Influence of iron on mineral status of two rice (*Oryza sativa* L.) cultivars

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Iron is an essential nutrient for plants. In aerobic conditions, Fe is highly unavailable for plant uptake, and Fe deficiency can be severe in plants grown in calcareous soils. In waterlogged soils, however, Fe availability increases and can reach toxic concentrations. Rice is an important staple crop worldwide and faces iron deficiency or excess, depending on the growth conditions. To contribute to the study of mechanisms involved in response to Fe deficiency and resistance to Fe excess, experiments were carried out with rice cultivars BR-IRGA 409 (I409, susceptible to Fe toxicity) and EPAGRI 108 (E108, resistant to Fe toxicity) grown in culture solutions and submitted to Fe excess, control concentration or deficiency (500, 6.5 or zero mg L^{-1} Fe, respectively). Analysis of shoot dry weight confirmed the resistance of E108 plants to Fe excess. Mössbauer spectroscopy analysis indicated the presence of four different Fe^{3+}compounds. The parameters obtained match those expected for ferrihydrite, lepidocrocite (and/or citrate) and Fe-nicotianamine. Mineral concentrations were determined using the PIXE (Particle Induced X-Ray Emission) technique. E108 plants had lower Fe concentrations than I409 plants when exposed to excess Fe. Except for lower Mn levels in roots and shoots, the excess of Fe did not result in lower nutrient concentrations in the susceptible cultivar compared to the resistant one. I409 plants seem to be affected directly by Fe toxicity rather than by secondary effects on mineral nutrition, whereas E108 plants seem to make use of the avoidance mechanism in the resistance to Fe overload. Both cultivars responded to Fe deficiency with allocation of P from roots to shoots. In addition to being more resistant to iron overload, E108 plants seem to be more efficient in inducing Fe deficiency responses.

Key words: ferritin, iron deficiency, iron toxicity; Mössbauer spectroscopy, *Oryza sativa*, PIXE

Influência do ferro no status mineral de duas cultivares de arroz (*Oryza sativa* L.)**:** O ferro é um nutriente essencial para as plantas. Em condições aeróbicas, é altamente indisponível para absorção pelas plantas, e sua deficiência pode ser severa em plantas cultivadas em solos calcáreos. Em solos alagados, no entanto, a disponibilidade de Fe aumenta e pode atingir concentrações tóxicas. O arroz é uma cultura básica mundialmente importante e enfrenta deficiência ou excesso de Fe, dependendo das condições de cultivo. Para contribuir com o estudo dos mecanismos envolvidos nas respostas a deficiência de Fe e resistência ao excesso de Fe, foram realizados experimentos com as cultivares de arroz BR-IRGA 409 (I409 – sensível à toxidez por Fe) e EPAGRI 108 (E108 – resistente à toxidez por Fe) submetidas a excesso de ferro, concentração-controle ou deficiência (500, 6.5 ou zero mg L^{-1} Fe, respectivamente). A avaliação do peso seco das partes aéreas confirmou a resistência da cultivar E108 ao excesso de Fe. A espectroscopia Mössbauer indicou a presença de quatro diferentes compostos de Fe^{3+}. Os parâmetros obtidos coincidem com os esperados para ferrihidrita, lepidocrocito

Abbreviations: DW – dry weight; E108 – EPAGRI 108 rice cultivar; I409 – BR-IRGA 409 rice cultivar; MA – mugineic acid; NA – nicotianamine; PIXE – Particle Induced X-Ray Emission; PS – phytosiderophore

(e/ou citrato) e Fe- nicotianamina. As concentrações de minerais foram determinadas pela técnica PIXE (Particle Induced X-Ray Emission). A concentração de Fe em plantas da cultivar E108 foi menor do que em plantas da cultivar I409, quando expostas a excesso de Fe. Exceto por concentrações de Mn menores em raízes e partes aéreas, o excesso de Fe não resultou em concentrações de nutrientes mais baixas na cultivar sensível do que na cultivar resistente. Plantas da cultivar I409 parecem ser afetadas diretamente pela toxidez de Fe, e não por efeitos secundários na nutrição mineral, enquanto plantas da cultivar E108 parecem valer-se de mecanismos de exclusão na resistência ao excesso de Fe. Ambas cultivares responderam à deficiência de Fe com alocação de P, das raízes para as partes aéreas. Além de ser mais resistente ao excesso de Fe, a cultivar E108 parece ser mais eficiente na indução de respostas à deficiência de Fe.
Palavras-chave: deficiência de ferro, espectroscopia Mössbauer, ferritina, *Oryza sativa*, PIXE, toxidez por ferro

INTRODUCTION

Iron (Fe) is essential for plant growth and development (Curie and Briat, 2003). In aerobic conditions, soil Fe is usually found as oxihydroxide polymers, which have very low solubility, limiting the Fe supply for plant uptake, especially in calcareous soils. Iron deficiency is therefore a yield-limiting factor with major implications for field crop production in many agricultural regions of the world (Hansen et al., 2006). Rice (*Oryza sativa*) plants are especially susceptible to low Fe supply, differently from other cultivated grass species, such as oats (Takahashi et al., 2001). In anaerobic and acidic soils, however, high concentrations of ferrous (Fe^{2+}) ions may lead to Fe toxicity due to excessive Fe uptake (Bienfait et al., 1985), which can result in yield reductions from 12 to 100% (Sahrawat, 2004). Excess Fe can be extremely toxic, as it reacts with oxygen and catalyses the production of free radical species. Iron toxicity can occur in rice cultivated under waterlogging.

In Brazil, a major portion of the rice is grown under lowland conditions (Fageria et al., 2003), and half of the rice production is done in Rio Grande do Sul State, where all rice is grown in waterlogged soils. Use of tolerant rice cultivars is an alternative for rice production on Fe toxic soils (Fageria et al., 2003); however, most elite rice cultivars grown in this geographical region are sensitive to Fe toxicity.

