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The effect of silicon on iron plaque formation and arsenic accumulation in rice genotypes with different radial oxygen loss (ROL)

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ABSTRACT Rice is one of the major exposure pathways of arsenic (As) in the human food chain which threatens over half the global population. A series of greenhouse pot investigations were conducted to examine the effects of Si application on iron plaque formation, As uptake and rice grain As speciation in indica and hybrid rice genotypes with different radial oxygen loss (ROL) patterns. The results demonstrated that Si significantly increased root (p<0.05) and grain (p<0.001) biomass. Indica genotypes with higher ROL induced greater Fe plaque formation compared to hybrid genotypes (p<0.005) and sequestered more As in Fe plaque. Silicon applications significantly increased Fe concentrations in iron plaque of different genotypes. Silicon application significantly decreased As concentrations in roots (p<0.005), straws (p<0.05) and husks (p<0.001) by 28-35%、15-35% and 32-57% respectively, whilst also reducing inorganic As (iAs) and DMA in rice grains. Indica genotypes with higher ROL accumulated lower concentrations of iAs in grains than hybrid genotypes with lower

ROL.

Key words: Arsenic, Iron plaque, Rice, Silicon.

1. Introduction

Arsenic (As) is a well-known carcinogenic metalloid and its exposure to humans is predominantly through drinking water and diet, which has led to increased health risks (Stone, 2008; Cui et al., 2013). Paddy soils have been contaminated with As due to the use of As-contaminated groundwater for irrigation, mining and other anthropogenic activities (Rahman et al., 2014; Jia et al., 2014; Shi et al., 2014); this has resulted in the accumulation of soil As and hence its transfer into rice (Abedin and Meharg, 2002; Seyfferthet et al., 2014). To exacerbate the problem, rice is largely cultivated under flooded conditions, the anaerobic environment significantly enhancing the mobilization and bioavailability of As (Pan et al., 2014). Under anaerobic conditions, arsenite is the predominant As species in soil solution (Takahashi et al., 2004; Xu et al., 2008). Arsenic species in rice comprise mainly of inorganic As (iAs), including arsenite and arsenate, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Zhao et al., 2010; Nookabkaew et al., 2013). The main As species in rice grain are iAs and DMA (Zhu et al., 2008a), with inorganic species being considered of greater toxicity than MMA and DMA (Williams et al., 2005; EFSA, 2009; Calatayud et al., 2013), and thereby creating a major exposure pathway via human ingestion (EFSA, 2009; Halder et al., 2012; Qu et al., 2015).

There are two main pathways for As uptake in rice (Zhao et al., 2013a). Firstly,

arsenate is a chemical analogue of phosphate, and arsenate can be assimilated in the rice root via the phosphate transporter protein system (Chen et al., 2013; Wu et al., 2015). Secondly, arsenite is a silicic acid analogue, and therefore it can be taken up by roots through the silicic acid transport system (Ma et al., 2008; Chen et al., 2012). Studies have also indicated that the addition of Si markedly decreased uptake and shoot As accumulation in rice (Guo et al., 2007; Wu et al., 2015). Furthermore, Seyfferth and Fendorf (2012) discovered that the addition of Si in soil pore-water significantly decreased As concentrations in rice grains. In addition, MMA also shares the same silicic transportation pathway and it is reported that Si can promote As methylation and hence affect the concentration and species of As in rice grain. This has been observed to reduce iAs concentrations by 59% whilst increasing DMA concentrations by 33% (Li et al., 2009). In addition, it has been reported that Si application reduced As concentrations in straw, flag, leaf and husk by half, with arsenite concentrations in brown and polished rice reduced by 22% and 33% respectively (Fleck et al., 2013). It has also been demonstrated the Si application strongly reduced the concentration of iAs, mainly arsenite, whilst increasing the concentration of DMA in both vegetative and reproductive tissues of rice (Liu et al., 2014). Nanoscale silica sol foliar application may also alleviate toxicity and accumulation of As in rice grains due to strengthening of their antioxidant defense capacity (Liu et al., 2014).

The oxygenation of plant roots by radial oxygen loss ROL (Colmer, 2003) and rhizosphere oxygenation by microbial activities, converts Fe^{2+} to Fe^{3+}, leading to the

