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REGULAR ARTICLE

Effects of simultaneous arsenic and iron toxicities on rice (*Oryza sativa* L.) development, yield-related parameters and As and Fe accumulation in relation to As speciation in the grains

Delphine Vromman · Stanley Lutts · Isabelle Lefèvre · Laurent Somer · Olivier De Vreese · Zdenka Šlejkovec · Muriel Quinet

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Abstract
Background and aim In numerous areas, rice cultivated under flooded conditions is exposed simultaneously to iron excess and arsenic contamination. The impact of these combined stresses on yield-related parameters and As distribution and speciation in various plant parts remains poorly documented.
Methods Rice (cv I Kong Pao) was exposed to iron excess (125 mg L^{-1} Fe_2SO_4), arsenic (50 and 100 μM $Na_2HAsO_4.7H_2O$) or a combination of those stressing agents in hydroponic culture until harvest. Plant growth, yield-related parameters, non protein thiols concentration and mineral nutrition were studied in roots and shoots. Arsenic speciation was determined by high-performance liquid chromatography-hydride generation-atomic fluorescence spectrometry.
Key Results Iron excess increased As retention by the roots in relation to the development of the root iron plaque but decreased As accumulation in the shoot. Arsenic concentration was lower in the grains than in the shoots. Iron stress reduced As accumulation in the husk but not in the dehusked grains. Iron excess decreased the proportion of extractable As(III) and As (V) in the grain while it increased the proportion of extractable As(III) in the shoot. Combined stresses (Fe+As) affected plant nutrition and significantly reduced the plant yield by limiting the number of grains per plant and the grain filling.
Conclusions Fe excess had an antagonist impact on shoot As concentration but an additive negative impact on several yield-related parameters. Iron stress influences both As distribution and As speciation in rice.

Keywords Iron plaque · Iron stress · Arsenic · *Oryza sativa* · Rice · Rice grain arsenic

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D. Vromman · S. Lutts (✉) · I. Lefèvre · L. Somer · O. De Vreese · M. Quinet
Groupe de Recherche en Physiologie végétale (GRPV), Earth and Life Institute – Agronomy (ELI-A), Université catholique de Louvain,
Croix du Sud 4-5, boîte L7.07.13,
1348 Louvain-la-Neuve, Belgium
e-mail: stanley.lutts@uclouvain.be

Z. Šlejkovec
Department of Environmental Sciences,
Jožef Stefan Institute, Jamova 39,
1000 Ljubljana, Slovenia

Introduction

Rice (*Oryza sativa*) is a staple crop for over half of the world's population. In numerous areas where it is cultivated, irrigation often carries polluted water to

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paddy rice, and this crop has to face multi-elemental stress imposition. This is especially the case in South and South-East Asia with arsenic and iron, which problem recently came out (Williams et al. 2007; Brammer and Ravenscroft 2009).

Build-up of As in soil associated with the use of As-contaminated irrigation water has been shown to lead to elevated levels of As in paddy soil and soil solution (Brammer and Ravenscroft 2009). Excessive accumulation of this element, particularly inorganic As, in rice poses a potential health risk to populations with high rice consumption (Zhao et al. 2010). Arsenic is a redox-sensitive trace element which can exist in four oxidation states and its chemical form in the environment strongly depends on its oxidation state and speciation. In soils, inorganic As is predominantly present as the pentavalent oxyanion arsenate (As(V)) or the trivalent oxyanion arsenite (As(III)) (Zhao et al. 2009). Soil microbes can methylate inorganic As to give monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Meharg and Hartley-Whitaker 2002; Lomax et al. 2012).

Accumulation of As in rice reduced plant growth and productivity, affects macro- and micro-nutrition and results in the generation of reactive oxygen species (ROS) (Hartley-Whitaker et al. 2001; Carbonell et al. 1998; Chen et al. 2005; Shaibur et al. 2006; Hossain et al. 2007; Panaullah et al. 2009). Arsenite reacts with sulfhydryl groups (−SH) of enzymes and tissue proteins, leading to inhibition of cellular functions and ultimately to death (Meharg and Hartley-Whitaker 2002) while As (V) is an analogue of phosphate and thus interferes with essential cellular processes such as oxidative phosphorylation and ATP synthesis (Tripathi et al. 2007). Plants roots are able to take up various As species through different mechanisms (for review, see Zhao et al. 2010). It is generally admitted that As(V) is readily reduced to As(III) *in planta* and that As(III) is the main form loaded into the xylem (Zhao et al. 2010). Complexation with thiol compounds (−SH) such as phytochelatins (PCs), and subsequent vacuolar sequestration constitute an important mechanism for As(III) detoxification in plants (Zhao et al. 2009, 2010; Moore et al. 2011; Wu et al. 2011).

In most As-affected areas of South and South-east Asia, groundwater is also rich in Fe (Rahman and Hasegawa 2011). Concomitance of both elements has also been reported in African areas (Zhu et al. 2008; Fageria et al. 2008). Iron is a major constituent of most soils and is also a redox sensitive metal element. In normal aerated soil conditions, Fe is mainly present in its oxidized insoluble form Fe(III). Under flooded conditions, as those commonly prevalent for rice culture, reduction processes of the ferric form to the soluble ferrous form (Fe(II)) occurs. Arsenic and Fe are thus quickly released from soil to soil solution during the flooded period because of the dissolution of Fe (hydro)oxides that also sequester As (Takahashi et al. 2004). Toxicities of As and Fe are thus directly linked to their biodisponibility in paddy soils (Yamaguchi et al. 2011).

The ability of rice to carry oxygen from the air down its stem through aerenchyma and discharge it through its roots, creates an oxidised zone around individual roots in which Fe is re-oxidised to the Fe(III) state and forms a coating called "iron plaque" (Liu et al. 2004; Hu et al. 2005). The Fe plaque may retain As and reduces its disponibility for the plant (Hansel et al. 2002; Hu et al. 2005; Liu et al. 2006; Garnier et al. 2010; Lee et al. 2013). Most of the As adsorbed by the Fe plaque consists in As(V) (70–80 %), the remaining being As(III) (Voegelin et al. 2007). Arsenic concentrations in the rice Fe plaques could be 5-fold higher than those recorded inside the root tissues (Liu et al. 2006). However, some studies showed that significant amount of As could nevertheless be taken up by rice plants in the presence of Fe plaque (Rahman and Hasegawa 2011; Seyfferth et al. 2011).

Iron is an essential element for all plants, and assumes many important biological roles in photosynthesis, respiration, nitrogen fixation, DNA synthesis, or as co-factor of key enzymes involved in plant hormone synthesis (Hell and Stephan 2003; Jeong and Connolly 2009). However, Fe excess can be highly damaging as it induces free radical production that irreversibly impairs cellular structure and damages membranes, DNA and proteins (Thongbai and Goodman 2000; Sahrawat 2004). In rice, iron-stressed plants present a stunted growth, limited tillering, reduced fertility and nutritional disorders (Thongbai and Goodman 2000; Sahrawat 2004; Becker and Asch 2005; de Dorlodot et al. 2005; Majerus et al. 2007). The most known iron toxicity symptoms in rice are "leaf bronzing" characterized by small brown-purple spots appearing from the tips towards the base of the older leaves, and the formation of an Fe plaque at the outer surface of the roots (Asch et al. 2005; Becker and Asch 2005; Majerus et al. 2007).

Several recent studies investigated the impact of Fe plaque on the As uptake by rice plants (Deng et al. 2010; Garnier et al. 2010; Seyfferth et al. 2011; Rahman et al. 2011; Lee et al. 2013). Nevertheless, the effect of combined Fe and As toxicities on plant growth and yield-related parameters is still poorly studied. Arsenic accumulation and speciation in rice grains has been studied and show considerable genotypic and geographic variation. Beside As(V) and As (III), the presence of various proportion of DMA(V) is also frequently mentioned (Ma et al. 2008; Carey et al. 2010; Lomax et al. 2012). However, only few data concerning the impact of the Fe plaque on As distribution, accumulation and speciation in the grains are available (Seyfferth et al. 2011). Conversely, Fe is an important element for human health and is present at rather low concentration in rice grains (Jeong and Guerinot 2008). The impact of Fe toxicity and the influence of As on Fe translocation to the grains have been poorly documented.

In this work, we aimed to investigate the impact of As and Fe stresses on rice growth and yield in order to see i) if the presence of Fe plaque reduces As translocation and speciation in the shoots and in the grains, ii) if As modifies Fe content in the grains and iii) how combined stresses affect yield-related parameters and plant nutrition. Arsenic and Fe excess were applied separately and simultaneously in order to precise the antagonist, additive or synergic effects of those two ion toxicities on rice growth and development.