Under anaerobic conditions, oxygen release from rice roots allows the formation of Fe and Mn depositions, the so-called Fe plaque, on the surface of rice roots. The Fe plaque may prevent excessive uptake of Fe^{2+}, Mn^{2+} and other toxic compounds formed in waterlogged soils, and could also have a role as a phosphorus reservoir (Zhang et al., 1999). Other authors, however, suggest that excess Fe may result in lower uptake of other essential nutrients, either due to the barrier created by the Fe coatings (Howeler, 1973) or due to chemical interactions in the soil (Neue et al., 1998). Sahrawat (2004) pointed out the existence of *"pseudo"* Fe toxicity (when Fe toxicity symptoms were caused by deficiency of other nutrients) and *"true"* Fe toxicity (caused by toxic concentrations of Fe, without any apparent deficiency of other plant nutrients).

Resistance to Fe overload in rice plants can be a consequence of Fe avoidance and/or tolerance to high internal Fe concentrations. Diverse genotypes vary widely in their ability to stand up to Fe overload, and it has been suggested that the ability to maintain high levels of essential nutrients under Fe toxic conditions contributes to the tolerance phenotypes (Luo et al., 1997).

We compared two rice cultivars, BR-IRGA 409 (I409) and EPAGRI 108 (E108), respectively susceptible and resistant to Fe toxicity, to help the investigation of mechanisms involved in resistance to Fe toxicity and responses to Fe deficiency in rice. Mössbauer spectroscopy was used to help to characterize the chemical forms in which Fe was present in plant tissues. To evaluate the influence of Fe nutrition on the uptake of other nutrients, elemental concentrations in rice roots and shoots were assessed using the Particle Induced X-Ray Emission (PIXE) method.

MATERIAL AND METHODS

Plant growth: Rice (*Oryza sativa* L. ssp. *indica*) seeds from cultivars E108 and I409 were provided by IRGA (Instituto Rio Grandense do Arroz, Brazil). After germination and growth in vermiculite and nutrient solution (Yoshida et al., 1976) for 14 d in an incubator

(28°C, first two days in the dark and remaining days with 16 h of light), plants were transferred to glass pots covered with aluminum foil and containing 500 mL of nutrient solution. Each pot harbored four plants, held by a styrofoam lid, and plants were kept for 10 d in control solution, modified from Yoshida et al. (1976) to contain 6.5 mg L^{-1} Fe as $FeCl_2.6H_2O$ combined to citric acid in a 2:1 (citric acid:Fe) ratio, pH 5.0 ± 0.1. Plants were then transferred to different treatments, with the following modifications from the Yoshida solution: excess Fe (500 mg L^{-1} Fe as $FeSO_4.7H_2O$, solution pH 4.2 ± 0.1), control (6.5 mg L^{-1} Fe as $FeSO_4.7H_2O$ plus 500 mg L^{-1} S as Na_2SO_4, solution pH 5.1 ± 0.1), and Fe deficiency (no Fe added, with addition of 0.3 mM Ferrozine – Sigma). All solutions were replaced every 3 d. Plants were cultivated in a growth room at 26 ± 1°C under a photoperiod of 16/8 h light/darkness. Preliminary experiments performed in our laboratory, in which rice plants were exposed to the control solution with pH adjusted to 4.2 or 5.1 for 10 d, resulted in no growth difference, neither in roots nor in shoots.

Dry weight: Plants submitted to the different treatments were separated into roots and shoots. Dry weights were determined after the samples (n = 12 for each treatment) were oven-dried at 65°C until constant weight.

Mössbauer spectroscopy: Samples (roots or shoots from four plants) were rinsed in distilled water, dried at 65°C, ground and pressed into pellets. Independent measurements were obtained from three pellets for each treatment.

The dried plants were sealed in a sample holder and the Mössbauer spectra were obtained using a conventional acceleration spectrometer. A 50-mCi source of $^{57}Co(Rh)$, kept at room temperature, was used. For low temperature measurements, samples of fresh tissue were ground in liquid nitrogen and the sample holder was placed in a liquid nitrogen cryostate (sample temperature 85 K). The velocity scale was calibrated with an Fe metal foil at room temperature. The hyperfine parameters were obtained by a least-square procedure assuming Lorentzian line shapes constrained to equal half widths (Bankcroft, 1973).

Analysis of PIXE: The concentrations of Fe, P, Ca, Zn, Mn, K, S and Cu were evaluated in roots and shoots by the PIXE technique. Samples (roots or shoots from four plants) were dried, ground and pressed into pellets. Measurements were obtained from three pellets for each treatment, obtained from three independent experiments.

Analysis of PIXE was carried out at the 3 MV Tandetron accelerator facility at the Physics Institute at the Universidade Federal do Rio Grande do Sul, Brazil. All measurements were performed using a 2 MeV proton beam with an average current of 5 nA. The acquisition time for each sample was of the order of 20 min. The characteristic X-rays induced by the proton beam were detected by a standard PIXE experimental set-up using a high-purity Germanium detector with an energy resolution of 180 eV at 5.9 KeV (Ge detector manufactured by EG&G Nuclear Instruments, GLP series, Oak Ridge, USA).

The detector was positioned at 45º with respect to the beam axis. The GUPIX code was used for data analysis (Campbell et al., 2000). Due to the thick beryllium window of the HPGe detector employed in the measurements, it was not possible to evaluate Mg levels.

The quantitative analysis was done based on a river sediment standard (National Institute of Standards and Technology – NIST 8704 – England). The final uncertainties quoted for the elemental concentrations were evaluated taking into account the discrepancy of the independent measurements and the uncertainties arising from the least-square fitting procedure given by the GUPIX code.

Statistical analyses: All data were subjected to t test or ANOVA followed by Duncan test for mean comparison ($P \leq 0.05$). All calculations were made using the SPSS Base 10.0 for Windows (SPSS Inc., USA).

