over other cereal crops [4, 5]. Furthermore, As-enriched

1 rice grain and straw has been widely used as domestic bird and cattle feed and subsequently the
2 metalloids have been introduced into the food chain [6, 7].

3 Paddy rice belongs to the wetland plants which generally grow in waterlogged soil, resulting in
4 oxygen (O_2) deficiency [8-10]. In order to cope with anoxic conditions, wetland plants develop
5 large volumes of root aerenchyma which is spongy tissue with large air spaces providing low
6 resistance for the exchange of O_2 or other gases between tissues above water and submerged tissues
7 [8, 11]. The process of Oxygen diffusion from aerenchyma to the rhizosphere occurs in roots of
8 wetland plants and is termed radial oxygen loss (ROL) [8, 12], which establishes an oxic rhizosphere
9 around the root tip [9, 13]. On the other hand, to prevent excessive oxygen loss from basal zones and
10 to enhance longitudinal O_2 diffusion towards the root tip, a barrier to ROL was induced in the basal
11 root zones of plants grown in anoxic condition [8]. ‘Tight’ barrier has low radial permeability to
12 oxygen in the root base of plants grown in anoxic condition while ‘partial’ barrier has high radial
13 permeability to O_2 when grown in oxic condition [8]. In particular, the “tight” barrier to ROL provides
14 a higher oxygen release rate at the root tip to detoxify phytotoxins which consequently ensures root
15 elongation; this is considered as the most tolerant form to flooding and contaminants [9, 14, 15].

16 Due to the fact that there are four main As species found in rice plants, arsenate ($As(V)$),
17 arsenite ($As(III)$), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and trimethylarsine
18 (TMA) [4, 16, 17]. the ROL process in the rhizosphere is likely to influence the As species and as a
19 result affect the tolerance and uptake of As in rice [12, 18]. Furthermore, it has been demonstrated
20 that ROL contributes to As tolerance in rice plants and that rice genotypes with high ROL
21 accumulate less As in shoots than in genotypes with low ROL [12, 18, 19].

22 The oxygenation of plants root by ROL and microbial activities may oxidize dissolved $Fe(II)$
23 and subsequently resulted in the formation of iron plaque on the surface of rice roots [20-22]. In
24 rhizosphere soil, iron plaque has an effect on the bioavailability of As and its subsequent uptake by
25 the rice roots [20, 23, 24], and iron plaque didn't intercept As entry into rice roots directly but rather
26 acted as “barrier” for As uptake and accumulation in rice plants [21, 22]. More recently Pan et al [24]
27 discovered that genotypes with higher ROL may possibly oxidize more arsenite in rhizosphere soils,
28 inducing additional Fe plaque formation and subsequently sequestering more As on rice root surfaces.

1 Iron plaque formation on roots is characterized as amorphous or crystalline mineralogy and was
2 constituted of of lepidocrocite (γ -FeOOH), goethite (α -FeOOH) and ferrihydrite ($\text{Fe}(\text{OH})_3 \cdot n\text{H}_2\text{O}$) [21,
3 25, 26]. Previous studies have suggested that Fe plaque plays an important role in the adsorption of
4 As on rice root surfaces, which sequester As and prevent its translocation to shoots [27, 28].

5 It has been recognized that Fe plaque and ROL are related to As tolerance and uptake in rice [10,
6 19, 20]. Nevertheless, previous studies have mainly focused independently on either the effects of Fe
7 plaque [27-29] or ROL [20]. In addition, most of the earlier studies have focused on the relationship
8 of As, root anatomy and Fe plaque conducted in solution cultures [12, 28, 29], which is substantially
9 different from the rhizosphere under natural environmental conditions [30-31]. It has been reported
10 that heavy metals induced the alteration of root anatomical structure and decreased root porosity and
11 caused the decrease of ROL [14, 20, 32]. And Wu et al [33] found the As treatments significant
12 affected total ROL. However, there is little study regarding the effects of As on spatial pattern of
13 ROL in rice.

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

21 **2 Materials and Method**

23 **2.1 Plant culture**

24 Three rice genotypes (Nanyangzhan, Yuxiangyouzhan, CNT 87059-3) were selected for this
25 study with the rates of ROL as follows 'Nanyangzhan' $5.3 \mu\text{mol O}_2 /(\text{g dry weight} \cdot \text{day})$;
26 'Yuxiangyouzhan' $17.5 \mu\text{mol O}_2 /(\text{g dry weight} \cdot \text{day})$; 'CNT 87059-3' $7.0 \mu\text{mol O}_2 /(\text{g dry}$
27 $\text{weight} \cdot \text{day})$ [19]. Rice seeds were germinated on moist filter paper and subsequently grown in the
28 Yoshida nutrient solution [10, 34]. After 30 days, 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 were 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). Arsenic was applied as an arsenate solution ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) 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 days 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 Yuxiangyouzhan) 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 2.25 mm, height 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 fixed at approximately 120 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$. Three roots from each treatment were measured for each genotype.