Materials and methods

Plant material and growth conditions

Seeds of rice (*Oryza sativa* L.) cv 'I Kong Pao' (IKP) were obtained from the IRRI (International Rice Research Institute, Philippines). The seeds were germinated on two layers of moistened Whatman N° 2 filter paper in a growth chamber at 25 °C under a 12 h daylight period (70 $\mu mol\,m^{-2}\,s^{-1}$). Fifteen-day-old seedlings were transferred into greenhouse and fixed on polystyrene plates floating on nutritive solution (1.43 mM NH_4NO_3, 323 μM NaH_2PO_4, 512 μM K_2SO_4, 750 μM $CaCl_2$, 1.64 mM $MgSO_4$, 9.45 μM $MnCl_2$, 0.07 μM $(NH_4)_6\,Mo_7O_{24}$, 18.76 μM H_3BO_3, 0.14 μM $ZnSO_4$, 0.14 μM $CuSO_4$, 35.59 μM $FeCl_3$, 77.41 μM $C_6H_8O_7$, Yoshida et al. 1976) in 25 L tanks. The greenhouse was heated to ensure a mean temperature of 24 °C and extra lighting was provided by PHILIPS HPLR 400 W bulbs to expose the plants to a 12 h daylength at a minimum photon flux density (PAR) of 250 $\mu mol\,m^{-2}\,s^{-1}$ at the top of the canopy. The solutions were renewed weekly. After 2 weeks of acclimation $FeSO_4 \cdot 7H_2O$ (final concentration of 0 and 125 mg L^{-1} of ferrous iron) and $Na_2HAsO_4.7H_2O$ (final concentration of 0, 50 and 100 μM arsenic) was added in order to obtain 6 different treatments: control, Fe 125 $mg\,L^{-1}$, As 50 μM, As 100 μM, Fe 125 $mg\,L^{-1}$+ As 50 μM and Fe 125 $mg\,L^{-1}$+ As 100 μM. The ferrous iron solution added to the Yoshida solution was bubbled with N_2 to ensure that iron remained in its soluble reduced form (de Dorlodot et al. 2005). The pH was adjusted every 2 days to 4.5 in all tanks. Redox potential (Eh) was maintained between 178 and 204 mV throughout the experiment. Plants were harvested after 4, 8, 16 and 23 weeks of treatment.

Plant growth, yield-related parameters and pollen viability

The shoot height, number of leaves and number of tillers were recorded every week for 5 plants per treatment. The heading date was assessed considering the appearance of the first panicle in each treatment. The number of panicles per plant and number of grains (full and empty) per panicle as well as the 1000 grains weight were recorded for 7 plants at final harvest.

To estimate pollen viability, spikelets were collected just before anthesis and fixed in formalin-acetic acid-alcohol (FAA; 70 % ethanol: acetic acid : formaldehyde, 18 : 1 : 1, by volume). Pollen grains of the stamens were then dispersed by squeezing on a glass slide in a drop of Alexander solution (Alexander 1969) and observed under a light microscope. Viable grains had a circular shape with red protoplasm, whereas aborted grains were oval and empty exhibiting a green coloration in their wall. Five spikelets and 200 pollen grains per spikelet were analysed per treatment.

Four plants per treatment were harvested after 4 and 23 weeks of treatment. Roots of those harvested plants were rinsed in demineralized water and gently blotted dry with a paper towel. For each plant, roots, shoots and panicles were separated and weighed. Samples were dried at 70 °C for 72 h and weighed again to measure the dry weight.

Mineral content determination and arsenic speciation

The mineral content was determined in roots and shoots after 4 and 23 weeks of treatment. Arsenic, Fe, P and S concentrations were also quantified in grains at final harvest. A distinction was made between empty grains and full grains. Full grains were dehusked and ion content was determined in the grain itself and in the husk separately. For each plant part and treatment, 3 replicates of c.a. 100 mg DW of pooled material was digested in HNO_3 65 %. The mixture was gently heated on a sand bath until complete dryness. The residue was redissolved with aqua regia (HCl 37 %: HNO_3 65 % 3:1) and filtered (Whatman, 11 μm). The solution was diluted to 10 mL with deionised water and analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, iCAp6500, Thermo Scientific).

For the As speciation by high-performance liquid chromatography-hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS), the harvested plant material was immediately frozen in liquid nitrogen and was subsequently freeze-dried. The samples were ground in a mortar and extracted overnight in methanol/water (1:1) and then centrifuged at 1,000*g* (10 min). The extraction step was repeated twice, and the obtained extracts were combined, dried on a rotary evaporator (45 °C, approximately 20 min), resuspended in 4 mL of Milli-Q water, filtered (0.45 μm Millipore PVDF filter) and maintained at 4 °C until the analysis. The chromatographic separation of the anionic As species (As(III), As(V), MMA and DMA) was performed on a Hamilton PRP-X100 anion exchange column using 15 mM KH_2PO_4, pH6.1 as a mobile phase. An on-line hydride generation step (2 M HCl, 3.0 mLmin^{-1} and 1.5 % $NaBH_4$ in 0.1 % NaOH, 3 mLmin^{-1}) was applied to generate volatile arsenic hydrides, which were detected in an Excalibur atomic fluorescence spectrometer (PS Analytical, Kent, UK). The cation exchange chromatography (Alltech Adsorbosphere SCX column, 2.5 mM pyridine, pH2.65) with an online UV decomposition was used to test for the presence of the cationic As species (arsenobetaine, arsenocholine, trimethylarsine oxide, and tetramethyl arsonium ion) (Šlejkovec and Van Elteren 1999). Non extractable As was calculated as total As minus extractable As species (sum of all species found).

Non-protein thiol quantification

The root and shoot of three plants per treatment were harvested after 4 and 16 weeks of treatment and frozen in liquid nitrogen. The total non-protein thiol concentration was determined according to De Vos et al. (1992) by grinding 200 mg FW of tissue in 2 mL of 5 % (w/v) sulfosalicylic acid + 6.3 mM diethylenetriaminepentaacetic acid (pH<1) at 0 °C with quartz sand in a mortar. The homogenate was centrifuged at 10,000*g* for 10 min at 4 °C. The clear supernatants were collected and used for the determination of thiols using Ellman's reagent. Three hundred microliters of supernatant were mixed with 630 μL of 0.5 M KH_2PO_4 and 25 μL of 10 mM 5,5-dithiobis 2-nitrobenzoic acid (final pH7.0). The absorbance at 412 nm was recorded after 2 min, and the thiol concentration was estimated using an extinction coefficient of 13,600 $M^{-1}cm^{-1}$.

Iron plaque analysis

Four types of extraction procedures to separate iron plaque from the roots were tested and compared. The roots of 20 plants per treatment were harvested after 4 weeks of treatment, 5 of them were rinsed with water for 1 min (control), 5 others were rinsed with 0.016 M KH_2PO_4 for 1 h, 5 root systems were rinsed with dithionine-citrate-bicarbonate (DCB; 0.03 M $Na_3C_6H_5O_7.2H_2O$, 0.125 M $NaHCO_3$, 0.6 g $Na_2S_2O_4$) for 30 min (Delfosse et al. 2005) and the last 5 were rinsed with 0.2 M ammonium oxalate pH3.0 for 2 h (Liu et al. 2004; Hu et al. 2005; Delfosse et al. 2005). After these treatments, roots were rinsed in demineralized water, gently blotted dry with a paper towel, dried at 70 °C for 72 h and digested as previously described to quantify minerals. The concentrations of Fe, As, P and S in the roots were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, iCAp6500, Thermo Scientific). Arsenic speciation was determined in roots rinsed with water and in roots rinsed with DCB as above described.

Statistical analysis

Normality tests were performed and log transformation of the raw data was made when required. An analysis of variance was performed on all data set

using SAS software (SAS 9.1 system for windows). Differences between means were score for significance using the Student Newman Keuls multiple range test by using 95 % confidence interval.

Results

Growth of rice plants exposed to arsenic and iron stress

Twenty five days old rice seedlings were exposed to As (V) (50 μM and 100 μM), Fe(II) (125 mgL^{-1}) or combined treatments until plant maturation. As shown on Fig. 1b, dry weight was significantly reduced in shoot of plants exposed to Fe and As from 4 weeks of treatment. Subsequent growth reduction increased with stress duration (data not shown). Shoot reduction was particularly marked in plants treated with 100 μM As and with combined As and Fe (Fig. 1b and c). Both stresses also significantly reduced the shoot length already after 2 weeks of stress (data not shown, F=283.37, P<0.0001 for Fe, F=263.62, P<0.0001 for As) and the mean tiller number from 3 weeks of treatment (Fig. 1c, F=37.50, P<0.0001 for Fe, F=42.73, P<0.0001 for As). After 16 weeks of treatment, at the beginning of flowering, the control plants produced 2–3 times more tillers than the stressed plants. The strongest reduction was observed in plants treated with 100 μM As either applied alone or combined with Fe.