RESULTS AND DISCUSSION

Dry weight: Shoots from E108, which is considered a slow growing cultivar (Cordeiro, 2005), reached lower final dry weights (DW) than shoots from I409, both under control and Fe excess conditions (Figure 1). A decrease in shoot DW as a result of the Fe excess treatment was seen only in the I409 cultivar. In E108 plants, there was a decrease in shoot DW only after 6 d of treatment, but the plants seemed to recover at the next sampling time, confirming the resistance of this cultivar to Fe overload (Nava and

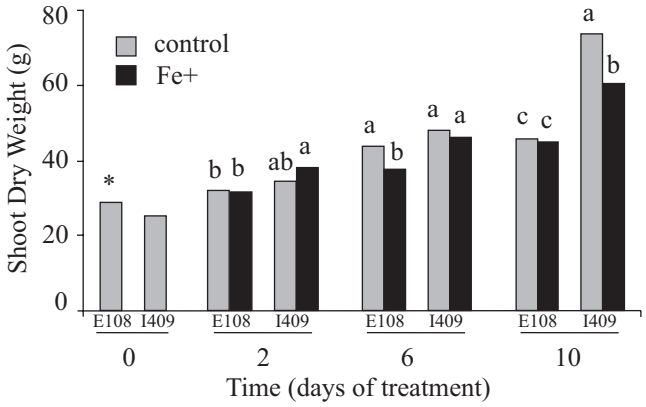

Figure 1. Dry weight from shoots of rice cultivars EPAGRI 108 (E108) and BR-IRGA 409 (I409) submitted to 6.5 mg L^{-1} of iron (control, gray bars) or 500 mg L^{-1} of iron (Fe+, black bars) for 2, 6 and 10 d. Means ($n = 12$) with an asterisk or different letters are significantly different using *t* test or Duncan test ($P \leq 0.05$).

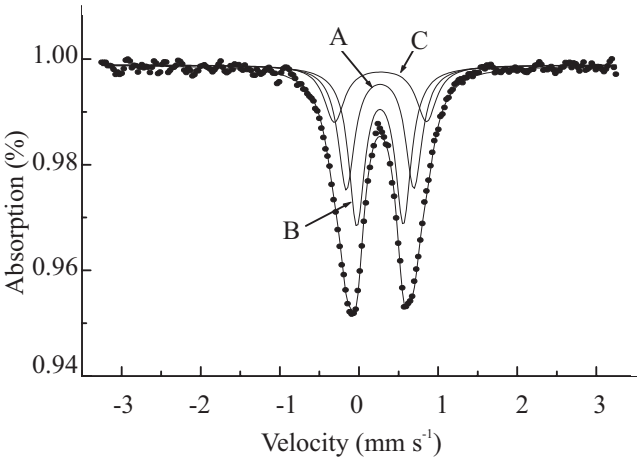

Figure 2. Mössbauer spectrum obtained from roots of rice cultivar I409 submitted to 500 mg L^{-1} of iron (Fe+). The data from this sample was fitted into three sub-spectra, represented by the curves A, B and C.

Bohnen, 2002). Our group has also seen a decrease in photosynthetic activity in plants from the same cultivars exposed to Fe excess, with E108 plants being able to fully recover their photosynthetic capacity after 6 d, while I409 plants were not able to recover (Ricardo J. Stein, personal communication).

Mössbauer spectroscopy: All spectra generated from the analyzed samples could be fitted to Fe^{3+} components. No parameters compatible with Fe^{2+} components were obtained. A typical spectrum, obtained from one of the samples, is shown in Figure 2. The data obtained at room temperature (298 K) was grouped in four components, named A to D (Table 1). Spectra were also obtained at low temperature (85 K) (Table 1, bold lines). It was possible to detect the same compounds in samples from the same cultivars/ treatments when analyzed at room temperature and at low temperature, even with small changes in the parameters in low temperature, such as increased isomer shift values, and variations in the proportions between the compounds. Variations in the isomer shift at lower temperatures would be expected due to the second-order Doppler shift (Guttlich et al., 1978). The proportions between the different compounds, estimated by their respective areas, were also expected to change in low temperature, since the recoil free fraction (*f* factor) from diverse compounds is affected differentially by the temperature decrease. The areas are proportional to the *f* factor, which increases significantly with the temperature reduction in some cases (Bauminger et al., 1987; Mohie-Eldin et al., 1995). The particle size also influences the *f* factor variations under low temperature, with smaller particles suffering larger variations (van der Kraan, 1973).

It is possible that compound A (Table 1) corresponds to ferrihydrite ($5Fe_2O_3.9\ H_2O$). Mössbauer parameters for ferrihydrite have been reported in studies with plants, such as pea roots and seeds, *Canavalia ensiformis*, rice and *Imperata cylindrica* (Goodman and DeKock, 1982; Yariv et al., 1988; Wade et al., 1993; Kilcoyne et al., 2000; Rodriguez et al., 2005). Ferrihydrite is the inorganic compound usually associated with Fe accumulation in ferritin (Chasteen and Harrison, 1999). Ferritins are large proteins with a central cavity that can store up to 4500 Fe atoms, which can be released when necessary (Yamashita, 2001). Therefore, these proteins are believed to play a critical role in the cellular regulation of Fe storage and homeostasis (Zancani et al., 2004). Since expression of some plant ferritin isoforms can be induced by Fe overload (Fobis-Loisy et al., 1995; Petit et al., 2001), we believe that iron storage inside ferritin could be related to Fe overload tolerance in some rice cultivars.

In roots, ferrihydrite is also possibly found in the Fe plaque. The Fe plaque has been shown to be formed by 63% ferrihydrite ($Fe_2O_3.nH_2O$), 32% goethite (α-FeOOH) and 5% siderite ($FeCO_3$) in the wetland plant *Phalaris*

Table 1. Parameters obtained from room temperature and low temperature (lines with bold characters) Mössbauer spectra for roots (R) and shoots (S) of rice plants from cultivars BR-IRGA 409 (I409) and EPAGRI 108 (E108) submitted to excess (Fe+), deficiency (Fe–) and control levels of iron (C). Isomer shift (δ, relative to Fe metal at room temperature) and quadrupole splitting (Δ_{EQ}) values are given in $mm.s^{-1}$. The numbers in brackets represent uncertainties in the final digit based on the standard deviations from the least-square fits to individual spectra. Areas are given as percentages of total area of each spectrum.