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45 2 **2.4 Plant analysis for total As**

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7 3 At maturity, rice plants were harvested and subsequently washed (deionized water), and
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9 4 separated into roots, straw and grains, which were then oven-dried at 50°C until a constant weight
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11 5 was obtained [36]. Prior to analysis, rice samples were ground to a powder using a mechanical
12
13 6 stainless steel mill. The digestion procedure followed that described by Liu et al [31]. Rice samples
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15 7 including roots, straw and grains were digested with 5 mL concentrated HNO₃ using a hot block at
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17 8 120°C until the extracts were clear. NIST (National Institute of Standards and Technology, USA)
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19 9 CRM 1568a rice flour was used to validate the analyses of As and Fe. The digested extracts and
20
21 10 DCB-extracts were analyzed for total As and Fe by Inductively Coupled Plasma Mass Spectrometer
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23 11 (ICP-MS, PerkinElmer, Elan 9000). Recovery of As from 1568a rice flour CRM following total
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25 12 digestion was between 102 – 108%.

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27 1328
29 14 **2.5 Statistical analyses**

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31 15 The statistical package SPSS 19.0 (SPSS Inc., USA) was used to analyze plant biomass, As
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33 16 concentrations and the rates of radial oxygen loss. Origin 8.0 was used to create the figures.

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37 18 **3 Results**38
39 19 **3.1 Spatial ROL pattern along lateral roots**

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41 20 Seedlings of two genotypes all demonstrated a remarkably “tight” ROL barrier pattern in
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43 21 adventitious roots in treatments without additional As. Due to the stress caused by As, the ratio of the
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45 22 ROL at root tip to the ROL at root base decreased with increasing concentrations of As in higher
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47 23 ROL genotype (Yuxiangyouzhan, Fig. 1). For lower ROL genotype (Nanyangzhan), the ratio of the
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49 24 ROL at root tip to the ROL at root base was lowest in 100 mg As kg⁻¹ treatment, but there was no
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51 25 significant difference between control and 50 mg As kg⁻¹ treatment (Fig. 1). It is suggested that
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53 26 spatial ROL patterns in these groups were shifted from the “tight” barrier towards the “partial”
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55 27 barrier type.

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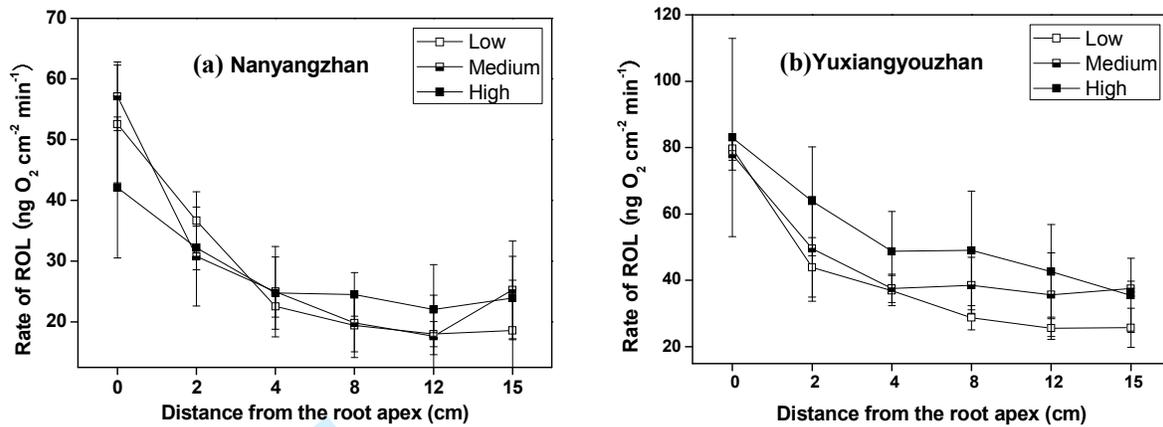


Fig. 1 Spatial patterns of radial oxygen loss (ROL) from lateral roots of two genotypes of rice grown in soils amended with different concentrations of arsenic (0, 50, 100 mg/kg). All data are shown as means \pm SD

3.2 Fe plaque formation on root surfaces

SEM X-ray analysis revealed large concentrations of Fe sequestered as Fe plaque. Besides iron, Na, K and Si were also detected (Fig. 2). An exemplary X-ray spectrum of Fe plaque on rice roots grown in high As concentrations is presented in Fig. 2b. Fe peak signals showed that increasing the As concentration leading to a slight increase in Fe plaque formation on root surfaces of genotypes.