Root growth decreased significantly with As 100 μM after 4 weeks of stress (Fig. 1a) but such a reduction was not significant anymore after 23 weeks

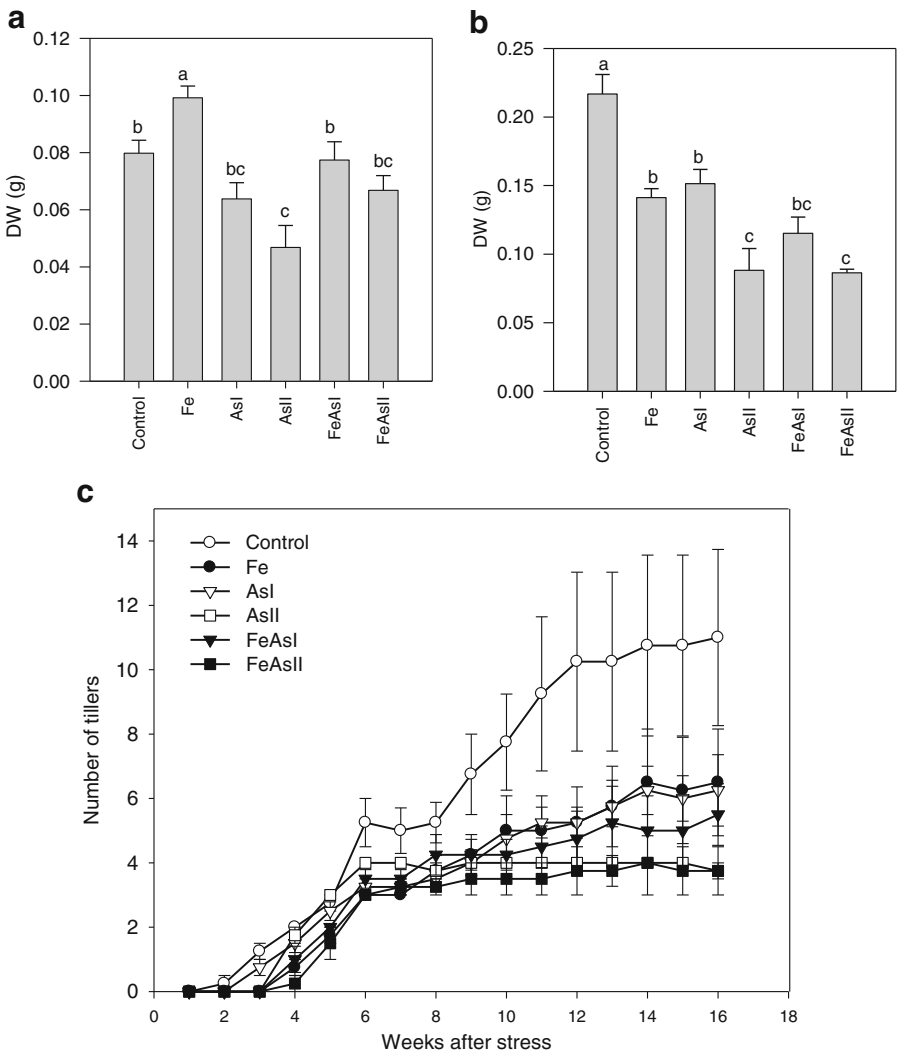


Fig. 1 Impact of As and Fe toxicities on rice (*Oryza sativa*) plants growth. Dry weight of roots (**a**) and shoot (**b**) after 4 weeks of treatment and (**c**) number of tillers. Plants were exposed to either 0 mgL^{-1} Fe(II) and 0 mM As(V) (control), 125 mgL^{-1} Fe(II) (Fe), 50 μM As(V) (AsI), 100 μM As(V) (AsII), 125 mgL^{-1} Fe(II)+50 μM As(V) (FeAsI) or 125 mg L^{-1} Fe(II)+100 μM As(V) (FeAsII). Bars represent SE of means. Values followed by a same letter are not statistically different at P<0.05 according to Student Newman Keuls multiple range test

of treatment (data not shown). The increase of root dry weight in response to the Fe treatments after 4 weeks of stress might be due to the presence of the Fe plaque (Fig. 1a). Iron plaque indeed appeared after 2 weeks of treatment and leaf bronzing symptoms started to be visible after 4 weeks of stress.

Reproductive parameters of rice plants exposed to arsenic and iron stress

Both As and Fe stresses delayed rice flowering (F= 32.73, P<0.0001): the first panicle appeared 140.7± 0.7 days after germination in the control plants and respectively 148.0±1.3, 147.7±0.5 and 145.7±1.0 in the Fe, As 50 μM and As 100 μM treated plants. Addition of Fe excess to the As treatment delayed the flowering by respectively 4.3 and 8.3 additional days compared to the plants grown with As 50 μM and As 100 μM alone. Arsenic and Fe treatments significantly reduced the number of panicles produced per plant (data not shown), the panicle DW (Fig. 2a), the number of spikelets and full grains per plant (Fig. 2b, c). Iron stress particularly decreased the panicle DW and the total number of full grains per plant either when applied alone or combined to As stress (Fig. 2a, c). However, neither Fe nor As stresses affected pollen viability; indeed, between 94 and 98 % of the pollen grains were viable whatever the treatments (detailed data not shown). The weight of 1000 grains was also reduced in Fe treated plants while As induced a reduction of 1000 grains weight at the highest dose only (Fig. 2d). Addition of Fe to As significantly reduced the weight of 1000 grains compared to As treatments alone.

Thiols concentrations of rice plants exposed to arsenic and iron stress

The As stress drastically increased the -SH content in both the root and the shoot after 4 weeks of treatment (Fig. 3). This increase was the highest in the root in response to 100 μM As (Fig. 3a). An increase of –SH content was also observed in the Fe-treated plants

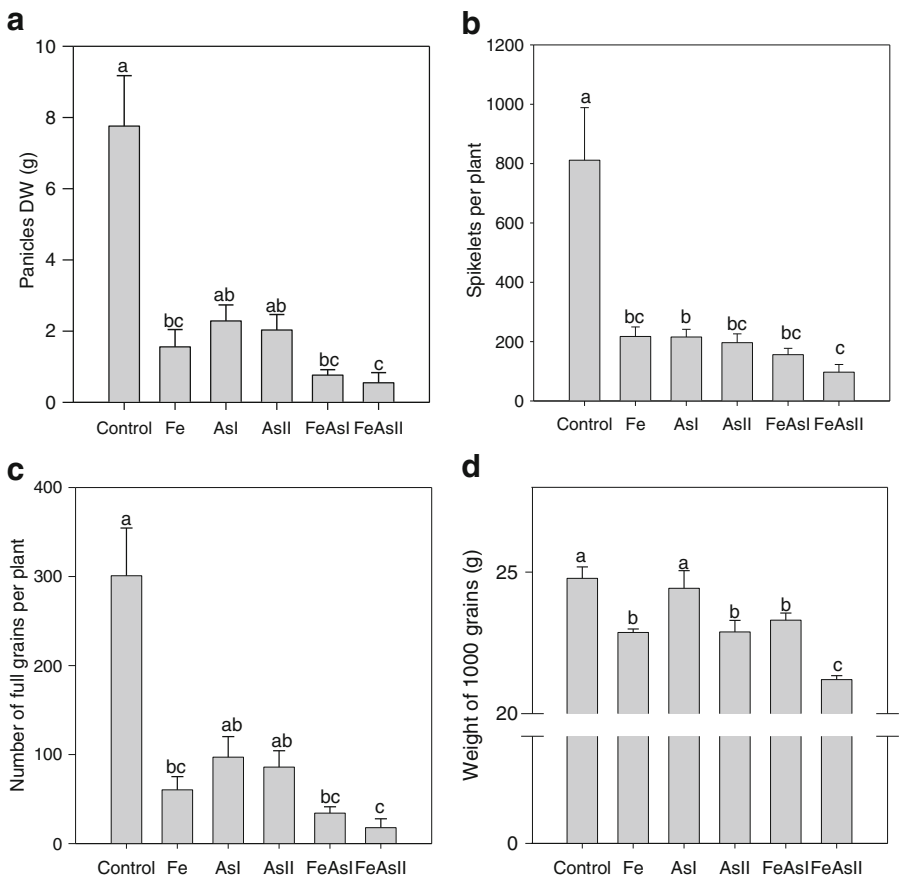


Fig. 2 Impact of As and Fe toxicities on yield related parameters of rice (*Oryza sativa*) plants. (**a**) panicles dry weight, (**b**) number of spikelets per plant, (**c**) total number of full grains per plant, (**d**) weight of 1000 grains. Plants were exposed during 23 weeks to either 0 mg L^{-1} Fe(II) and 0 μM As(V) (control),125 mg L^{-1} Fe(II) (Fe), 50 μM As(V) (AsI), 100 μM As(V) (AsII), 125 mg L^{-1} Fe(II)+50 μM As(V) (FeAsI) or 125 mg L^{-1} Fe(II)+100 μM As(V) (FeAsII). Bars represent SE of means. Values followed by a same letter for a same parameter are not statistically different at P<0.05 according to Student Newman Keuls multiple range test