formation of Fe-plaque around roots (Mei et al., 2009; Wu et al., 2012). Root ROL rates are considered the key biotic factor controlling Fe-plaque formation (Li et al 2011; Wu et al., 2012) and the plaque is mainly comprised of ferric hydroxides (63%), goethite (32%) and minor concentrations of siderite (5%), whose structure is characterized as amorphous or crystalline iron (oxyhydr)oxides (Liu et al., 2004a). Due to adsorption or coprecipitation mechanisms, iron plaque can sequester metals, metalloids (eg. As) and anions such as carbonate and silicate on rice roots (Liu et al., 2004a,b; Liu and Zhu, 2005). It has been widely demonstrated that iron plaque plays an important role in mediating As accumulation and alleviating As toxicity in rice plants (Ultra et al., 2009; Wu et al.,2012； Lee et al., 2012); the iron plaque serving as a barrier to prevent As translocation from roots to shoots (Liu et al., 2004a,b). Lee et al., (2012) reported that As addition induced iron plaque formation on roots, the Fe formation decreasing As uptake by roots and shoots, indicating that iron plaque can sequestrate As and reduce As uptake in rice. Furthermore, Wu et al (2012) discovered that higher rates of ROL contributed to increases in Fe-plaque which subsequently sequestered more As on rice roots. Nevertheless, these previous studies have focused on Fe plaque formation and Si concentration independently affecting As accumulation, and showing limited evidence as to the effects of Si on Fe plaque formation and As accumulation in rice genotypes with different ROL.

The aims of the present study were 1) to investigate the effects of Si on Fe plaque formation in rice genotypes with different ROL; 2) to determine the effect of Si on As sequestration in Fe plaque of rice genotypes with different ROL; 3) to

94 determine the effect of Si on As concentrations and speciation in rice genotypes with
95 different ROL.
96
97 **2. Materials and methods**
98 *2.1 Materials*
99 Rice seeds from four genotypes were obtained from Hunan Agricultural
100 University, and included hybrid subspecies Xiangfengyou 9 ('XFY-9'), T-you207
101 ('TY-207') and indica subspecies Xiangwanxian 17 ('XWX-17'), Xiangwanxian 12
102 ('XWX-12'). The ROL of XFY-9, TY-207, XWX-17 and XWX-12 were 9.55, 15.41,
103 19.76 and 27.00(需要单位吗) respectively (Wu et al., 2015). All seeds were surface
104 disinfected with a 30% H_2O_2 solution for 15 min, and subsequently washed repeatedly
105 in deionized water. The seeds were then germinated in culture dishes on moist filter
106 paper. Germinated rice seedlings were then cultured in a nutrient solution for 2 weeks.
107
108 *2.2 Pot investigation under waterlogged conditions*
109 Paddy soils (pH 6.6, 9.4 mg As /kg) were collected (0-20 cm depth) from a paddy
110 field near the campus of Central South University, Changsha, P.R. China. Soils were
111 returned to the laboratory and air dried at room temperature, then ground and sieved
112 to <2 mm. Nutrients were then thoroughly mixed by hand into the soil as follows, P as
113 $CaH_2PO_4 \cdot H_2O$ at 0.15 g/kg P_2O_5, K as KCl at 0.2 g/kg K_2O, and N as CO $(NH_2)_2$ at
114 0.2 g/kg N (Wu et al., 2011). Arsenate solution ($Na_2HAsO_4 \cdot 12H_2O$) was then applied
115 at 60 mg As/kg to all treatments with the exception of the control. Silicon was then
116 added as a SiO_2 colloid (63-200μm) (Seyfferth and Fendorf, 2012) as follows: Control,
117 no Si, no As; Treatment A, arsenate only (Si0); Treatment B, arsenate and 10 mg Si/kg
118 (Si10); Treatment C, arsenate and 20 mg Si/kg (Si20); Treatment D, arsenate and 40

mg Si/kg (Si40). All treatments were subsequently thoroughly hand-mixed and allowed to equilibrate for two weeks. After equilibration, seedlings (three seedlings per pot) from the four rice genotypes were transferred to polyethylene pots (20 cm diameter, 20 cm high) which had been previously filled with 3.5 kg of the individual soil treatments. All treatments were carried out in triplicate. After transplantation, the seedlings were grown under waterlogged conditions, the water level being maintained at 2–3 cm above the soil surface. The pots were placed randomly in a greenhouse (25℃ day, 20℃ night, relative humidity 70%) and natural light was supplemented with sodium light (1200 Lux), providing a photoperiod of 12 hr light/12 hr dark. Plants were harvested after maturity.

2.3 Extraction of Fe plaque

Rice plants were harvested at maturity and washed thoroughly using tap water followed by deionized water. Plants were then divided into roots and shoots. Iron plaque on root surfaces was determined by DCB-extraction (Wu et al., 2012). Root tissue (1.0 g) was extracted using 30 ml of DCB solution for 30 min at room temperature; the solution was prepared using 0.03 M sodium citrate ($Na_3C_6H_5O_7.2H_2O$) and 0.125 M sodium bicarbonate ($NaHCO_3$), with 0.6 g of sodium dithiomite ($Na_2S_2O_4$). After incubation, roots were rinsed three times with deionized water and the washings were added to the DCB extract. Deionized water was then added to the extracting solution to obtain 100 ml solution prior to analysis. The Fe concentrations in extracts were measured by Atomic Absorption Spectrometry (AAS, TAS-990, Beijing Puxi Instruments Co., P.R. China). Arsenic concentrations in extracts were determined with hydride generation atomic fluorescence spectrometry (HG-AFS, AFS-8230, Beijing Jitian Instruments Co., China).