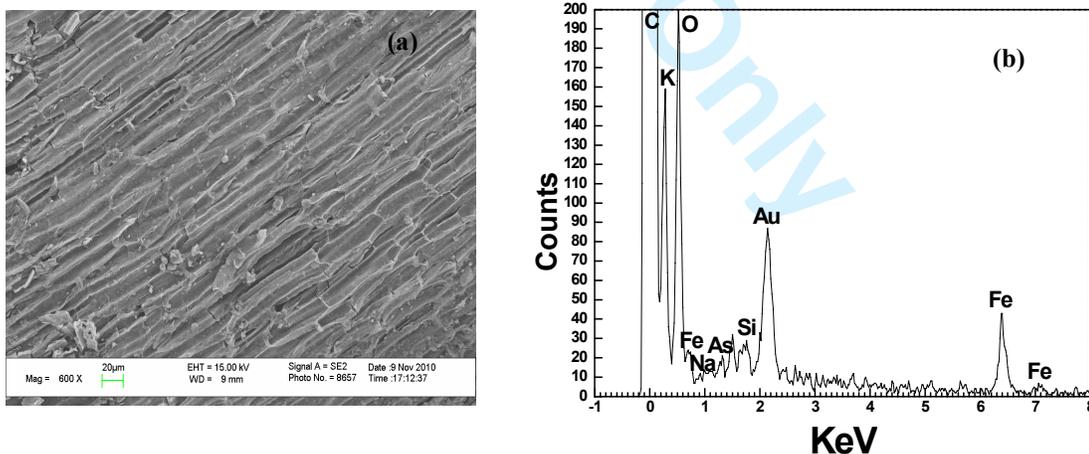


Fig. 2 Iron plaque on rice roots: (a) is the surface of iron plaque on rice roots; (b) is an exemplary X-ray spectrum.

3.3 Arsenic uptake in rice plants

Root As concentrations of genotypes in 50 and 100 mg As kg⁻¹ treatments were significantly higher than control treatment ($P < 0.05$) (Fig. 3a).