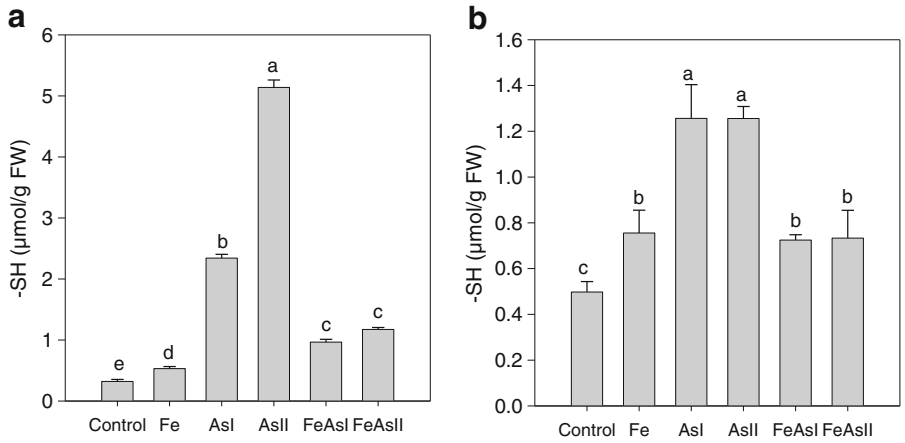


Fig. 3 Impact of As and Fe toxicities on thiols (−SH) content in the root (**a**) and the shoot (**b**) of rice (*Oryza sativa*) plants after 4 weeks of treatment. Plants were exposed to either 0 mgL^{-1} Fe(II) and 0 µM As(V) (control), 125 mgL^{-1} Fe(II) (Fe), 50 µM As(V) (AsI), 100 µM As(V) (AsII), 125 mgL^{-1} Fe(II)+50 µM As(V) (FeAsI) or 125 mgL^{-1} Fe(II)+100 µM As(V) (FeAsII). Bars represent SE of means. Values followed by a same letter for a same parameter are not statistically different at $P<0.05$ according to Student Newman Keuls multiple range test

compared to the control ones. The combination of As and Fe treatments strongly reduced the –SH concentration compared to the As treatments alone (Fig. 3). A similar –SH concentration distribution was observed after 12 weeks of stress (data not shown).

Iron content of rice plants exposed to arsenic and iron stress

The Fe content in the shoot of Fe stressed plants was higher than in control plants after 4 weeks of treatment (Fig. 4a). Combined Fe and As treatments significantly increased the Fe content in the shoot compared to the As treatments alone (Fig. 4a).

The Fe concentration was more than 200 higher in the roots compared to the shoots whatever the treatment (Fig. 4a, b). The addition of Fe excess to the nutrient solution drastically increased the Fe concentration in the root of treated plants (Fig. 4b), consisting most probably mainly in Fe plaque. The root Fe content was similar in the plants treated with Fe alone or combined with As. The stress duration did not affect the root Fe concentration (Fig. 4b). To extract the different phase of the iron plaque, roots were rinsed either with i) KH_2PO_4 that induced desorption of the elements retained on the iron plaque, ii) ammonium oxalate that extracted the amorphous phase and iii) DCB that extracted all the iron plaque (Hossain et al. 2009). Table 1 shows the Fe concentrations remaining in the roots after those rinsing procedures and the percentage of Fe removed by the different rinsings. Around 30 % of Fe present in the roots of Fe treated plants was adsorbed on the iron plaque. The rinsings with DCB and ammonium oxalate removed most of the iron from the roots of plants treated with Fe either alone or combined to As (Table 1). As shown on Fig. 5, rinsing procedures did not harm the superficial root cell layers integrity.

The Fe treatment, either in the absence or in the presence of As, significantly increased the Fe content in the empty grains and in the husks (Fig. 4c). Iron treatment, however, had no impact on the Fe concentration of dehusked full grains which remained similar to values recorded for control plants.

Arsenic content of rice plants exposed to arsenic and iron stress

In the absence of iron stress, the As concentration in the shoot increased both with the As concentration in the growth solution and the exposure duration (Fig. 6a). Interestingly, the addition of Fe to the As treatments strongly reduced the As content in the shoot (Fig. 6a). The presence of Fe also directly affected the As speciation. As shown in Fig. 6b, the Fe treatment decreased the proportion of non-extractable As and increased obviously the proportion of extractable As(III) compared to the As stressed plants. As a result, the extractable As (III) proportion in the shoot was higher than the extractable As(V) proportion, whatever the treatment. Both extractable As(III) and As(V) concentrations remained

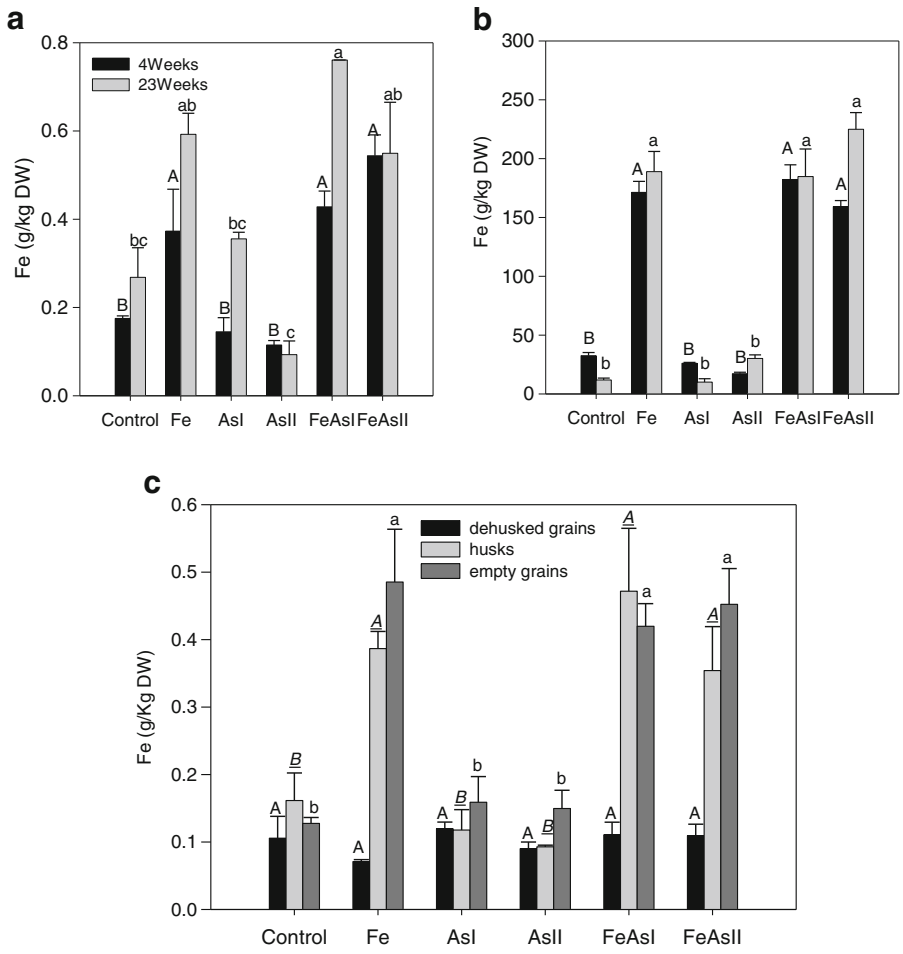


Fig. 4 Impact of As and Fe toxicities on Fe content in rice (*Oryza sativa*) (**a**) shoot, (**b**) root and (**c**) grains. Roots were rinsed with water for 1 min. A distinction was made between empty grains, dehusked full grains and husks. Note that ordinate scales are not the same for shoots and roots. Plants were exposed during 4 weeks (**a**, **b**) and 23 weeks (**a**, **b**, **c**) to either 0 mgL^{-1} Fe(II) and 0 μM As(V) (control), 125 mgL^{-1} Fe(II) (Fe), 50 μM As(V) (AsI), 100 μM As(V) (AsII), 125 mgL^{-1} Fe(II)+50 μM As(V) (FeAsI) or 125 mg L^{-1} Fe(II)+100 μM As(V) (FeAsII). Bars represent SE of means. Values followed by a same letter for a same parameter are not statistically different at $P<0.05$ according to Student Newman Keuls multiple range test

nevertheless lower in plants treated with As + Fe compared to plants treated with As alone. Neither MMA nor DMA were detected in the analyzed shoot.

The As concentration in roots was higher than in the shoot and increased with the As concentration in the nutrient solution but not with the treatment

Table 1 Impact of As and Fe toxicities on Fe and As content in rice (*Oryza sativa;* cv. IKP) roots rinsed with water (control rinsing), with KH_2PO_4, with DCB or with NH_4-Oxalate. Plants were exposed during 4 weeks to either 125 mgL^{-1} Fe(II) (Fe), 125 mgL^{-1} Fe(II)+50 μM As(V) (FeAsI) or 125 mgL^{-1} Fe(II)+100 μM As(V) (FeAsII)

Element	Treatment	Water (control)	KH_2PO_4		DCB		NH_4-Oxalate	
		Means ± SE[a]	Means ± SE[a]	%[b]	Means ± SE[a]	%[b]	Means ± SE[a]	%[b]
Fe (g/kg DW)	Fe	171±9[a]	119±15[a]	30	39±10[a]	77	2.5±0.1[a]	99
	FeAsI	182±12[a]	127±7[a]	31	28±7[a]	85	1.5±0.4[a]	99
	FeAsII	159±5[a]	146±12[a]	8	20±1[a]	87	1.6±0.2[a]	99
As (g/kg DW)	FeAsI	6.9±0.3[b]	4.8±0.2[b]	31	1.2±0.2[b]	83	0.19±0.03[b]	89
	FeAsII	15.8±0.4[a]	13.3±0.9[a]	16	2.2±0.2[a]	86	0.38±0.07[a]	83

[a] Values followed by a same letter for a same treatment and element are not statistically different at $P<0.05$ according to Student Newman Keuls multiple range test.