144

145 *2.4 Plant analysis for total As*

146 Plant samples were harvested at maturity and washed carefully using deionized
147 water and divided into root, straw, husk and grain. Straw from below the irrigation
148 water was removed to avoid contamination. Half the roots, straw and grains were
149 oven-dried at 70℃ to a constant weight, while the other half was freeze dried for As
150 speciation determination. Grains and husks were further divided, ground using a
151 pestle and mortar, and stored at -20℃ prior to analysis.

152 After dry weight determination, samples were ground using a mechanical mill,
153 and 0.5g sample was weighed into a conical flask (100ml) with 5ml concentrated
154 nitric acid. The samples were left to digest overnight at room temperature, then placed
155 on an electric hot plate (120℃) until the solution became clear. After digestion, the
156 samples were filtered (0.45 μm) into colorimetric tubes and diluted to 20 ml with
157 deionized water. The total As concentration (root, straw, husk and grain) was
158 determined using HG-AFS (AFS-8230, Beijing Jitian Instruments Co., China) (Zhu et
159 al., 2008b). A certified reference material (bush branches and leaves, GBW07603)
160 was used for quality control purposes, with recovery of As ranging from 80.4% to
161 89.5%.

162

163 *2.5 Plant analysis for As speciation*

164 For determination of As species in grain, samples were ground under liquid N_2 to
165 ensure stabilization of As species (Zhu et al., 2008b). Milled grain samples (1.0 g)

were added to centrifuge tubes (50 ml), and 20 ml HNO_3 (1%) was added and heated to 95℃ for 1.5 h. After the samples had cooled to room temperature, the extracting solution was centrifuged at 5000r/min for 10 min and the supernatant filtered (0.22 μm). Arsenic speciation was determined using HPLC-HG-AFS (HPLC, Shimadzu LC-15C Suzhou Instruments Co., China; HG-AFS, AFS-8230, Beijing Jitian Instruments Co., China) (Zhu et al., 2008b; Shi et al., 2013).

2.6 Data analysis

All data was analyzed in EXCEL 2007. Analysis of variance for plant biomass, As and Fe concentrations in different genotypes and Si treatments were determined by SPSS 19.0. All figures were created in Origin 8.0.

3. Results

3.1 Effect of Si on plant growth

Root, straw and grain biomass, from the four rice genotypes grown in different treatments, is presented in table 1. Significant differences are observed in root biomass between genotypes (p<0.05) (Table 1). In the control treatment, root biomass was ranked as XWX-12 < TY-207 < XWX-17 < XFY-9, ranging from 9.25 g/pot to 15.8 g/pot. Under different Si concentrations, XFY-9 and XWX-12 developed the largest root biomass, whilst TY-207 and XWX-17 displayed the greatest root biomass in Si20 treatment (Table 1). Silicon application significantly increased root biomass of XFY-9, XWX-12 and TY-207 (p<0.05), but had no significant effect on

XWX-17 (p>0.05). Root biomass of XFY-9 was significantly greater than the other three genotypes in Si40 treatment (p<0.05) (Table 1).

Significant genotypic differences were also observed in straw biomass (p<0.001). In control treatments, straw biomass from the four genotypes ranged from 17.8 g/pot to 29.4 g/pot (XWX-12 < TY-207 < XFY-9 < XWX-17). Straw biomass from XWX-17 was the greatest between the four genotypes in Si40 treatment (p<0.05) (Table 1).

Results showed that there were significant differences in grain biomass between genotypes (p<0.001). In control treatments, grain biomass of the four genotypes was ranked as follows, XWX-12 < TY-207 < XFY-9 < XWX-17, with the largest value being 14.8 g/pot and 6.4 g/pot. Silicon significantly increased grain biomass of the four genotypes (p<0.001) (Table 1). Genotypes XFY-9, XWX-17 and XWX-12 obtained the largest grain biomass within Si40 treatment, with values being 17.0, 19.1 and 12.9 g/pot respectively; the grain biomass of TY-207 was greatest in Si-20 treatment (18.2 g/pot).

3.2 Effect of Si on Fe plaque formation

In control treatments, As concentrations in Fe plaque were below the detection limit. Significant differences (p<0.005) in Fe concentrations in Fe plaque were observed between the genotypes. In control treatments, Fe concentrations of the four genotypes ranked as follows, XFY-9 < XWX-17 < TY-207 < XWX-12. The results demonstrated that Si had a significant effect on Fe concentrations in plaque formation (p<0.05). Silicon additions increased Fe concentrations in Fe plaque (Figure 1a) and

had a significant effect on As concentrations in the Fe plaque. However, with increasing Si, As concentrations firstly increased but then decreased (Figure 1b).