	Fe^{3+} component A			Fe^{3+} component B			Fe^{3+} component C			Fe^{3+} component D		
	δ	Δ_{EQ}	Area	δ	Δ_{EQ}	Area	δ	Δ_{EQ}	Area	δ	Δ_{EQ}	Area
I409 R Fe+	0.37(1)	0.85(2)	0.36	0.37(1)	0.59(2)	0.44	0.37(1)	1.16(2)	0.20	-	-	-
I409 R Fe+	**0.49(1)**	**0.95(2)**	**0.17**	**0.50(1)**	**0.55(1)**	**0.74**	**0.47(1)**	**1.32(4)**	**0.09**	**-**	**-**	**-**
I409 R C	-	-	-	0.40(1)	0.49(1)	0.58	0.37(1)	0.97(1)	0.42	-	-	-
I409 R Fe–	0.42(1)	0.75(2)	0.33	0.30(1)	0.65(2)	0.53	0.34(1)	1.24(3)	0.14	-	-	-
I409 S Fe+	0.38(1)	0.73(5)	0.39	-	-	-	0.39(1)	1.14(5)	0.18	0.40(1)	0.40(5)	0.43
I409 S Fe+	**0.50(1)**	**0.74(3)**	**0.33**	**-**	**-**	**-**	**0.51(1)**	**1.12(4)**	**0.33**	**0.51(1)**	**0.40(4)**	**0.34**
I409 S C	-	-	-	-	-	-	-	-	-	-	-	-
I409 S Fe–	-	-	-	-	-	-	-	-	-	-	-	-
E108 R Fe+	0.44(1)	0.66(1)	0.36	0.32(1)	0.65(1)	0.46	0.38(1)	1.09(2)	0.19	-	-	-
E108 R Fe+	**0.51(1)**	**0.81(3)**	**0.14**	**0.51(1)**	**0.55(2)**	**0.72**	**0.51(1)**	**1.17(2)**	**0.14**	**-**	**-**	**-**
E108 R C	-	-	-	0.40(1)	0.55(3)	0.62	0.39(1)	0.96(3)	0.38	-	-	-
E108 R Fe–	-	-	-	0.32(2)	0.61(5)	0.69	0.38(2)	1.13(5)	0.31	-	-	-
E108 S Fe+	0.38(1)	0.63(2)	0.45	-	-	-	0.41(1)	1.05(2)	0.30	0.40(1)	0.34(4)	0.25
E108 S Fe+	**0.50(1)**	**0.78(2)**	**0.45**	**-**	**-**	**-**	**0.49(1)**	**1.18(5)**	**0.16**	**0.51(1)**	**0.45(3)**	**0.39**
E108 S C	0.44(8)	0.65(8)	0.13	-	-	-	0.34(4)	0.96(4)	0.25	0.34(8)	0.31(4)	0.62
E108 S Fe–	-	-	-	-	-	-	-	-	-	-	-	-

arundinacea (Hansel and Fendorf, 2001). In rice, Bacha and Hossner (1977) had previously shown that the crystalline phase of Fe compounds in the root coatings was lepidocrocite. In longer experiments, Chen et al. (1980) identified both goethite (α-FeOOH) and lepidocrocite (γ-FeOOH) in rice root coatings. The Mössbauer spectra of goethite and lepidocrocite are not significantly different (Murad and Schwertmann, 1984).

Compound B has hyperfine parameters compatible with lepidocrocite. This Fe compound could actually be located in the Fe plaque, since we detected its presence only in root samples and not in shoots. At low temperature, the parameters obtained for the same compound are similar to those reported for ferric Fe citrate (Ambe, 1994). Citrate is an important ligand for Fe in plants. Ferric Fe citrate is considered the major Fe form in the xylem, and Fe deficiency has been shown to induce increases of citrate concentrations in the phloem (Curie and Briat, 2003; Hell and Stephan, 2003). Therefore, compound B could correspond to a combination of Fe^{3+} complexed to lepidocrocite and/or citrate, but we are unable to distinguish the proportions between the two compounds.

We were unable to determine the identities of compounds C and D based on comparisons between the hyperfine parameters obtained in our experiments and Mössbauer parameters for Fe^{3+} compounds available in the literature. Compound C has similar parameters to those obtained by Kilcoyne et al. (2000) for one compound found in roots and leaves of rice plants. These authors reported the presence of two major compounds in rice plants and considered the parameters consistent with Fe being in the form of oxyhydroxide or hydrated oxide species, suggesting similarity to ferrihydrite and lepidocrocite. However, we considered the parameters not similar enough with those reported in the literature for these two compounds. Compound C seems to be a major Fe component in rice plants, being found in all samples from which we were able to obtain spectrum. The values of quadrupole splitting are similar to those reported for diferric-peroxo intermediates formed in the interior of the ferritin shell (Chasteen and Harrison, 1999). However, the

isomer shift values reported by those authors are higher than the ones found in our work and by Kilcoyne et al. (2000). It remains to be elucidated the identity of such a prevalent Fe form from rice plants.

Compound D was present only in shoot samples. We may expect to find, in these samples, Fe complexed to NA, the most important chelator of free Fe in plant cells (Hell and Stephan, 2003). Although Fe^{2+}-NA complexes are more stable, it has been shown that NA can form complexes with Fe^{3+} as well (von Wirén et al., 1999). We did not find in the literature Mössbauer parameters for Fe^{3+}-NA complexes, but only for Fe^{3+}-mugineic acid (Mino et al., 1983), a phytosiderophore derived from NA. Both NA and MA (mugineic acid) have six ligands for Fe complexation and both chelate Fe in a similar way. Their structural differences (hydroxyl/amino substitutions on carbon 3 and the presence of a hydroxyl group on carbon 2 of MA) could account for variations in the Mössbauer parameters.