[b] percentage of Fe or As removed in the root by the different rinsings.

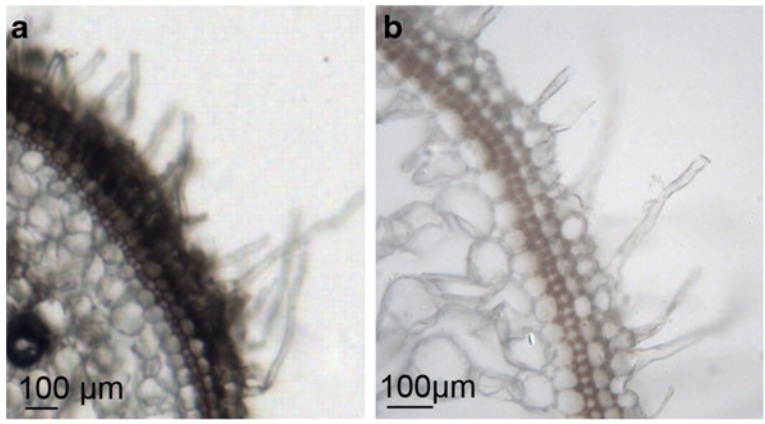


Fig. 5 Transversal sections of rice (*Oryza sativa*) roots treated with 125 mgL^{-1} Fe(II) (**a**) before and (**b**) after rinsing with DCB for 30 min

duration (Fig. 6c). The As concentration significantly increased in the roots when Fe was added in the nutrient solution (Fig. 6c) resulting probably from the retention of As on the Fe plaque. The KH_2PO_4 rinsing removed only a small part of the As present in the roots of plants grown with combined treatments mainly in plants treated with 100 µM As (Table 1). In contrast, DCB and ammonium oxalate rinsing removed most of the As content remaining in As + Fe treated roots (Table 1). The presence of Fe affected also the As speciation (Fig. 6d). More than 50 % of the As present in the roots of the As treated plants was non-extractable and the remaining part mainly consists in extractable As(III) while more than 99 % of the As present in the root rinsed with water of plants treated with both Fe and As was non-extractable (assumed to mainly consist in As bound to the Fe plaque). The concentration of extractable As(III) and As(V) in roots of plants treated with As + Fe rinsed with water were respectively 35±1 and 4.4±0.4 µg/g DW for 50 µM As and 34±4 and 8.3±0.7 µg/g DW for 100 µM As. After removal of the Fe plaque by the DCB rinsing, the concentration of non extractable As decreases by 7.6 times compared to the roots rinsed with water but was similar to the one observed in the roots of plants treated with As alone (fig. 6d). Roots of plants treated with As + Fe after Fe plaque removal contained less extractable As(III) than roots of As-stressed plants but an equal concentration of extractable As(V). No MMA neither DMA were detected in the roots whatever the treatment.

The As concentration was significantly lower in dehusked full grains than in husks and in empty grains whatever the treatment. The As content in the grains was not proportional to the As concentration in the nutrient solution (Fig. 6e). Addition of Fe excess to As-containing nutrient solution strongly decreased As content in husks and empty grains but had no significant impact on dehusked full grains (Fig. 6e). Most of the As present in the dehusked full grains was non-extractable and the remaining part mainly consisted in extractable As(V) (Fig. 6f). The proportion of non-extractable As increased with the As concentration in the solution. The husks of plants treated with As alone contained more extractable As(V) and As(III) than the dehusked full grains. The iron treatment decreased the concentration of extractable As(V) in the dehusked full grains and the concentration of both extractable As(III) and As(V) in the husks.

Mineral content of rice plants exposed to arsenic and iron stress

The P concentration was higher in the roots than in the shoots whatever the treatment (Fig. 7a, c). The Fe treatment, either alone or combined with As, increased the P content in the roots rinsed with water (Fig. 7a). The root rinsing with DCB and ammonium oxalate reduced the P concentration remaining in the roots whatever the treatment (Table 2). After ammonium oxalate rinsing, the lowest P concentration was observed in the roots treated with 100 µM As and Fe. In contrast with root, both As and Fe stresses reduced the P content in the shoots, the strongest reductions were observed in plants treated with Fe and combined treatments. The shoot P content was not affected by the stress duration (Fig. 7c). Phosphorus content was significantly higher in dehusked grains compared to husks and empty grains (F=179.72; P<0.0001). Iron stress decreased the P content in dehusked full grains

and in empty grains mainly when combined with As stress. Arsenic treatment alone did not change P content in grains.

When Fe was added to the nutrient solution, the S concentration increased in the roots rinsed with water after 4 weeks of treatment (Fig. 7b). Rinsing with KH_2PO_4 reduced the S concentration in the roots of Fe treated plants, either alone or combined to As, by 40–50 % and ammonium oxalate removed up to 70 % of the S present in the roots rinsed with water (Table 2). The S concentration in the shoot increased with the treatment duration (Fig 7d). The As treatment increased the S concentration in the shoot after 4 weeks of stress but the difference was no more significant after 23 weeks of stress (Fig. 7d). The S content was higher in husks and empty grains than in dehusked full grains (F=25.48; P<0.0001). Iron and As treatments did not affect the S content in the dehusked full grains and the empty grains but both treatments reduced the S content in the husks when applied alone. The S content in the husks was higher in plants treated with both As and Fe compared to plants treated with As alone.

In general, all stresses reduced the Mg, Ca and K concentrations but the Fe impact was the most obvious (Fig. 8). The Mg and Ca content reduction were similar in the plants treated with Fe alone and in those treated with both Fe and As (Fig. 8a, b) while the reduction was more important in the combined stresses compared to Fe alone for K in the roots (Fig. 8c). The Mg concentration was higher in the shoots than in the roots whatever the treatment (Fig. 8a) while Ca concentration was higher in the shoots compared to the roots at the exception of the Fe treatments (Fig. 8b) and the K content was higher in the shoot than in the roots for all treatments at the exception of the control plants (Fig. 8c).

Discussion

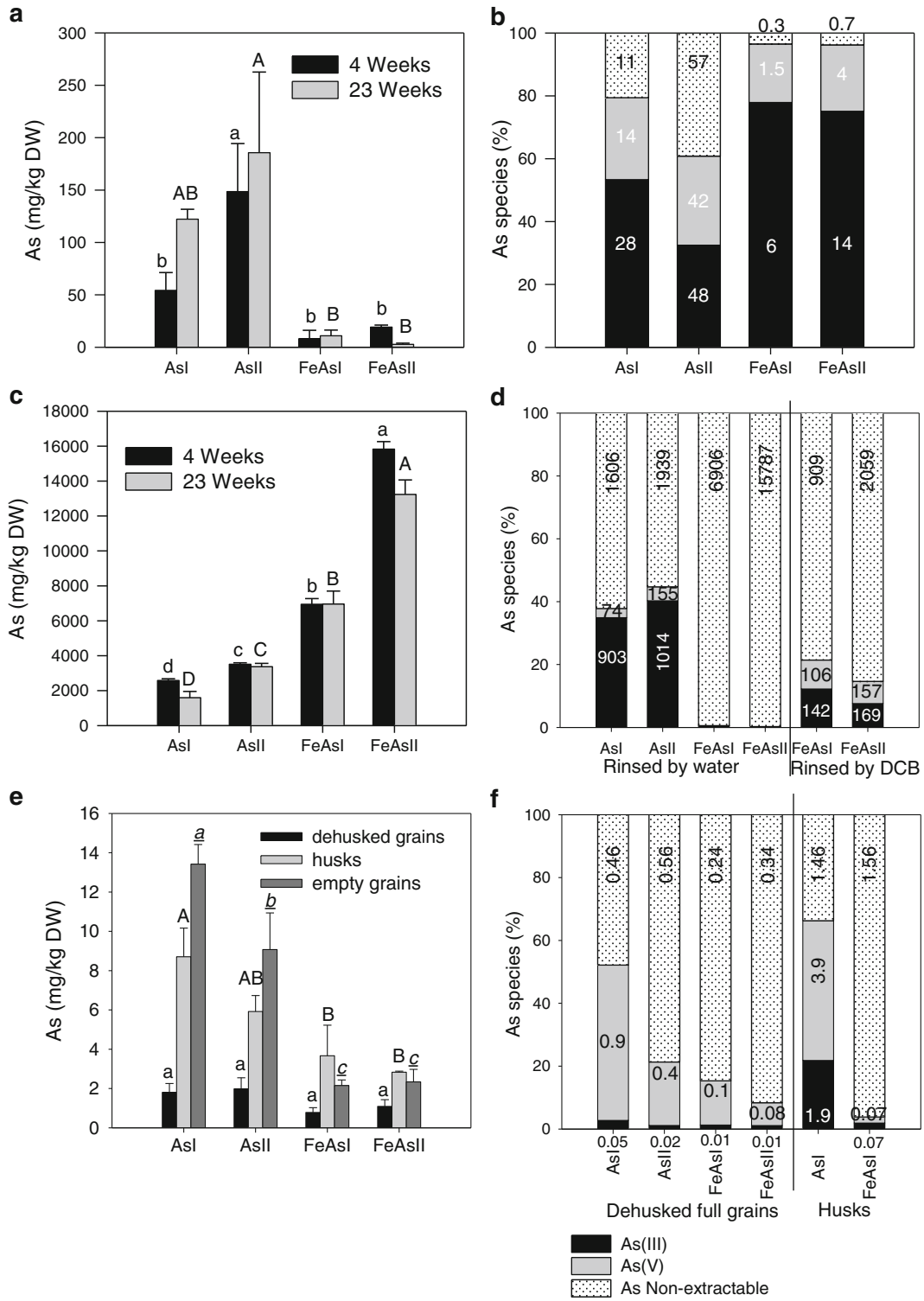
Fe plaque reduces As translocation and modifies As speciation in the vegetative plant organs