Both Fe and As concentrations were higher in DCB-extracts from indica than that of the other hybrid genotypes (Figure 2), demonstrating that indica genotypes with higher ROL contribute to further Fe plaque formation. Compared to treatments without Si, Si application influenced the sequestration of As on Fe plaque both in indica and hybrid genotypes (Figure 2a, b). Arsenic was positively correlated with Fe concentrations in Si0 treatment (Figure 2a), however, with the increasing Fe concentrations, As concentrations increased only marginally in hybrid genotypes, and negatively correlated with Fe concentrations in indica genotypes in Si treatments (Figure 2b).

3.3 The effect of Si on total As in rice plants

Total As concentrations in rice plants cultivated in control treatments were below the detection limit (Table 3). There were significant genotypic effects on root As concentrations in rice ($p<0.05$) (Figure 3a). Application of Si significantly reduced root As concentrations in rice plants ($p<0.005$), and with increasing Si concentrations, root As concentrations in the four genotypes decreased, reaching the lowest concentrations in Si40 treatment (Figure 3a). In Si40 treatment, root As concentrations from the four genotypes were reduced to their lowest. Compared to Si0 treatment, root As concentrations decreased 30%, 35%, 28% and 16% in the four treatments respectively.

Genotypes had no significant effect on straw As concentrations in rice plants (p>0.05) (Figure 3b). Genotypes XWX-17 and XWX-12 grown in Si40 treatment however differed significantly in husk total As concentrations (p<0.01) (Figure 3c).

Genotypes had a significant effect on total grain As concentrations (p<0.001) (Figure 3d). In Si0 treatment, As concentrations in grains ranked as follows, XWX-12 < XWX-17 < TY-207 < XFY-9. A significant effect on reducing grain As concentrations in XWX-17 grown in Si10, Si20 and Si40 treatments was also observed compared to Si0 treatment (Figure 3d).

3.4 The effects of Si on As species

The predominant As species in rice grain were As (III) and DMA; DMA accounted for 91%-95% of total As concentrations in grains (Figure 4). All Si treatments reduced DMA accumulation in rice grains of genotype XWX-12 (Figure 4). The Si40 treatment decreased total grain As of XWX-12 by 20% compared to Si0 treatment. Results also demonstrated that MMA was not detectable in the grain. Silicon addition decreased DMA accumulation but had no significant effect on iAs accumulation.

4. Discussion

4.1 Effect of Si on rice biomass

Rice is a typical Si-accumulating crop, and Si application, regardless of Si solution, minerals or foliar-fertilizer, is considered to have positive and consistent

254 effects on rice health and yield (Seyfferth and Fendorf, 2012; Detmann et al., 2012; Liu
255 et al., 2014), due to alleviation of both biotic and abiotic stress factors (Ma, 2004;
256 Guo et al., 2005; Epstein, 2009). Emerging evidence has shown that Si can enhance
257 rice resistance to As toxicity (Fleck et al., 2013). Silicon increased shoot length and
258 biomass of rice seedlings cultivated in As-amended hydroponic solutions (Guo et al.,
259 2007; Guo et al., 2009; Tripathi et al., 2013) and this has been demonstrated in our pot
260 experiment indicating that Si significantly increased the biomass of roots and grains
261 of different rice genotypes (Table 1). Recent studies have also reported that Si
262 markedly increased rice grain and straw yield (Li et al., 2009; Fleck et al., 2013; Liu
263 et al., 2014), which is consistent with our results, but we also observed that with
264 certain Si treatments the opposite effect can occur (Table 1). Recent research by Lee
265 et al (2014) demonstrated that Si addition to As-contaminated soil increased As
266 concentrations in soil solution due to competition between of Si and As for adsorption
267 sites; this increased the bioavailability of As and increased its toxicity to rice.
268
269 *4.2 Effect of Si on Fe plaque formation*
270 Iron plaque is abundantly formed on the root surfaces of common aquatic plants
271 such as paddy rice (Chen et al., 2005; Wu et al., 2012). The formation of the plaque is
272 considered to be a “barrier” to As uptake and subsequent above-ground accumulation
273 in rice (Liu et al., 2004a; Wu et al., 2012). Previous studies have demonstrated that
274 there are many factors influencing the formation of Fe plaque such as radial oxygen
275 loss (ROL), genotype, pH, Eh and presence of microorganisms (Taylor et al., 1984;

