



## Irrigated rice genotype performance under excess iron stress in hydroponic culture

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**ABSTRACT** – Iron stress is a major stress factor in irrigated rice. The complete mechanism underlying iron metabolism and transport is still unknown. Irrigated rice (*Oryza sativa* L.) cultivars were exposed to Fe<sup>2+</sup> stress in hydroponic conditions, aiming to assess genotype performance under stress as well as to develop a protocol for genotype selection. The experimental design was completely randomized, using a triple factorial scheme 2 x 5 x 6 (time x dose x genotype). The trait shoot length and nine days under stress were favorable for genotype discrimination under iron stress. The genotypes BR IRGA 409 and BRS AGRISUL were, respectively, the most sensitive and tolerant genotypes to iron stress. According to the genotype performance, hydroponics can be recommended as an efficient cultivation technique for the selection of iron stress-tolerant rice genotypes.

**Key words:** *Oryza sativa* L., Fe<sup>2+</sup>, controlled conditions, plantlet traits.

### INTRODUCTION

Irrigated rice covers over 50% of the lowland soils in southern Brazil, reaching approximately three million hectares (FAOSTAT 2005). The artificial flooding in this area is highly beneficial for rice. However, some unfavorable conditions such as iron toxicity can occur under specific conditions. This condition is caused by an increase in Fe<sup>2+</sup> concentrations in the soil solution.

Under iron stress, plant roots tend to be shorter and thicker. The symptoms may appear in any

developmental phase, but are commonly seen at maximum tillering and beginning of flowering (Sousa et al. 2006). The growth of the injured plants is then stunted, they have less tillers and produce small panicles with a high proportion of sterile spikelets, resulting in lower yields (Yoshida 1981).

The intensity of symptoms caused by iron toxicity under flooding is determined by different pH, organic matter and iron oxide contents and reactivity conditions in the soil. It was observed that the critical iron content in the soil solution can range from 30 mg L<sup>-1</sup> to over 500

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mg L<sup>-1</sup> (Sousa et al. 2006). The iron in the soil is mostly observed as ferric ion (Fe<sup>3+</sup>) combined in oxides such as Fe(OH)<sup>2+</sup>, Fe(OH)<sub>3</sub> and Fe(OH)<sub>4</sub><sup>-</sup> (Vahl 2004). The increase in the concentration of this element in the soil solution occurs by a reduction of ferric oxides - Fe<sup>3+</sup> (insoluble), to ferrous oxides - Fe<sup>2+</sup> (soluble), by anaerobic microorganisms, which use organic matter as electron source -  $\text{Fe(OH)}_3 + 3\text{H}^{2+} + \text{e}^- \leftrightarrow \text{Fe}^{2+} + 3\text{H}_2\text{O}$

(Camargo and Tedesco 2004). According to Vahl (2004), under low fertility conditions this problem can be aggravated, mainly in the presence of phosphorus or potassium, or even of respiration inhibitors such as H<sub>2</sub>S.

To minimize the iron toxicity effects in iron-rich soils soil management techniques are recommended, combined with lime application, soil drainage, later beginning of flooding irrigation, drainage during the cultivation period and/or anticipation of phosphate and potassium fertilization (SOSBAI 2007). These measures characterize positive alternatives, but are often economically unviable for being costly and labor intensive. It is also only a temporary solution to the problem.

The species and varieties differed largely in terms of tolerance to iron toxicity. The search for genotypes more adapted to the irrigated rice system is therefore an efficient alternative to circumvent this problem. Plant cells can limit the damaging effects caused by this metallic ion by storing the excess iron in iron-protein complexes called phytoferritin (Arosio and Levi 2002). By the aerenchyma in the rice plant the atmosphere oxygen can be diffused effectively to the rhizosphere, where the ferrous ions can be excluded by oxidation, since the Fe<sup>2+</sup> reaching the root surface can be precipitated and are not absorbed by the plant (Vahl 2004).

Plant reactions to Fe<sup>2+</sup> can be detected and measured in laboratory tests based on different traits, where differences in plantlets can indicate tolerant and sensitive genotypes (Ferreira et al. 1997, Camargo and Freitas 1992, Fageria et al. 1981).

Hydroponics is an efficient technique to isolate biotic and abiotic factors in the soil; particularly, factors can be investigated with higher accuracy, lower costs and in less time. This is useful when the results obtained in controlled conditions are highly correlated with those from field experiments (Camargo and Oliveira 1981). The great advantage of this method is that it is non-destructive, so the selected plants can be advanced to the next generation for progeny tests (Spehar and

Makita 1994).