It is well known that As increase in the growth environment induces an As increase in plant tissues both at root and shoot levels (Hossain et al. 2009; Panaullah et al. 2009). Our results clearly show that Fe application in the nutrient solution may drastically reduce the As concentration in the shoot compared to plants treated with an equivalent As dose without Fe plaque (Fig. 6a). All the

Fig. 6 Impact of As and Fe toxicities on total As content (**a**, **c** and **e**) and As species (**b**, **d**, **f**) in rice (*Oryza sativa*) (**a**, **b**) shoot, (**c**) root rinsed with water, (**d**) root rinsed either with water or DCB and (**e**, **f**) grains. Note that ordinate scales vary according to the considered organs. A distinction was made between empty grains, dehusked full grains and husks. For As species, the values on the histograms represent the concentration of each As species in mg/kg DW. Plants were exposed during 4 weeks (**a**, **b**, **c**, **d**) and 23 weeks (**a**, **c**, **e**, **f**) to either 0 mgL^{-1} Fe(II) and 0 μM As(V) (control), 125 mgL^{-1} Fe(II) (Fe), 50 μM As(V) (AsI), 100 μM As(V) (AsII), 125 mgL^{-1} Fe(II)+50 μM As(V) (FeAsI) or 125 mgL^{-1} Fe(II)+100 μM As(V) (FeAsII). Bars represent SE of means. Values followed by a same letter for a same parameter are not statistically different at P<0.05 according to Student Newman Keuls multiple range test

plants treated with Fe developed an iron plaque at the root surface. The comparison of the Fe and As concentrations in the roots of plants exposed to combined treatments before and after rinsing with DCB and ammonium oxalate (Figs. 4b and 6c, Table 1) suggests an almost complete dissolution of the Fe plaque and the As it contains. The As content remaining in the rinsed roots of plants treated with both Fe and As was then lower than in the corresponding As treated plants without Fe plaque suggesting that the presence of the Fe plaque has limited the As uptake inside the roots. A similar retention of As by the Fe plaque was previously reported (Hansel et al. 2002; Hu et al. 2005; Liu et al. 2006; Garnier et al. 2010). Nevertheless, according to Seyfferth et al. (2011), Fe precipitated discontinuously on rice roots and thus As may freely enter the root apoplasm without being attenuated by Fe oxide precipitates at the young root surface.

According to Moore et al. (2011), Fe plaque presence affects the As localization in the roots. In plants exposed to both Fe and As, Fe plaque is localized outside the plasma membranes of the epidermal cells and As retention thus occurs outside the tissue. In contrast, in plants exposed to As in the absence of Fe excess, a very high As concentration was detected in the pericycle cell vacuoles where a very high S concentration was also observed (Moore et al. 2011). Our data also suggest that a high proportion of the non-extractable As present in the water rinsed roots of plants exposed to the combined As + Fe treatment consists in As adsorbed on Fe oxide at root surface (Fig. 6d). In plants treated with As alone, the increase in -SH compounds (Fig. 3) suggests a complexation of As with PCs in the roots. Indeed, complexation with -SH compounds, mainly PCs, and subsequent vacuolar sequestration is considered to be an important mechanism for As(III) detoxification in plants (Zhao et

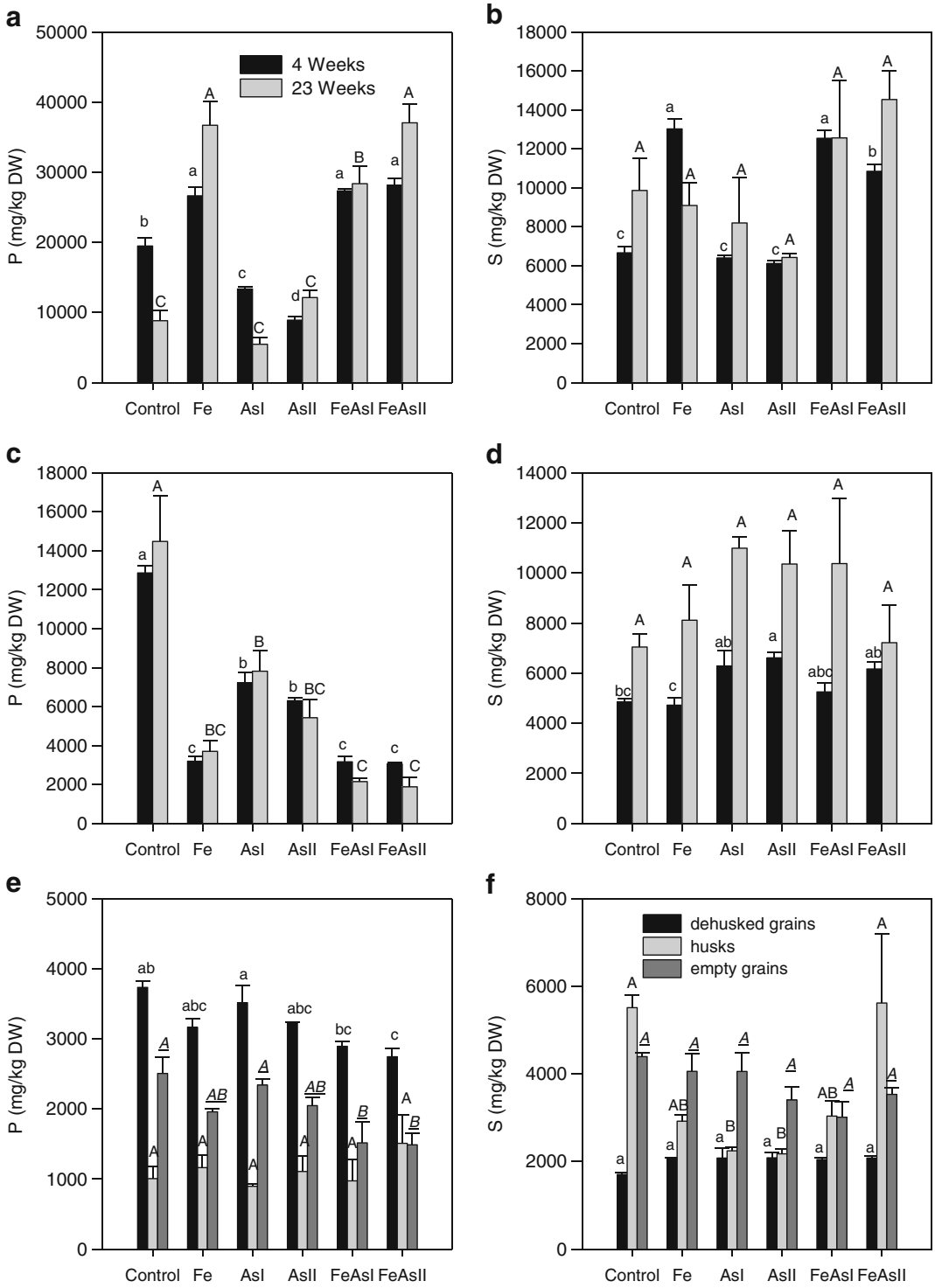


al. 2009, 2010; Moore et al. 2011; Wu et al. 2011). Mir et al. (2007) and Lombi et al. (2009) reported that As-PC complexes are not directly observable through routine HPLC-based speciation analysis because of dissociation

of the complex during extraction and analysis. The concomitant increase of extractable As(III) in the roots of plants treated only with As compared to plants treated with Fe and As (Fig. 6d) lends further support to the hypothesis of As-SH complexation. We observed more extractable As(III) than As(V) in the roots of As-treated plants as did Wu et al. (2011) while Seyfferth et al. (2011) observed more As(V) than As(III). Nevertheless some non-extractable As remained inside the roots after removal of the Fe plaque by DCB treatment and this concentration was similar to the one observed in roots of As stressed plants (Fig. 6d). According to Mir et al. (2007), while methanol–water extractant maintains the integrity of As species in the samples, it has a limited extraction efficiency and this efficiency varies widely depending on plant matrix. Low extraction efficiency could be due to a number of factors such as the insoluble forms of As, the chemical and/or physical bonding of As to the plant matrix, and the trapping of As compounds inside the plant vascular tissues (Mir et al. 2007). Lombi et al. (2009) compared As speciation in rice grains by HPLC-ICP-MS and XANES (X-ray absorption near-edge spectroscopy) and showed some discrepancies although, both techniques yielded similar results regarding the inorganic As species. It is known that hyperaccumulating plants can store toxic metals in photosynthetically inactive cell structures such as vacuoles and cell walls or specific cell types like epidermis and trichomes (Rascio and Navari-Izzo 2011). In *Pteris cretica* almost the same amount of As was found bound in cell walls as in cytosol (Feng et al. 2011). It could thus be possible that in rice root, a part of the non-extractable As is bound to cell walls but this hypothesis has to be verified. Moreover, according to Pal and Rai (2010), exposure of plants to cadmium results in two types of PCs: low molecular weight (LMW) Cd-PC and more stable high molecular weight (HMW) PC-CdS with excess of sulphide (S^{2-}). Some complexes with high ratio of S^{2-} /Cd appeared to be CdS crystallites surrounded by HMW PC peptides (Dameron et al. 1989; Reese et al. 1992). Since As reacts readily with S^{2-} some analogy could be expected and some part of non extractable As might consist in insoluble HMW PC-As but the presence of such complexes has still to be proven in rice.