276 Emerson et al., 1999; Wu et al., 2012). Iron oxides are able to combine with silicate to
277 form iron silicate and deposit on root surfaces (Liu et al., 2004a, b; Liu and Zhu,
278 2005). Results from the present study have indicated that Si application can
279 significantly increase Fe plaque formation (p<0.05). Seyfferth and Fendorf (2012)
280 discovered that Si concentrations, in root Fe plaque of three rice genotypes, were
281 higher in Si treatments than controls (no Si), which is further confirmed in our
282 investigation. Liu et al (2006) observed that Fe plaque mainly consisted of oxides, but
283 also contained ferrihydrite and goethite. Silicon acid limits the adsorption of As(III)
284 and As(V) on ferrihydrite by occupying the absorption sites (Luxton et al., 2008; Liu
285 et al., 2014) and this may explain the decrease in As observed in DCB-extracts with
286 increasing Si concentrations (Figure 2b).
287
288 *4.3 Effect of Si on As uptake by rice*
289 Previous studies have revealed the suppressing effect of Si on As uptake and
290 accumulation in rice, regardless of whether the investigation was hydroponic (Guo et
291 al., 2005, 2007) or pot experiments (Seyfferth and Fendorf, 2012; Fleck et al., 2013;
292 Wu et al., 2015). Numerous studies have indicated that As(III) is the predominant
293 species in waterlogged soils due to the absence of O_2 (Takahashi et al., 2004; Xu et al.,
294 2008; Zhao et al., 2013a) and As(III) concentrations can exceed 87% of the total As in
295 soil solution (Liu et al., 2014). As a chemical analogue of silicic acid, As(III) is
296 taken up into rice roots via the Si transport channel (Ma et al., 2008). This
297 consequently leads to competition between Si and arsenite for uptake and

translocation in rice roots. However, Si application has been shown to reduce the regulation of Si transporter genes (Ma et al., 2006; Liu et al., 2014) and Fleck et al (2013) reported that Si application decreased As concentrations in brown and polished rice by 22% by inhibiting As(III) uptake and transportation to shoots. Furthermore, external Si increased the P concentration in nutrient solution and soil pore water in hydroponic and soil pot experiments respectively (Guo et al., 2007; Fleck et al., 2013). In addition, while As(V) has similar physicochemical properties with phosphate and As(V) shares the same phosphate transport pathway (Abedin et al., 2002), increased P may inhibit As uptake in rice. Although some studies have reported that Si addition increased As concentrations in soil solution, by displacing soil adsorption retention sites and inhibiting As adsorption on Fe plaque (Luxton et al., 2008; Liu et al., 2014), the effects were much lower than the inhibitory effect of Si on As uptake and translocation in rice roots (Liu et al., 2014). The competitive relationship between Si and As was determined by Lee et al (2014), who observed that higher Si/As ratios induced greater inhibitory effects on As uptake.

4.4 Effect of Si on As speciation in rice grain

Previous investigations have demonstrated that iAs and DMA were the predominant As species in rice grain (Zavala et al., 2008; Zhu et al., 2008a), which are consistent with the results of the present study (Figure 4). Not only did the addition of Si reduce total As concentrations in XFY-9 and XWX-12, but it reduced iAs by 16

and 20% respectively (Table 4), this result being consistent with Li et al (2009) who observed a reduction iAs by 59% following Si application. Arsenite is the main As species found in rhizospheres of waterlogged soils (Takahashi et al., 2004; Xu et al., 2008; Zhao et al., 2013a). As a result of competition between Si and As(III) in the silicic acid transport system, and the reduced expression of the Si transporter genes (Ma et al., 2006; Liu et al., 2014), Si reduced the uptake and translocation of As in rice plants (Liu et al., 2014). Furthermore, rice grain iAs concentrations were lower in genotypes with higher ROL than genotypes with lower ROL under the same Si treatments. This indicates that both genotype and environmental conditions have an influence on As speciation.

In the present study Si reduced DMA concentrations in rice grain, whereas Li et al (2009) and Liu et al (2014) observed that Si application increased DMA concentrations in rice grain. Conflicting evidence from Fleck et al (2013) reported that Si application to soil didn't have any influence on DMA concentrations in rice grains. Recent research indicates that rice plants lack the ability to methylate As, and DMA is derived from methylation by soil microorganisms (Zhao et al., 2013a); differences in microbial communities and soil type will affect this process considerably (Zhao et al., 2013b). Additionally, Fleck et al (2013) demonstrated that Si addition increased soil pH, and Li et al (2009) found that increasing soil pH reduced MMA and DMA uptake significantly in rice. Additionally, in paddy soils it is believed that DMA can exist as neutral molecules and negatively charged anions. Former species of DMA are most likely to be regulated by the silicic acid transport

342 system (Kersten and Daus, 2014), therefore external Si additions may inhibit the
343 uptake of DMA. Consequently, Si addition may have decreased DMA concentrations,
344 whilst preferential uptake of As(III) by roots may have further reduced DMA uptake
345 (Liu et al., 2014) as the affinity for As(III) is stronger than DMA (Abedin et al.,
346 2002).
347
348 **5. Conclusion**
349 Four rice genotypes which differed in their radial oxygen loss (ROL), two hybrid
350 subspecies Xiangfengyou 9 ('XFY-9') and T-you 207 ('TY-207'), and two indica
351 subspecies Xiangwanxian 17 ('XWX-17') and Xiangwanxian 12 ('XWX-12'),
352 were selected to investigate the effects of varying silicon concentrations on As uptake,
353 iron plaque formation in rice and As speciation in grains. Results demonstrated that in
354 most treatments, Si additions increased grain biomass. There were significant
355 genotypic differences in the concentrations of iron plaque formed, with indica
356 producing more iron plaque than hybrid genotypes and sequestering higher As
357 concentrations. Silicon applications significantly increased Fe concentrations in iron
358 plaque of the genotypes. Arsenic concentrations in roots, husks and grains of indica
359 genotypes were lower than hybrid genotypes. The main As species in rice grains were
360 As(III) and DMA, with DMA accounting for > 90% of total As. Indica genotypes
361 accumulated lower concentrations of iAs than hybrid genotypes. Silicon addition
362 decreased both inorganic arsenic and DMA concentrations in grain. This study is
363 potentially a step forward to understanding As uptake mechanisms in rice and