Based on the above information, the purpose of this study was to evaluate the performance of irrigated rice genotypes in response to iron toxicity in hydroponic culture and also to assess the viability of this technique as an auxiliary tool in rice breeding programs for Fe<sup>2+</sup> stress tolerance.

## MATERIAL AND METHODS

The experiment was performed in the Laboratório de Duplo-haplóides e Hidroponia of the Centro de Genômica e Fitomelhoramento - FAEM/UFPEL. Six irrigated rice genotypes were used, three of them sensitive (BR IRGA 409, BR IRGA 410 and IRGA 417) and three tolerant (BR IRGA 414, IRGA 419 and BRS AGRISUL) in their response to iron toxicity in the field, according to experimental results (SOSBAI 2007).

The experimental design was completely randomized with three replications. The experimental unit consisted of 10 plantlets distributed in a triple factorial scheme 2 x 5 x 6 (time x dose x genotype). Seeds from genotypes were germinated in containers (gerbox) filled with germitest paper, in a growth chamber at 25 °C for 72 hs. Uniform seedlings with 5 mm radicles were transferred to nylon nets placed on top of pots containing 1.5 L nutrient solution, containing 4732.30 μmol L<sup>-1</sup> Ca(NO<sub>3</sub>)<sub>2</sub>; 1640 μmol L<sup>-1</sup> MgSO<sub>4</sub>.7H<sub>2</sub>O; 1050.7 μmol L<sup>-1</sup> KNO<sub>3</sub>; 168.65 μmol L<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 351.35 μmol L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>; 9 μmol L<sup>-1</sup> MnSO<sub>4</sub>.H<sub>2</sub>O; 0.15 μmol L<sup>-1</sup> CuSO<sub>4</sub>.5H<sub>2</sub>O; 0.15 μmol L<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O; 15 μmol L<sup>-1</sup> NaCl; 0.1 μmol L<sup>-1</sup> Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O; 18 μmol L<sup>-1</sup> H<sub>3</sub>BO<sub>3</sub>. Iron was supplied as Fe EDTA, in the amount required to achieve concentrations of 0, 80, 160, 320, and 640 mg L<sup>-1</sup> (equivalent to 0; 1,440; 2,880; 5,760; and 11,520 μmol L<sup>-1</sup> Fe, respectively). Since iron is a micronutrient, it is required in small amounts during the initial developmental phases of rice. Considering the short evaluations periods of this study, a large portion of the nutrient was supplied by the iron stored in the seeds, and no deficiency was observed in the control treatment regarding this micronutrient.

The pH of nutrient solution was daily adjusted to 4.0, by addition of HCl and/or NaOH 1 mol L<sup>-1</sup>, with constant aeration and light (1.700 lx). The pots were



kept in a water-bath at  $27 \pm 1$  °C.

Seven and nine days after beginning the experiment (168 and 216 h, respectively), the plantlets were evaluated for root (RL) and shoot (SL) length (cm), number of roots (NR), first (FLL) and second (SLL) leaf length, coleoptile length (CL), first leaf insertion (FLI), and distance of insertion from first to second leaf (DIFS). Root (RDM) and shoot (SDM) dry matter (mg) was measured after drying the plants at 60 °C to constant weight. These evaluations after seven and nine days were performed to develop a method for selecting iron-tolerant rice genotypes and assess the correlation with field performances at early stages.

Plantlets were evaluated at early (seven days) and full (nine days) V<sub>2</sub> stages, according to Conce et al. (2000). This stage is characterized by the formation of a collar on the second leaf of the main stem.

For variance analysis, the data were transformed using square root of the traits RL, SL, NR, FLL, and SDM. The sources of variation and their interactions were tested at 5% probability and a fitting of the polynomial regression was performed to explain the performance of genotypes regarding the quantitative factor Fe<sup>2+</sup> dose based on the traits SL and RL. These procedures were performed using the *SAS Learning Edition* (2002) software. Afterwards, the coefficients of phenotypic ( $r_p$ ) and genetic ( $r_g$ ) correlation were estimated. Results of the two periods (seven and nine days of excess iron) were compared to identify the best period to analyze the plantlets. Then, the associations between RL and SL with the other traits (NR, FLL, FLI, CL, DIFS, SLL, RDM, and SDM) were evaluated at the plantlet stage. The correlated genetic gain (CGG) based on the genetic correlation matrix was calculated based on a selection index of 10% (Falconer 1989). The estimated values were expressed as percentage of the mean. To estimate the genetic dissimilarity, the generalized distance of Mahalanobis ( $D^2$ ) between genotype pairs was obtained with the standardized means using the GENES software (Cruz 2001). Based on the genetic dissimilarity matrix, a dendrogram was constructed according to the UPGMA (*Unweighted Pair Group Method with Arithmetic Mean*) procedure using software NTSYS pc 2.1 (Rohlf 2000). After the dendrogram was obtained, the cophenetic correlation coefficient was calculated by Mantel's test. Also, clusters were defined as those at a larger than the mean dissimilarity (Sokal and Rohlf

1962).