Iron compounds were not detected by Mössbauer spectroscopy in shoots of both cultivars under Fe deficiency, probably due to the decreased concentration of this metal in leaves. The same occurred in shoot samples from the I409 cultivar under control levels of Fe. However, it was possible to obtain a spectrum from shoots of E108 plants under control levels of Fe, although the spectrum obtained was not very well defined, possibly due to the low amount of Fe in these samples as compared with those from plants under Fe excess. As a consequence, higher error estimates are seen (between brackets, Table 1) for this treatment. Another difference between the two cultivars was seen in roots of plants submitted to Fe deficiency: I409 roots have compounds A, B and C, while compound A seems to be absent from E108 roots, with a consequent increase in the proportions of compounds B and C. Such differences in Fe speciation may impact in the way plants from both cultivars cope with Fe stress, either deficiency or overload.

Analysis of PIXE

Iron deficiency: Under Fe deficiency, there was a decrease in Fe content in both roots and shoots after 10 d of treatment (Figures 3A,B). Iron levels were lower in shoots than in roots (Figure 3B, note different scale from Figure 3A).

Phosphorus concentrations decreased in roots of both cultivars and increased in shoots of rice plants under Fe deficiency (Figure 4A). Phosphorus was preferentially translocated to shoots, but the total amounts present in the plants were almost the same, irrespective of the treatment (data not shown).

Increased Ca concentrations were seen under Fe deficiency in roots of both cultivars, mostly in E108 (Figure 4B).

Fe deficiency resulted in lower Zn concentrations in roots of I409 plants than in roots from control plants and in an over two-fold increase in Zn concentrations in shoots of E108 plants as compared with shoots from control plants (Figure 4C). Enhancement of Zn uptake under Fe deficiency, either as a divalent cation or complexed to siderophores (Zhang et al., 1998), could explain its higher concentrations found in shoots of E108 plants. Several iron transporters are able to transport also Zn and other metals (Korshunova et al., 1999; Eckhardt et al., 2001; Gross et al., 2003; López-Millán et al., 2004). Induction of iron transporters under Fe deficiency could result in increased Zn uptake.

Manganese concentrations decreased under Fe deficiency in roots and shoots of plants from both cultivars (Figure 4D). Although Mn can be transported by plant Fe transporters (Korshunova et al., 1999; Eckardt et al., 2001; López-Millán et al., 2004), we did not detect increased Mn concentrations in Fe-starved plants. Our nutrient solutions had pH adjusted to 5.0, which may have been too low to allow Mn transport by rice Fe transporters (Korshunova et al., 1999).

Higher K concentrations were seen in I409 plants under iron deficiency than in control plants, particularly in roots, what was not observed in E108 plants (Figure 4E).

Iron deficiency resulted in lower S concentrations in roots and shoots of both cultivars (Figure 4F). This is probably a consequence from the Fe requirement for S assimilation (Hell and Stephan, 2003).

Copper concentrations decreased in roots of both cultivars and in shoots of I409 plants under Fe deficiency (Figure 4G). In Poaceae, Fe deficiency induces the synthesis and exudation of phytosiderophores (PS), which bind Fe but are also able to bind Cu. The complex Cu-PS can be transported into root cells by the Fe-PS transporter, although with lower affinity (Briat et al.,