mitigating the health risks posed by As contamination in paddy fields.

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Table 1. Biomass of roots, straws and grains from the four genotypes grown in the different treatments (g/pot; mean ±SD, n=3).

Genotype	Treatment	Root	Straw	Grain
XFY-9	Control	15.8±2.73	22.0±4.34	7.80±0.64
	Si0	16.9±1.50	24.1±3.78	10.6±0.80
	Si10	14.1±1.69	21.8±2.06	4.43±1.78
	Si20	15.7±5.64	29.4±1.63	14.3±0.92
	Si40	25.1±2.60	26.3±3.62	17.0±0.79
TY-207	Control	9.7±0.11	19.7±0.68	11.2±1.78
	Si0	17.5±2.52	22.6±6.26	11.5±1.06
	Si10	18.8±7.06	24.2±6.82	9.19±1.68
	Si20	22.3±2.04	28.8±5.75	18.2±2.42
	Si40	17.9±3.14	26.5±3.44	15.5±1.73
XWX-17	Control	11.5±2.06	29.4±1.30	14.8±2.85
	Si0	17.6±8.82	27.1±12.0	12.6±2.03
	Si10	14.0±4.28	24.9±5.96	14.5±4.12
	Si20	17.8±6.13	32.6±9.59	10.5±0.99
	Si40	14.6±2.97	35.6±4.68	19.1±4.96
XWX-12	Control	9.25±0.94	17.8±3.18	6.40±1.81
	Si0	12.4±2.43	18.1±4. 80	8.47±1.16
	Si10	12.1±4.19	15.8±6.00	11.3±1.09
	Si20	12.7±1.85	16.2±7.01	12.1±0.78
	Si40	13.2±1.63	18.3±3.59	12.9±0.95
Analysis of variance				
Genotype (G)		P<0.05	P<0.001	P<0.001
Si		P<0.05	NS	P<0.001
G×Si		NS*	NS	P<0.001

555

556 Table 2. Iron and As concentrations in Fe plaque of rice roots subjected to different
557 treatments.

Genotypes	Treatments	Fe (mg/kg)	As (mg/kg)
XFY-9	Control	616±75.2	ND[a]
	Si0	835±139	20.8±7.5
	Si10	1119±160	24.4±10.2
	Si20	1240±298	21.5±16.9
	Si40	1160±158	19.1±6.4
TY-207	Control	925±141	ND
	Si0	900±154	21.3±9.7
	Si10	1080±248	26.6±12.7
	Si20	881±86.9	21.8±7.2
	Si40	962±301	18.4±5.7
XWX-17	Control	873±307	ND
	Si0	1130±707	36.9 ±28.1
	Si10	1430±603	40.5±20.8
	Si20	1210±626	32.1±22.7
	Si40	1380±191	25.4±5.8
XWX-12	Control	985±118	ND
	Si0	1450±44.2	25.5±5.6
	Si10	1490±593	31.3±15.0
	Si20	1500±255	24.1±7.1
	Si40	1660±49.8	27.1±4.5
Analysis of variance			
Genotype(G)		P<0.005	NS
Si		P<0.05	P<0.001
G×Si		NS[b]	NS