## RESULTS AND DISCUSSION

Significant differences were detected for the factors time (T), genotype (G) and dose (D) and for all interactions among these factors, for both traits RL and SL (Table 1). A regression fitting was performed to show the performance of each genotype as a function of Fe dose and time of evaluation (Figure 1) because a triple significant interaction was detected in the analysis of variance (Table 1). According to the mathematical model presented, an

**Table 1.** Summary of the analysis of variance for the traits root (RL) and shoot (SL) length in irrigated rice genotypes evaluated in plantlets exposed to different Fe<sup>2+</sup> doses for different periods

Source of variation	DF	MS	
		RL	SL
Time (T)	1	1.420*	1.185*
Dose (D)	4	27.766*	43.151*
Genotype (G)	5	0.120*	0.243*
T x D	4	0.983*	0.405*
G x D	20	0.040*	0.192*
T x G	5	0.055*	0.055*
T x G x D	20	0.042*	0.058*
Error	120	0.016	0.025
General Mean	-	4.330	9.260
CV%	-	6.622	5.518

DF= Degrees of freedom; MS= Mean square; RL= Root length (cm); SL= Shoot length (cm); CV= Coefficient of variation; \*Significant at 5% probability; ns= non significant

increase in the Fe<sup>2+</sup> dose leads to a reduction in RL and SL for all genotypes at both evaluation times.

The presence of iron in the nutrient solution, for both evaluation times (seven and nine days), was associated with a deposition of this element on the root surface, a typical mechanism of adaptation and response of rice to excess concentrations of Fe<sup>2+</sup> in the nutrient solution (Vahl 1991, Vahl 2004). The roots had a dark brown color directly proportional to the iron concentration in the solution, which is a commonly reported visual symptom of indirect iron toxicity in plants. Also, higher iron concentrations reduced root development, associated with shorter and thicker roots and less branching, i.e., reduced formation of secondary roots. In these same conditions, the genotypes had an intense shoot color, with darker green shades than the control (0 mg L<sup>-1</sup> Fe), possibly related to iron accumulation. The plant shoot growth was also stunted,