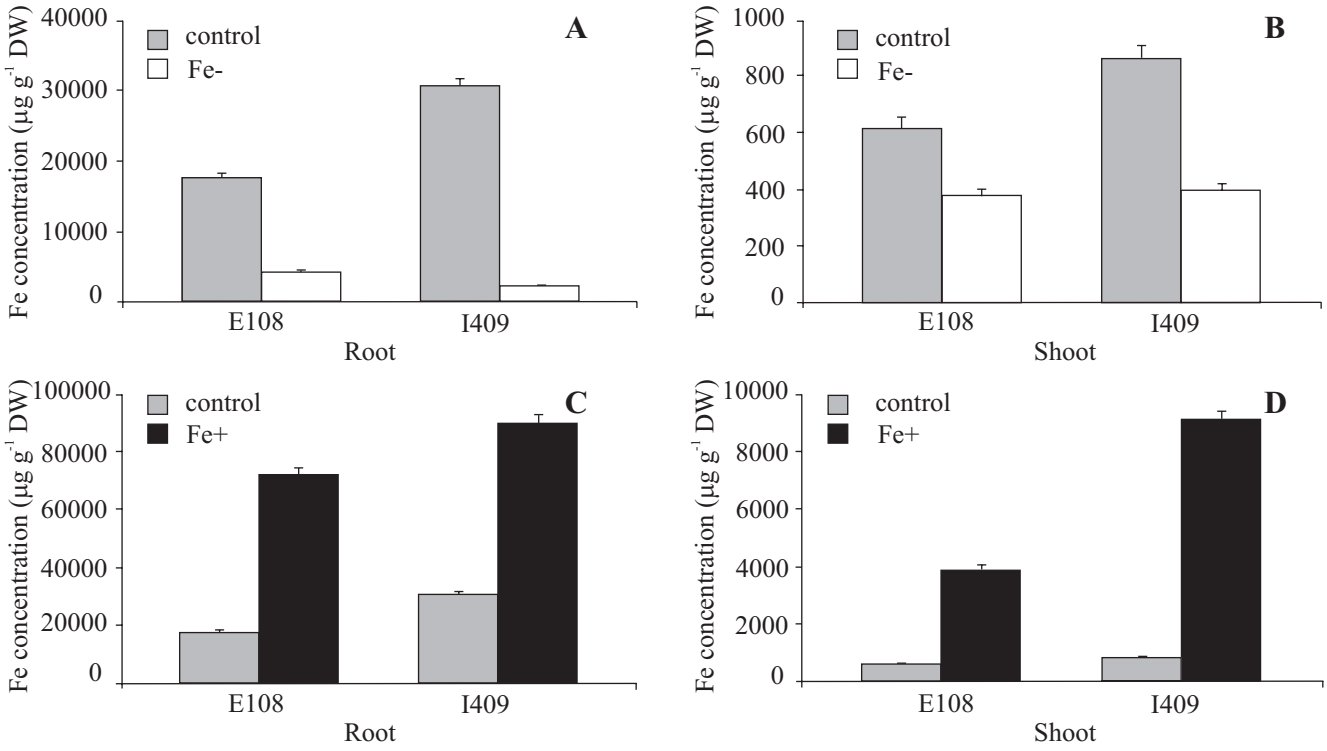

Figure 3. Iron concentration in roots and shoots of rice cultivars EPAGRI 108 (E108) and BR-IRGA 409 (I409) submitted to iron deficiency (Fe–, white bars) (**A,B**) 6.5 mg L^{-1} of iron (control, gray bars) or 500 mg L^{-1} of iron (Fe+, black bars) (**C,D**) for 10 d. Error bars stand for the overall uncertainties of the final elemental concentrations, taking into account the variations between independent measurements and the fit uncertainties given by the GUPIX code (n = 3).

1995). In the present work, however, such tendency was not detected. Since ferrozine can form a water soluble complex with Cu^{+} (Kundra et al., 1974), it is possible that lower amounts of Cu were also available for absorption by the plants in the Fe-starvation treatment, where ferrozine was added to the nutrient solution, than in the control treatment.

The two cultivars tested responded similarly (trends to increase or decrease) to Fe deficiency in relation to most evaluated nutrients, except for Zn concentrations, which responded in opposite ways (and shoot K concentration, with lower impact – Figures 5A,B). It is interesting to note that most of the highest increases in nutrient concentrations were seen in E108 plants, whereas the highest decreases occurred in I409 plants (except for the equivalent reduction in P levels in both cultivars). These results may be indicative of a superior fitness of E108 plants when dealing with Fe deficiency. Higher Zn concentrations in shoots of Fe-deficient E108 plants could be an indication that this cultivar may be more effective in inducing Fe starvation responses. This could be related to their lower Fe contents under control conditions, in relation to I409 plants. Under Fe deficiency, E108 plants would deplete their Fe reserves and trigger special Fe uptake mechanisms earlier than I409 plants. Interestingly, compound A was not detected by Mössbauer spectroscopy in E108 roots under Fe deficiency, while it was present in I409. This compound has similar parameters to ferrihydrite, which could be present either in the Fe plaque or in the interior of ferritin, two possible forms of iron storage.

Iron overload: As expected, plants cultivated in 500 mg L^{-1} of Fe had higher Fe concentrations than those grown under control Fe levels, both in roots and shoots (Figure 3C,D). Higher Fe concentrations were found in roots than in shoots (note different scales).

Under Fe excess, higher concentrations of Fe were detected in I409 plants, more susceptible to Fe toxicity, both in roots and shoots, with shoot concentrations up