The low proportion of extractable As(III) and As(V) may reflect a restriction of As root-to-shoot translocation explaining the reduction of As in the shoot of plants developing an Fe plaque (Fig. 6a). We indeed observed that the As concentration in the root was about 13–47 times higher than in the shoot of As-treated plants. Rahman et al. (2011) reported that As concentration in the shoots of rice seedlings was about 23–49 times lower than those in roots and that the As accumulation in shoot was not affected significantly by the additional iron. We nevertheless observed a lower As content in the shoot of plant showing a Fe plaque (Fig. 6a). Rahman et al. (2011) mainly worked on young seedling, while we considered adult plants maintained under stress conditions until harvest. Our data also demonstrate, for the first time to the best of our knowledge, that Fe plaque has a strong influence on As speciation in the shoots. Less non-extractable As was observed in the shoot compared to the roots (Fig. 6a, b) and the proportion of extractable As(III) increased in the shoot of plant treated with As + Fe (Fig. 6b). The concentration of extractable As(III) and As(V) remained nevertheless higher in plants exposed to As alone compared to plants treated with As + Fe (Fig. 6b). This could be directly related to a higher –SH content in the shoot of plants exposed to As alone comparatively to Fe+As (Fig. 3b) and suggests that in the As treated shoots PCs may constitute a major pathway of As(III) sequestration. We observed both As(III) and As(V) in the shoot but neither DMA nor MMA were detected. Wu et al. (2011) observed only As(III) and As(V) in the xylem sap of hydroponically-grown rice plants while methylated As species (MMA and DMA) were recorded in flooded soils conditions. These observations are in accordance with the hypothesis of Lomax et al. (2012) that methylated As-species originates from soil microbial activity and are not the result of

Fig. 7 Impact of As and Fe toxicities on P (**a**, **c**, **e**) and S (**b**, **d**, **f**) content in rice (*Oryza sativa*) root rinsed with water (**a**, **b**), shoot (**c**, **d**) and grains (**e**, **f**). A distinction was made between empty grains, dehusked full grains and husks. Plants were exposed to either 0 mgL^{-1} Fe(II) and 0 μM As(V) (control), 125 mgL^{-1} Fe(II) (Fe), 50 μM As(V) (AsI), 100 μM As(V) (AsII), 125 mgL^{-1} Fe(II)+50 μM As(V) (FeAsI) or 125 mgL^{-1} Fe(II)+100 μM As(V) (FeAsII). Bars represent SE of means. Values followed by a same letter for a same parameter are not statistically different at $P<0.05$ according to Student Newman Keuls multiple range test. Note that ordinate scales vary according to the considered organs



plant metabolism. Under our fully controlled environmental conditions, the nutrient solution thus probably does not contain the microorganisms able to produce such As-organic species.

Table 2 Impact of As and Fe toxicities on P and S content in rice (*Oryza sativa;* cv. IKP) roots rinsed with water (control rinsing), with KH_2PO_4, with DCB or with NH_4-Oxalate. Plants were exposed during 4 weeks to either 125 mgL^{-1} Fe(II) (Fe), 125 mgL^{-1} Fe(II)+50 μM As(V) (FeAsI) or 125 mgL^{-1} Fe(II)+ 100 μM As(V) (FeAsII)

Element	Treatment	Water (control)	DCB[a]		NH_4-Oxalate	
		Means ± SE[b]	Means ± SE[b]	%[c]	Means ± SE[b]	%[c]
P (g/kg DW)	Fe	27±1[a]	7±1[a]	74	2±0.1[a]	92
	FeAsI	27±0.3[a]	5±1[a]	81	1.7±0.2[ab]	94
	FeAsII	28±1[a]	4.8±0.4[a]	83	1.4±0.1[b]	95
	Treatment	water	KH_2PO_4[a]		NH_4-Oxalate	
		Means ± SE[b]	Means ± SE[b]	%[c]	Means ± SE[b]	%[c]
S (g/kg DW)	Fe	13±0.5[a]	7.4±0.5[a]	43	3.9±0.3[a]	70
	FeAsI	12.5±0.4[a]	6.3±0.4[a]	50	4±0.3[a]	68
	FeAsII	10.8±0.4[a]	6.5±0.3[a]	40	3.9±0.1[a]	64

[a] P content was not quantify after KH_2PO4 rinsing because KH_2PO_4 solution contains P. Similarly, S content was not quantify after DCB rinsing because DCB solution contains S

[b] Values followed by a same letter for a same parameter are not statistically different at $P<0.05$ according to Student Newman Keuls multiple range test

[c] percentage of P or S removed in the root by the different rinsings

As and Fe stresses do not modify As and Fe concentrations in the dehusked grains but affect As speciation

Our data demonstrate that Fe plaque not only influence As accumulation and speciation in the shoots but also in the grains. In our results, the concentration of As in the shoot was about 68–94 times higher than in the dehusked grains of plants treated with As alone while it was about 3–14 times higher in the shoot compared to the dehusked grains in plants exposed to combined stresses. We thus observed a lower translocation from the shoot to the grains (0.014–0.011) compared to the translocation from the root to the shoot (0.076–0.055) in plants treated with As alone. This observation is in contradiction with the results of Bhattacharya et al. (2010) who observed a higher efficiency in translocation of As from shoot to grain (0.099–0.393) compared to the translocation from root to shoot (0.040–0.108). The presence of -SH compound in the shoot in our experiment (Fig. 3b) may partly explain the low translocation of As to the grain for plants exposed to As alone.

Iron treatment reduced the As content in the empty grains and in the husks but not in the dehusked grains (Fig. 6e). We also observed that the accumulation of As in the seeds was not proportional to the As concentration in the solution. The As concentration observed in our grains was similar to the values observed by Xu et al. (2008) in their greenhouse study (1–2.5 mgkg^{-1}) but remains higher than the limited range of 0.3–0.6 mgkg^{-1} observed by Panaullah et al. (2009) in rice grown in paddies containing 10–70 mgkg^{-1} As in soil and levels as high as 2500 μgL^{-1} in water. In our experiment, the combined treatment (125 mgL^{-1} Fe+100 μM As) allowed nevertheless to obtain an As content in the dehusked grains of 0.52 mg Kg^{-1} which was lower than the OMS threshold of 1 mgKg^{-1} but remained higher than the value of 50 μgkg^{-1} considered by Williams et al. (2007) and Zhu et al. (2008) as the maximum value consistent with no major risk for the consumer. More As was accumulated in the empty grains and the husks than in the dehusked grains mainly in the plants treated with As alone (Fig. 6e). Similarly, a higher concentration of Fe was also observed in the empty grains and the husks compared to the dehusked grains in plants treated with Fe in both absence or presence of As (Fig. 4c). Significant variations in total As concentrations in different fractions of raw rice have been reported in literature (Rahman and Hasegawa 2011; Seyfferth et al. 2011). The order of As concentrations in rice fractions is husk > bran > whole rice > polish rice (Rahman and Hasegawa 2011). Elemental mapping show a striking accumulation of As on the periphery of rice grain in spots probably corresponding to the ovular trace (Zhao et al. 2010; Carey et al. 2012). This distribution pattern explains why the rice bran (comprising mainly