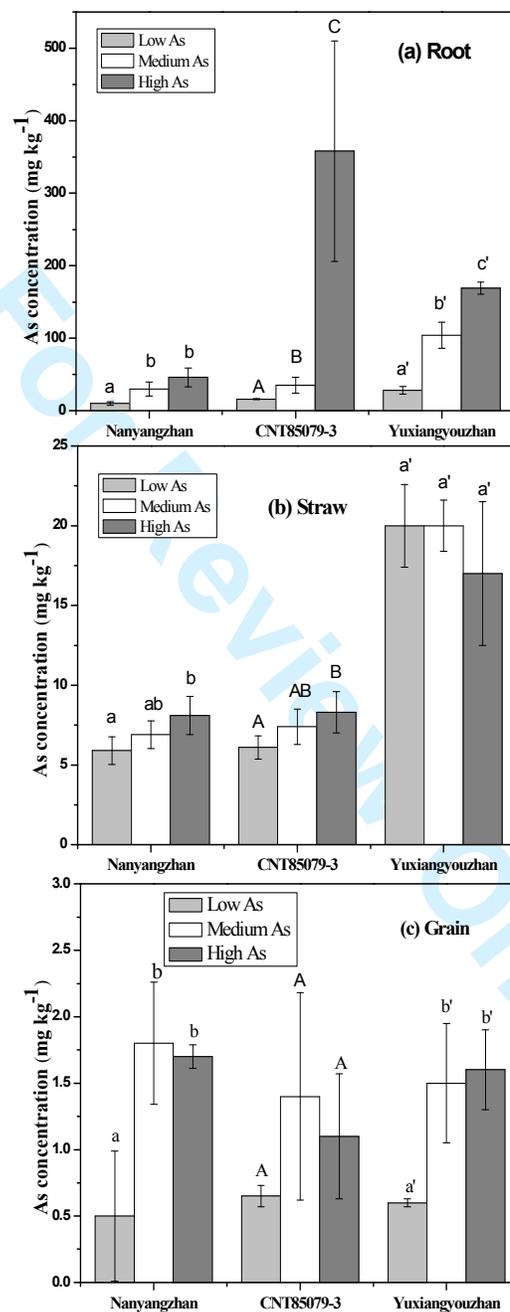


Fig. 3 Arsenic concentrations (mg/kg) in roots, straw, and grains of three rice genotypes including Nanyangzhan, CNT87059-3 and Yuxiangyouzhan grown in soils amended with different concentrations of As (0, 50, 100 mg/kg). Data are means \pm SD. Different letters in each treatment indicate that they were significantly different between three genotypes at $P < 0.05$ determined by Tukey's HSD test.

Especially, the As concentrations in roots of medium ROL genotype CNT85079-3 in 100 mg As kg⁻¹ treatment were significantly higher than 50 mg As kg⁻¹ treatment (Fig. 3a). Arsenic concentrations in higher ROL genotype Yuxiangyouzhan straw from the three As treatments were the highest among all genotypes, but there were no significant differences in As concentrations from straws between As treatments ($P > 0.05$) (Fig. 3b). Grain As concentration from higher ROL genotype Yuxiangyouzhan and lower ROL genotype Nanyangzhan differed significantly under control and 50 mg As kg⁻¹ treatments (Fig. 3c). Arsenic concentration from grain of lower ROL genotype was significantly higher ($P < 0.05$) than that of higher ROL genotype CNT85079 and Yuxiangyouzhan when grown in the 100 mg As kg⁻¹ treatment (Fig. 3c), with lower ROL genotype showing 1.7 mg/kg and higher ROL genotype showing 1.1 mg/kg. Regardless of arsenic quantity, As concentration in the different rice parts followed the trend: root > straw > grain.

4 Discussion

The present study showed no significant reduction in plant biomass when subjected to different As levels, which was different with other studies [37, 38], showing lower shoot biomass of rice subjected to high arsenate treatment. However, stimulation of rice growth by arsenate addition has also been reported for previous studies [19, 39, 40], possibly because of the different growth conditions. Moreover, arsenate addition may displace phosphate from the soil in certain situations, increasing plant P availability [41], which increased the nutrient for rice growth.

In anoxic and low redox potential environments, wetland plants enhance the accumulation of potentially toxic, and reduced solutes, such as Fe²⁺, Mn²⁺ and Pb²⁺ [15]. In order to survive in these adverse environments, wetland plants can develop adaptations, such as radial oxygen loss (ROL), and subsequently the formation of Fe plaque on root surfaces [9, 15].

Studies have observed significant correlations between ROL and As tolerance and accumulation in rice [10, 20]. Liu et al [14] found significant positive correlations between ROL production and metal tolerance in three species of mangrove. Furthermore, Deng et al [42] found that ROL was significantly correlated with Fe/Zn tolerance in 10 species of wetland plants. Besides differences in ROL production, the distribution of ROL along the root length is another important factor in flooding

1 and contaminant tolerance [8, 9, 13]. In the present study, rice plants in all As treatments were typical
2 “tight” barrier type and increasing the As concentration led to the spatial ROL pattern changing
3 somewhat from “tight” to “partial” barrier form. These results are in accordance with heavy metal
4 tolerance in other wetland plants. For example, it was discovered that heavy metals inhibited the
5 growth of mangrove seedlings and led to changes in the ‘tight’ barrier spatial pattern of ROL leading
6 to metal tolerance in mangrove seedlings [14, 15]. This study has demonstrated that As tolerance in
7 rice plants may also be varied with As concentration in the environment. Cheng et al [32] suggested
8 that ROL from the root tip might be a potential biomarker of environmental pollution, and decreased
9 ROL in wetland plants and rice being mainly attributed to the alternation of root anatomical structure
10 and reduced root porosity induced by toxins [14, 20, 32]. Less permeable roots induced by the
11 presence of pollutants appears to be a defense response to prevent excessive toxins entering the root
12 and possible fungal infection [43, 44].