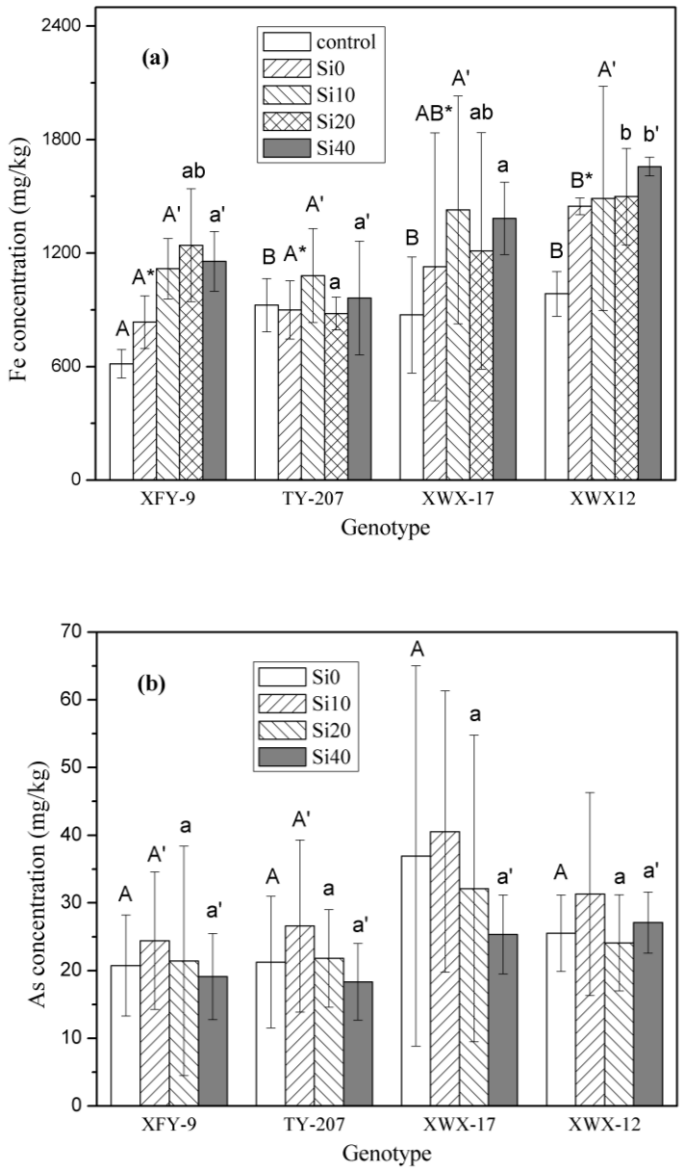

Fig. 1. Fe (a) and As (b) concentrations in Fe plaques of rice roots grown in different silicon treatments (mg/kg, mean ±SD).

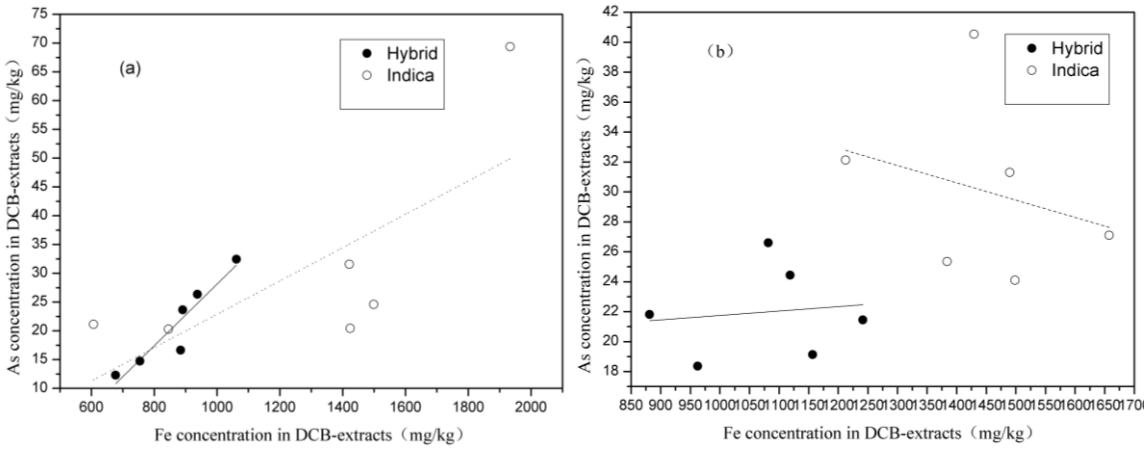

Fig. 2. Relationship between As and Fe concentrations in DCB-extracts (mg/kg; mean ±SD) for no Si treatment (a) and Si treatments (b).