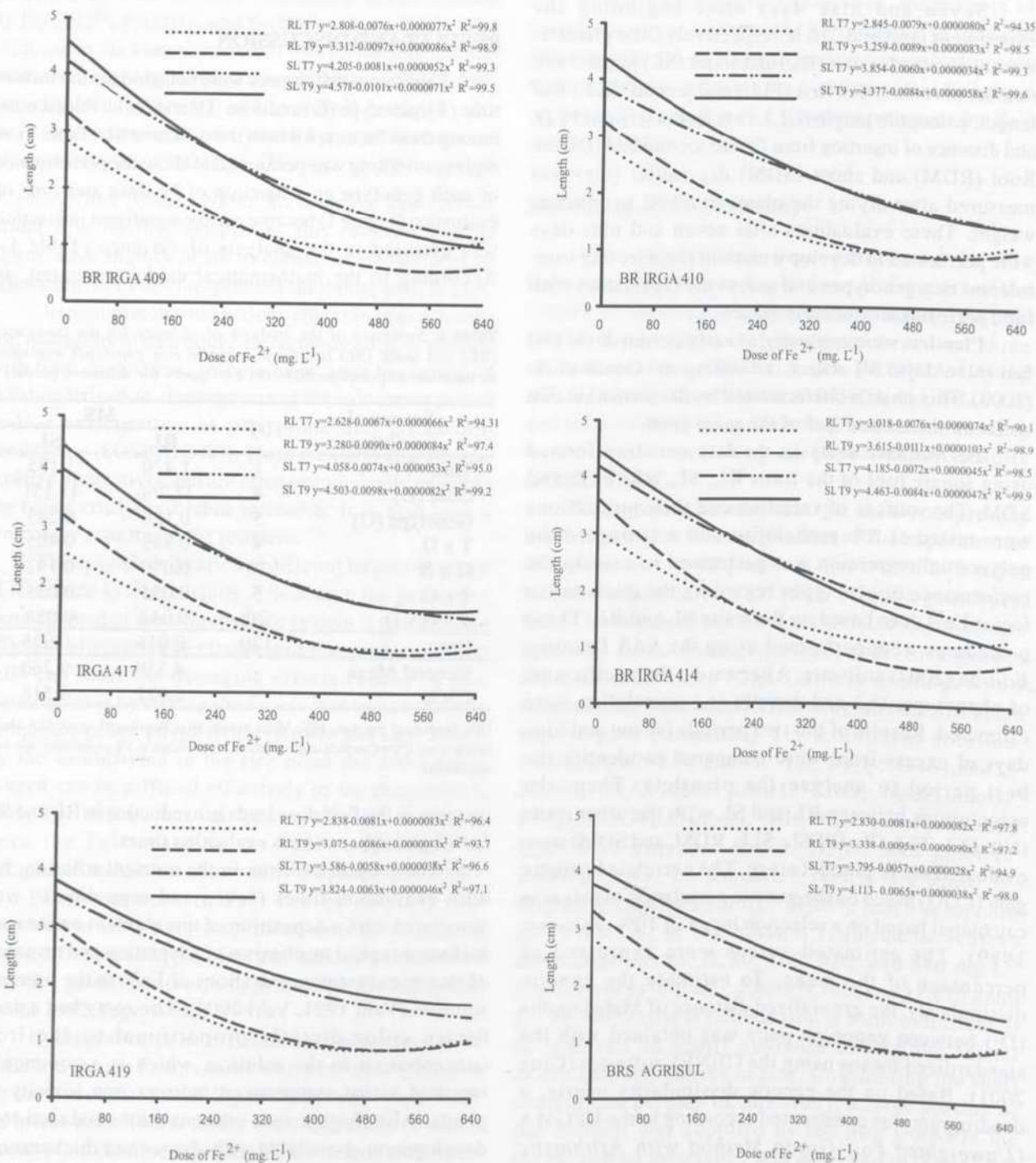


Figure 1. Representation of fitted regression equations (root square transformed data), generated by rice genotype performance regarding the traits root (RL) and shoot (SL), for the iron doses after seven and nine days in nutrient solution



markedly different from the control, but without the typical symptoms of leaf bronzing and yellowing.

In a comparison of the exposure periods of rice genotypes to toxic iron concentrations, it was observed that minimal shoot length ( $y = -b/2c$ ) is reached with lower doses after nine than after seven days (critical dose), suggesting a higher damage provoked by a longer exposure period of plant to the solution (Figure 1). Only genotype IRGA 414 performed differently, with a higher critical dose after nine ( $y=893.6 \text{ mg L}^{-1}$  of  $\text{Fe}^{2+}$ ) than after seven days ( $y=800.0 \text{ mg L}^{-1}$  of  $\text{Fe}^{2+}$ ).

The individual performance of each genotype versus exposure period indicated that the critical doses for SL were lower for the genotypes described as sensitive than for the tolerant genotypes, after both exposure periods. However, after seven days, the critical dose of genotype BR IRGA 410 displayed was higher than of BRS 414, described as tolerant, and the critical dose for genotype IRGA 419 was lower than the one observed for BR IRGA 409, described as sensitive. After nine days, the critical dose for genotype IRGA 419 was again oddly lower than for BR IRGA 409. Nevertheless, when one considers the  $b$  parameter of the regression equation for both periods, the reductions of shoot length with increases in the  $\text{Fe}^{2+}$  dose in the solution were higher for the sensitive genotypes. Lower reductions were observed for the tolerant genotypes, except for BR IRGA 410 (sensitive) which had a higher regression coefficient than BRS 414 (tolerant) after seven days. Lower reductions in shoot length observed with increases in  $\text{Fe}^{2+}$  doses in the solution may be related to the fact that increases in  $\text{Fe}^{2+}$  concentrations in the solution often result in shoot iron accumulation (Fageria et al. 1981). This situation would lead to concentrations higher than necessary for maximum growth without affecting the normal rice growth rate of plants (luxury consumption). On the other hand, higher concentrations of this element can lead to higher reductions in plant development (Fageria et al. 1976), as can be observed for the sensitive genotypes in this study.