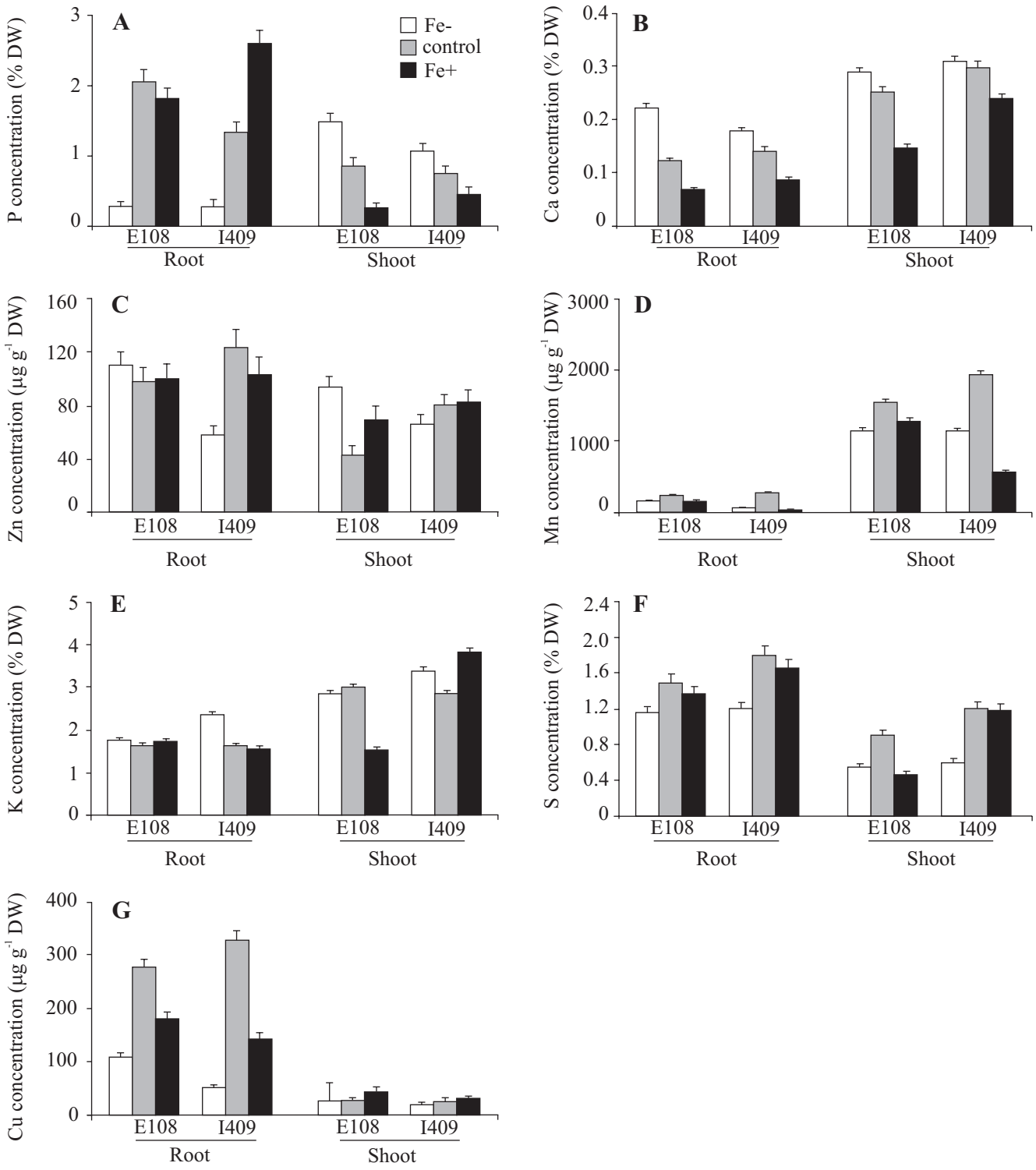

Figure 4. Nutrient concentrations [P (**A**), Ca (**B**), Zn (**C**), Mn (**D**), K (**E**), S (**F**), and Cu (**G**)] in roots and shoots of rice cultivars EPAGRI 108 (E108) and BR-IRGA 409 (I409) submitted to iron deficiency (Fe–, white bars), 6.5 mg L^{-1} of iron (control, gray bars) or 500 mg L^{-1} of iron (Fe+, black bars) for 10 d. Error bars stand for the overall uncertainties of the final elemental concentrations, taking into account the variations between independent measurements and the fit uncertainties given by the GUPIX code ($n = 3$).

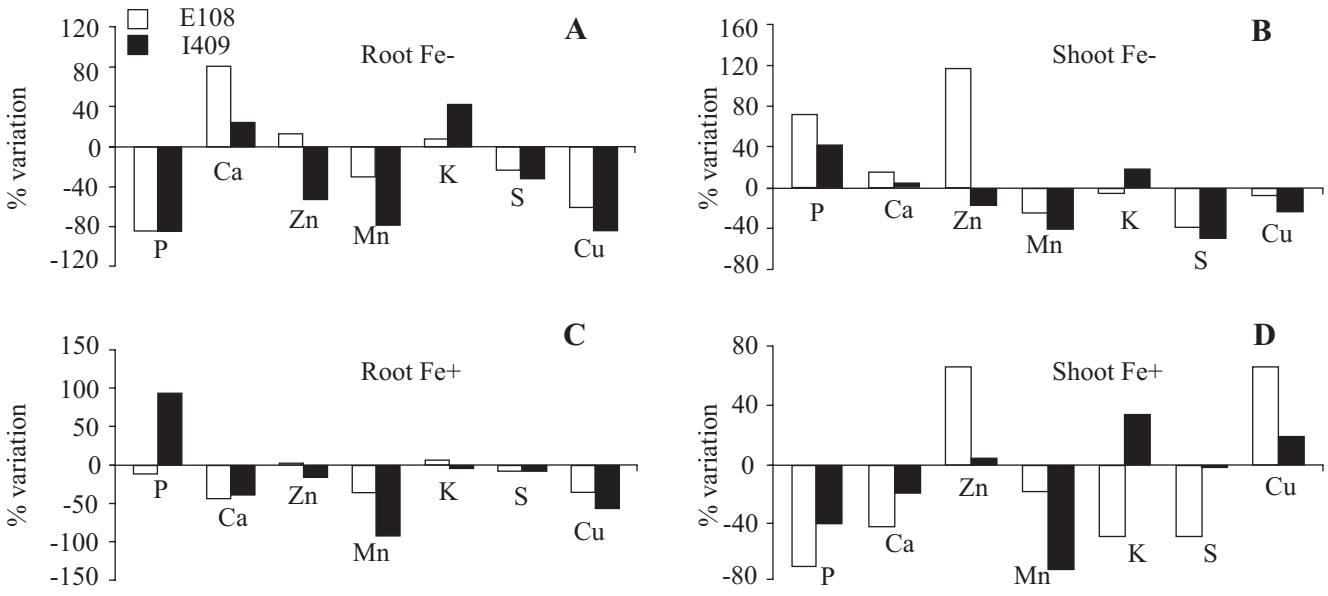

Figure 5. Nutrient concentrations variation in roots and shoots of rice cultivars EPAGRI 108 (E108, white bars) and BR-IRGA 409 (I409, black bars) submitted to iron deficiency (Fe–) (**A,B**) or 500 mg L^{-1} of iron (Fe+) (**C,D**) in relation to the control treatment (6.5 mg L^{-1} of iron).

to 2.5 times higher than in E108 plants after 10 d of treatment. Silva et al. (2003) have also seen higher concentrations of Fe in roots of I409 plants. We suggest, therefore, that E108 plants are more resistant to excess Fe due to the possible induction of avoidance mechanisms, allowing the plant to keep lower Fe amounts in its tissues and decreasing Fe translocation to shoots.

Phosphorus concentration increased considerably in roots of I409 plants submitted to excess Fe but not in roots of E108 plants (Figure 4A). Nava and Bohnen (2002) have also reported higher amounts of P in roots grown in solution with higher Fe concentration only in I409 plants and not in E108 plants. Higher amounts of Fe oxides accumulated in the roots can adsorb anions such as phosphate (Howeler, 1973; Zhang et al., 1999).

In shoots, the P amounts decreased in both cultivars under Fe excess, mostly in E108 plants (Figure 4A), suggesting that there was limited P translocation to the shoots. Possibly, there was limited uptake of apoplast P into the simplast. Therefore, our shoot data seems to agree with Howeler (1973), who states that P precipitation in the root's apoplast results in lower P absorption by the plant.