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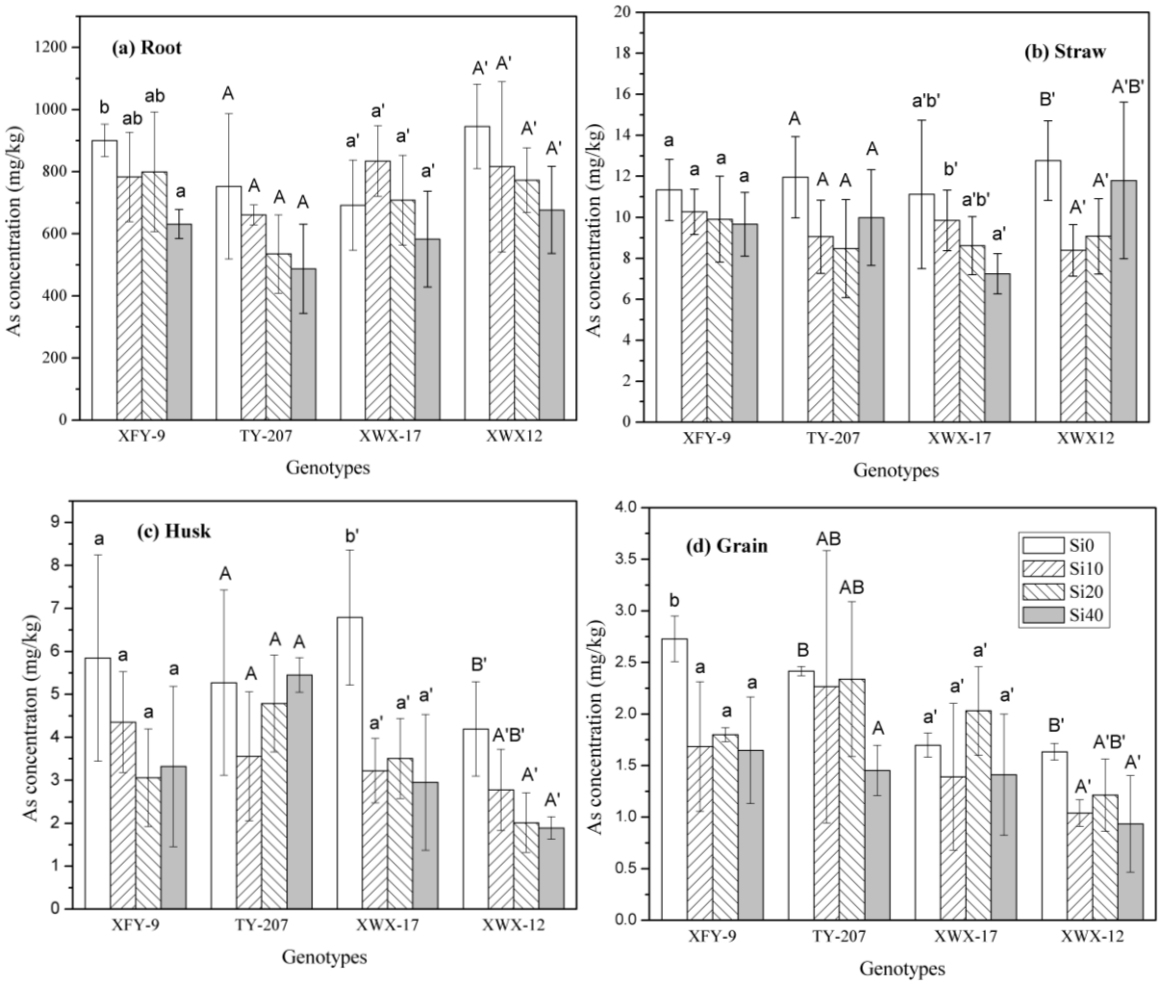

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612 Fig.3. Total As in rice roots (a), straws (b), husks (c) and grains (d) grown in
613 different silicon treatments (mg/kg; mean ±SD).

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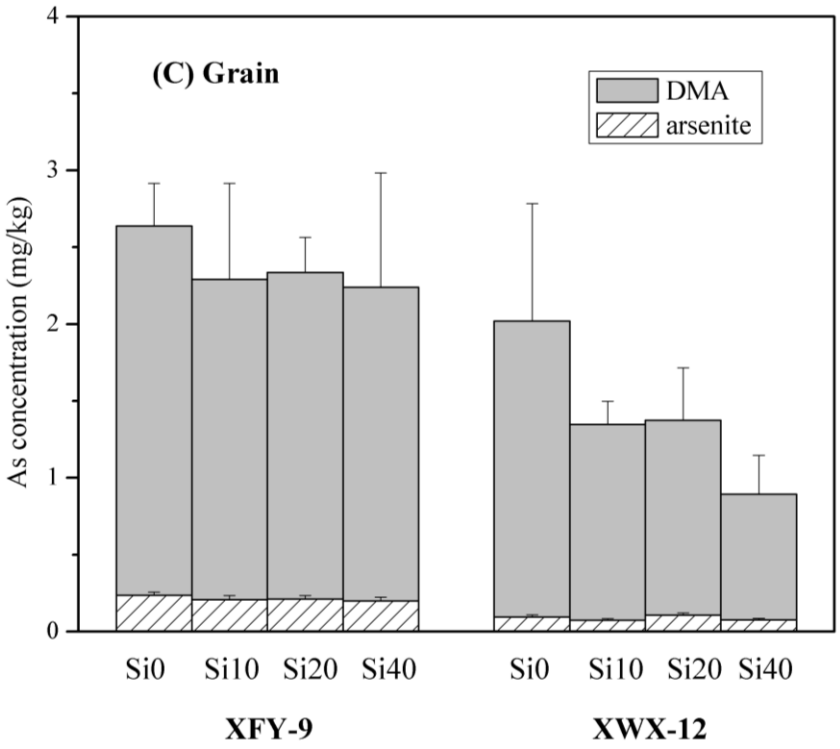

Fig. 4. Arsenic species in grains of XFY-9 and XWX-12 genotypes grown in different silicon treatments (mg/kg; mean ±SD).