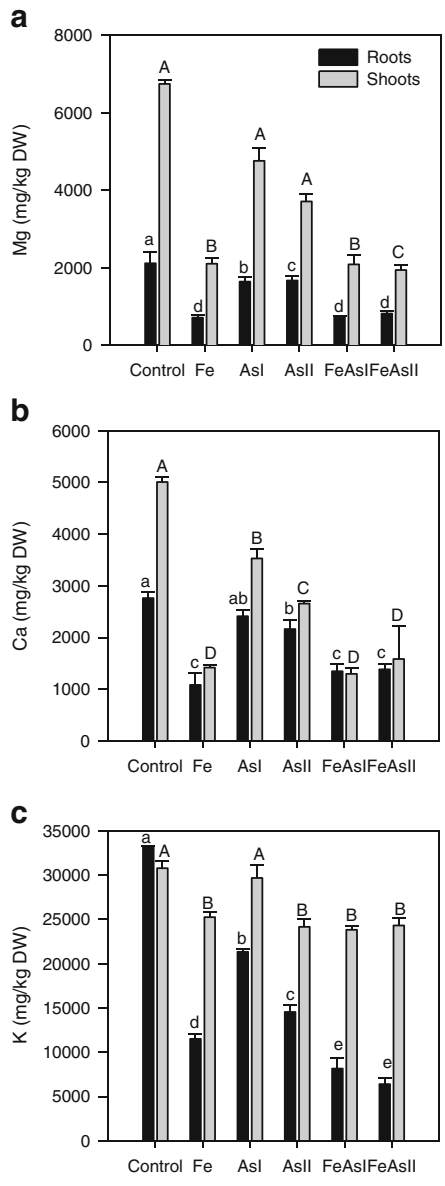


Fig. 8 Impact of As and Fe toxicities on (**a**) Mg, (**b**) Ca, (**c**) K content in rice (*Oryza sativa*) roots and shoots. Plants were exposed during 4 weeks to either 0 mgL^{-1} Fe(II) and 0 μM As(V) (control), 125 mgL^{-1} Fe(II) (Fe), 50 μM As(V) (AsI), 100 μM As(V) (AsII), 125 mgL^{-1} Fe(II)+50 μM As(V) (FeAsI) or 125 mgL^{-1} Fe(II)+100 μM As(V) (FeAsII). Bars represent SE of means. Values followed by a same letter for a same parameter are not statistically different at $P<0.05$ according to Student Newman Keuls multiple range test

pericarp, vascular trace, aleurone and embryo) is more enriched in As than the polished white rice (endosperm). Iron was also distributed in highest concentration in the bran layer, with a small concretion present in the germ and little, if any, present in the endosperm (Seyfferth et al. 2011), explaining why Fe stress do not increase the Fe content in dehusked full grains. The so-called "empty grains" contains the husks, which comprise active transpirating tissues exhibiting a direct connection to the xylem as well as a high stomatal density. In contrast, the grain itself is mainly, if not only, supplied by the phloem sap: a tight regulation of As concentration in the phloem may thus explain a lower accumulation in "full dehusked grains" than in "empty grains". Zhao et al. (2012) showed indeed that As is transported to the grains mainly through the phloem. Our data also suggest that Fe excess is not sufficient to significantly increase Fe content in rice grain.

Total As concentration in rice is not the only determinant of its toxicity. Arsenic toxicity mostly depends on its speciation, and As(III) is more toxic than As(V), trivalent arsenic containing DMA and MMA are more toxic than their pentavalent As-containing analogues (Rahman and Hasegawa 2011). We observed that the Fe treatment increased the proportion of non- extractable As in both husk and dehusked grains (Fig. 6f). The increase of non-extractable As in the husks of plant treated with both As and Fe (Fig. 6f) is correlated with an increase of S content in the husks (Fig. 7f). An hypothesis is that part of the non extractable As could be linked with non extractable high molecular weight PCs as was observed for Cd in tomato (Reese et al. 1992). In our experiment, more than 50 % of the As present in the dehusked grains was non-extractable; the remaining As consisted in extractable As(V) and a small amount of As(III) (Fig. 6f). As(V) was the main As species observed in the husks followed by non-extractable As and As(V) (Fig. 6f). In their experiment, Lombi et al. (2009) observed between 32 and 42 % of non extractable As in rice grains and a large majority of As extracted was inorganic As. No MMA neither DMA were observed in our grains in contrast to other studies (Zhu et al. 2008). According to Seyfferth et al. (2011), As in rice grains was present in bran layers as oxidized As (As(V) and DMA) and in the germ as a mixture of As(V) and As(III), but it was not detected from the endosperm. In contrast, Wu et al. (2011) observed that rice grain contained predominantly DMA and As(III), with As(V) being a minor species. Zhao et al. (2010) reported that MMA was occasionally found at minor levels. As speciation in rice grain thus varies with the genotypes, growing seasons and fractions of rice grain (Rahman and Hasegawa 2011).

Combined As and Fe stresses drastically reduce rice yield

Both As and Fe treatments reduced plant growth and plant yield (Figs. 1 and 2). Arsenic stress alone affects more the vegetative growth than Fe stress alone while the opposite is true for the yield-related parameters. Yield reduction in response to either Fe or As toxicities were previously reported (Becker and Asch 2005; Shaibur et al. 2006; Panaullah et al. 2009; Hossain et al. 2009) but the impact of a combined stress on yield is poorly documented. It is noteworthy that Fe excess had an antagonist impact on shoot As concentration (Fig. 6a) but an additive impact on several yield-related parameters such as panicle DW (Fig. 2a), number of full grains per plant (Fig. 2c) and the mean grain weight (Fig. 2d).

Several processes corresponding to different plant developmental stages may thus be involved in the yield reduction observed in the plants stressed with both As and Fe such as reproductive structures morphogenesis, fertilization and grain filling. The decrease of fertilization may not be linked with a reduction of pollen viability since this parameter was not affected neither by As nor by Fe stresses in our conditions. Ghanem et al. (2009) showed that toxic ions, such as Na^+, may be retained by the cell layer just below the anther tapetum and be excluded from the tapetum tissue which plays an important role in male sporo-and gametogenesis. They also showed that the accumulation of toxic ions in the female parts of the flowers could explain the abortion rate they observed by hampering germination of pollen. It would thus be useful to observe the As and Fe accumulation in the flower parts. The combined stresses also delayed rice flowering compared to single stresses. This delayed flowering may reduce the grain maturation period and thus partly explain the lowest grain weight.

Arsenic and Fe excess compromise plant nutrition

Arsenic and Fe stresses affected several aspects of the plant physiology that ultimately could also contribute to the yield decrease. The grain filling could be affected by a reduction of the photosynthetic activity since the raw materials requested for the grain filling are provided by photosynthesis. We indeed observed a strong decrease of the stomatal conductance in the plants treated with both As and Fe after 4 and 12 weeks of growth comparatively to controls and Fe had a higher impact on plant water status than As under our experimental conditions (Lutts, unpublished results).

Both As and Fe stresses are known to result in nutritional disorders (Carbonell et al. 1998; Thongbai and Goodman 2000; Sahrawat 2004; Chen et al. 2005; de Dorlodot et al. 2005; Shaibur et al. 2006; Hossain et al. 2007). Combined stresses induced a strong reduction of P, Mg, Ca in the shoot and of Mg, Ca, K, in the roots (Figs. 7 and 8). Arsenic and Fe stresses induced thus a multi-element deficiency in addition to As and Fe toxicities. The reduction of P content in rice shoot may be explained by a lower translocation from the roots due to the sequestration of P in the Fe plaque as revealed by the high concentration of P in the roots and the low values after DCB rinsing (Fig. 7, Table 2). Incorporation of P on the Fe plaque was previously reported and its presence may also reduce the capacity of Fe plaque to incorporate As (Garnier et al. 2010; Moore et al. 2011). It is worth to notice that such a decrease in P nutrition did not affect the grain under our experimental conditions. The reduction of Mg, Ca, K in the plant on the other hand may not be a consequence of Fe plaque retention. The perturbation of the root absorption mechanisms could explain the nutritional disorder and the physical barrier due to the Fe plaque may slow the passage of other elements independently of immobilisation. These deficiencies may in particular be explained by a competition with Fe and As for the root absorption sites. It is well known that As(V) is taken up by phosphate transporters and As(III) influx in rice roots could be mediated through the silicic acid transporters (Tripathi et al. 2007; Ma et al. 2008; Wu et al. 2011). Numerous studies also reported that a large number of transporters related to iron acquisition, transport and sequestration show a broad specificity for numerous cations, iron and zinc being more especially mediated by the same group of transporters (Krämer et al. 2007; Jeong and Connolly 2009). On the same way, Mn^{2+} and Fe^{2+} could be charged by the same transporters (OsIRT1 and OsIRT2) (Kerkeb and Connolly 2006). Our recent micro-array analysis showed that Fe stress affects the expression of genes coding for transporters and receptors involved not only in iron metabolism but also in Ca, K, Cu, Mn, Zn, P and S metabolism (Quinet et al. 2012). Phosphate, sulfate and metal transporters were also affected by As stress as revealed by the transcriptomic analysis of Chakrabarty et al. (2009).

Conclusion

This study investigated the impact of As and Fe toxicities on plant development and yield-related parameters of rice plants grown in hydroponic solution. The presence of Fe allowed the development of a Fe plaque on the rice roots that reduced As uptake by the roots and the subsequent translocation to the shoots but did not reduce the As content in the dehusked grain. Fe excess, however, modifies As speciation in both shoot and grains. Combined stresses (Fe+As) significantly reduced the plant yield by limiting the number of grains per plant and the grain filling. Moreover, As and Fe toxicities induced a multi-element deficiency. Fe excess has thus an antagonist impact on shoot As concentration but an additive negative impact on several yield-related and nutritional parameters.

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