Calcium concentrations decreased under the excess Fe treatment in roots and shoots, in both cultivars, reaching deficiency levels in E108 shoots (Figure 4B). The formation of the Fe plaque has been considered to induce Ca deficiency in rice plants (Howeler, 1973).

The most evident difference observed in Zn concentration after 10 d under Fe excess was a higher Zn concentration in shoots of E108 plants than in the control treatment (Figure 4C). Zhang et al. (1998) showed that the Fe plaque can lead to higher or lower Zn concentrations in shoots, depending on its size. In the present work, the Fe plaque did not seem to be acting either as a Zn reservoir or preventing Zn uptake.

Manganese concentrations in roots were low even in control plants, but much lower levels were achieved under Fe excess, in both cultivars, with I409 plants reaching the lowest levels in roots and shoots (Figure 4D). We found no reports on Mn deficiency levels in roots of rice plants. Precipitation of Mn in the Fe plaque may have resulted in its lower absorption by the sensitive cultivar, where the highest Fe concentrations were found. Negative interactions between Fe and Mn have been reported in plants (Fageria, 2003).

The potassium concentration decreased in E108 shoots under Fe excess in relation to the control

treatment, but increased in I409 shoots (Figure 4E). Smaller K absorption rates under Fe excess have been reported (Vahl et al., 1993; Neue et al., 1998). Our data, however, could support lower K absorption rates under excess Fe only in the E108 cultivar, but not in the I409, where it would be expected due to its higher susceptibility to excess Fe toxicity.

Sulphur concentrations were lower in shoots of E108 plants exposed to excess Fe for 10 d than in control plants, what did not happen in I409 plants (Figure 4F).

Lower Cu concentrations were seen in roots of plants from both cultivars when submitted to Fe overload. It has been suggested that the Fe plaque could act as a Cu reservoir in plants, increasing Cu absorption, depending on the Fe and Cu amounts in the environment (Ye et al., 2001). Since we observed the opposite, it is possible that our experiment resulted in a larger Fe plaque than the one reported by Ye et al. (2001), being able to act as a barrier to Cu absorption, such as proposed for Zn (Zhang et al., 1998).

From the above it follows that the two cultivars tested seemed to respond differently to Fe excess, especially in the shoots (e.g.: 71% reduction in Mn concentration in I409 and only 18% reduction in E108; 49% reduction in K concentration in E108 but 34% increase in I409; 49% reduction in S concentration in E108 and negligible reduction of 2% in I409) (Figure 5D). In roots, Fe overload impacted in opposite ways in the P concentration, with a 93% increase in I409 and a 12% decrease in E108. However, the differences between cultivars did not confirm the expected decreased nutrient absorption in the susceptible cultivar, since most of the lower ion concentrations were seen in the resistant one. Manganese was the only nutrient that reached very low concentrations in I409 plants under Fe excess (reductions of 92 and 71% in roots and shoots, respectively, in relation to the control treatment, whereas E108 suffered reductions of 36 and 18% in roots and shoots – Figure 5C,D).

Critical levels of nutrients: To the best of our knowledge, there are no reports assigning deficiency, normal and excess levels of minerals to rice plants at the same growth stage of the plants we used in our experiments. Based on available reference tables, relative to older plants (tillering stage), Ca was the only element which reached deficiency levels in leaves under Fe overload: less than 0.2% DW (Fageria, 1984) or less than 0.15% DW (Howeler, 1983; Sahrawat et al., 1996). Average Ca concentration in shoots of E108 plants after 10 d of exposure to Fe excess was 0.147% DW (Figure 4B). Levels of Fe, S, Mn and Cu were higher than the limits considered for mineral excess in several treatments, including control treatments, which may be an indication that younger rice plants have higher concentrations of these minerals than plants at the tillering stage. Shoot nutrient concentrations considered toxic for older plants were detected for Fe (>300 $\mu g\ g^{-1}$ DW) in all treatments, including the Fe deficiency treatment (Figure 3). Phosphorus and Mn concentrations also reached potentially toxic concentrations (0.8% and >1000 $\mu g\ g^{-1}$ DW, respectively – Fageria, 1984) in shoots of both cultivars under control and Fe deficiency (Figures 4A,D). Westfall et al. (1973) showed that the concentrations of nutrients in the rice plant vary with plant part and leaf age during the panicle differentiation stage. Phosphorus concentrations were highest in the culm plus sheaths, followed by the second youngest leaf. Considering that the same distribution pattern could be found in younger plants as the ones from our experiments, our average values, obtained for complete shoots, could be higher than the actual average leaf P content. The opposite could happen in relation to Ca concentrations, which were extremely low in the culm plus sheaths in the experiments reported by Westfall et al. (1973).

CONCLUSIONS

Except for Mn, no other nutrients seemed to have impaired uptake due to Fe toxicity in the susceptible cultivar and not in the resistant one. Therefore, I409 plants seem to be mostly affected directly by Fe toxicity rather than by secondary effects of Fe excess on mineral nutrition.

The E108 cultivar seems to rely mostly on avoidance of Fe uptake into the plant and decreased translocation to shoots, besides being able to maintain higher Mn levels in roots and shoots. E108 plants also seem to be more efficient in coping with Fe deficiency, with lower reductions in Fe concentrations in roots and shoots after 10 d of treatment, as well as increased Zn uptake and overall better nutritional status. The better performance

of E108 plants in relation to Fe nutrition may be related to the differences observed in the distribution of Fe among the diverse compounds detected by Mössbauer spectroscopy (especially absence of compound A in E108 roots under Fe deficiency and a different pattern of Fe speciation in shoots of control plants).

Considering that each rice genotype known as resistant to Fe overload and/or less susceptible to Fe deprivation may be the result of a unique combination of factors leading to the overall phenotype, a case-by-case research effort will be needed before a thorough understanding of the mechanisms regulating Fe homeostasis in rice can be reached. The use of diverse techniques, such as the combination of PIXE and Mössbauer spectroscopy analysis with physiological and gene expression studies, may be one promising approach.

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