13 Flooded environments may chemically reduce Fe and Mn compounds, leading to accumulation
14 of high concentrations of these bioavailable elements and hence potentially increase phytotoxic
15 concentrations of metals and metalloids [11, 45]. In paddy soils, phosphate, pH, soil texture, water
16 content, organic matter and microorganism activity may affect the redox conditions in the rice
17 rhizosphere [46, 47]. Yamaguchi et al [48] found that speciation of As near rice roots depends on
18 spatial and temporal redox variations in the soil matrix. Besides soil chemical properties (pH, Eh,
19 metallic oxides, organic matter) [48], microorganisms play critical roles in As transformation and
20 mobility in paddy soils, such as through ArsM (As(III) S-adenosylmethyltransferase) and interactions
21 with iron oxides or organic matter [3, 49]. Furthermore, rice plants lack the ability to methylate As
22 and soil flooding and addition of organic matter may thus increase microbial methylation of As [3].

23 Iron plaque formed on the roots of wetland plants can immobilize and prevent the uptake of
24 phytotoxic metals in some species, acting as a metal filter [11, 35]. There are several papers related
25 to iron plaque formation and heavy metal tolerance and uptake in wetland plants including rice [15,
26 27, 28]. It was reported that iron plaque can sequester As on rice roots, which subsequently reduced
27 As accumulation in aboveground biomass [27, 28, 31], enhancing arsenite and decreasing arsenate
28 uptake in rice [29]. Garnier et al [50] demonstrated that the formation of Fe plaque on the roots of

1 rice plants by micro-aeration, significantly limited the uptake of As by rice plants grown in paddy
2 fields. Furthermore, it was also found that genotypes with higher ROL could oxidize more arsenite in
3 rhizosphere soils, and induce additional Fe plaque formation, which subsequently sequestered more
4 As on rice root surfaces [24].

5 Rice is a prolific extractor of arsenic over other cereal crops [5]. However, present data shows
6 that straw arsenic content of genotype Yuxiangyouzhan slightly decreased with increase in soil
7 arsenic content from 8 to 50 and 100 ppm. Besides grain arsenic content of all the genotypes is more
8 or less same at both 50 and 100 ppm soil arsenic level. It may due to the genotypic difference from
9 other studies [4, 5] or the increased iron plaque formation in the high As treatment which sequestered
10 more As in iron plaque, decreased As uptake to aboveground parts of rice. The results from this study
11 have demonstrated that iron plaque concentrations increased slightly with increasing As
12 concentration in soils. It may indicate that As tolerance in rice plants varies with As concentration in
13 the environment, which needed further study.

14 Moreover, As toxicity depends not only on its total contents, but also on its chemical speciation,
15 with inorganic As considered much more toxic than organic forms such as DMA and MMA [10]. Wu
16 et al. [19] found that there were genotypic differences in levels of DMA and inorganic As (As_i) in the
17 grains, with higher ROL genotype having higher inorganic As in grains than lower ROL genotype. It
18 may be the reason for the differences of As accumulation among genotypes and lower ROL genotype
19 accumulating more total As in grains.

21 5 Conclusions

22 1) The ratio of ROL in root tips to that in the root base slightly decreased with increasing As
23 concentration, suggesting that the spatial ROL patterns in these groups may be shifted from the
24 “tight” barrier towards the “partial” barrier form.

25 2) Iron plaque was clearly visible on rice roots, and increasing the As concentration led to a
26 slight increase in Fe plaque formation on root surfaces.

27 3) In high As treatments, As concentration in grain of genotype Nanyangzhan with a ROL of 5.3
28 $\mu\text{mol O}_2 / (\text{g dry weight} \cdot \text{d})$ was significantly higher ($P < 0.05$) than that of genotype CNT85079 with

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4 1 a ROL of 7.0 $\mu\text{mol O}_2 /(\text{g dry weight} \cdot \text{d})$, with Nanyangzhan showing 1.7 mg/kg and CNT87059-3
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6 2 showing 1.1 mg/kg in high As treatment.
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砷对水稻根部渗氧分布和铁膜形成的影响

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摘要: 研究了砷对三种水稻品种 (玉香油占、CNT85079 和南洋占) 生物量、根表铁膜形成、根部渗氧径向分布以及砷在植物体内积累的影响, 结果表明随着砷处理浓度的增加, 水稻根表铁膜的含量增加; 水稻根尖渗氧率与根基部渗氧率的比值随着砷处理浓度的增加而降低, 水稻根部渗氧模式从“紧密型”向“疏松型”转变; 与对照相比, 50 和 100 mg/kg 砷处理能显著增加水稻根部的砷积累 ($P < 0.05$); 低渗氧能力水稻品种 (南洋占) 谷粒砷含量显著高于高渗氧能力水稻品种 (CNT85079, $P < 0.05$).

关键词: 砷; 铁膜; 水稻; 渗氧

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