Based on the jointly evaluated critical dose criteria and regression coefficient, the genotypes with higher sensitivity to  $\text{Fe}^{2+}$  were BR IRGA 409 and IRGA 417, and the most tolerant was BRS AGRISUL, confirming the tolerance levels described for field reactions (SOSBAI 2007). Iron toxicity has been described in the field since the end of the 1970's. Until then, traditional or intermediate cultivars were used. When modern

cultivars were adopted, iron toxicity symptoms started to appear (Sousa et al. 2006). The cultivar BR IRGA 409 was the first released short stature high-yielding genotype, but with sensitivity to iron toxicity, which became a strong limitation for a continued use by farmers (SOSBAI 2007).

The performance of cultivars regarding RL under iron stress suggests that a longer exposure (nine vs. seven days) increases the critical dose. Based on this response, there is consequently no agreement between the tolerant/sensitive description and performance in the experiment. Also, considering the regression coefficient values ( $R^2$ ) for trait RL, it can be observed that in general, the coefficient values of sensitive genotypes were higher than of tolerant genotypes for both exposure periods. It is evident that a ranking based on RL does not fit with the description based on field experiments. Trait SL is therefore more effective in discriminating irrigated rice cultivars under iron stress in hydroponic culture. This performance can be explained by the great accumulation of this chemical element on the rice shoots when grown at high iron concentrations. This situation has been reported for rice (Fageria et al. 1981, Vahl 1991, Dynia and Moraes 1998), since roots take up iron or chelated iron through active transport, and these compounds are oxidated to the ferric form and translocated to leaves in the form of electrostatic complexes with citrate (Taiz and Zeiger 2004). In leaves, iron has the function of a micronutrient, and is found in the cytochrome heme molecules (iron-porphyrin complexes), in chloroplasts and mitochondria, and is biochemically involved in redox reactions (electron transport), photosynthesis and respiration (Raven et al. 2001).

The analyses of the parameters critical dose and equation coefficient for the trait SL indicate that the nine day exposure to iron was better to discriminate the genotypes. This can be explained by a better agreement between plant growth performance at higher  $\text{Fe}^{2+}$  levels and their field response descriptions. After seven days of exposure, the plantlets were at the beginning of second leaf expansion and despite their already efficient root system, there was still a considerable use of seed-stored compounds when compared to plantlets after nine days of exposure. At this later stage, plants were forced to use their own energy producing mechanisms more effectively, and more prone to be affected by stressing factors.



Root and shoot length are reported in the literature as traits used to test rice genotype response to  $\text{Fe}^{2+}$  in nutrient solution (Ferreira et al. 1997, Fageria et al. 1981). However, it is important to study other traits at the plantlet stage, to allow the indirect selection of tolerant genotypes, especially when using highly heritable traits. A correlation analysis was performed between plantlet traits and RL and SL, based on results of nine day of exposure to excess iron, to capture the strongest phenotypic expression of genotypes regarding  $\text{Fe}^{2+}$  toxicity. In this evaluation, the analysis of variance confirmed the presence of significant differences for the factors genotype (G) and dose (D) for all evaluated traits, except for SDM that did not differ for the factor genotype (Table 2). Interactions between the factor genotype (G) and dose (D) were also observed for all traits except SDM (Table 2), which did not differ significantly among the genotypes, although trait SL was shown to be the most efficient in characterizing the studied genotypes regarding  $\text{Fe}^{2+}$  tolerance.

Phenotypic ( $r_p$ ) and genetic ( $r_G$ ) correlations, and the correlated genetic gain (CGG%) between the traits RL and SL with the other plantlet traits (NR, FLL, CL, DIFSL, SLL, and RDM) were established as well as the associations between the statistically significant traits for each  $\text{Fe}^{2+}$  dose (Table 3).

The genotypes performance for trait SL differed at the doses 0, 80, 320 and 640  $\text{mg L}^{-1}$  of  $\text{Fe}^{2+}$ . At 0  $\text{mg L}^{-1}$  of  $\text{Fe}^{2+}$ , the genetic and phenotypic correlations between SL and the traits FLL and DIFSL were positive, showing high and medium expression, respectively. With an

increase in SL there is therefore a decrease of 15.80% in FLL, as well as a greater distance between the position of the first and the second leaf on the plantlet, with an increase of 43% in this trait when selected based on SL. The fact that some genetic correlations have values above one, according to Falconer (1989), can be a consequence of non-additive and/or higher order genic interactions.

With the addition of iron to the nutrient solution, positive correlations are observed, with medium to high values, between SL and the traits RL, NR, DIFSL, SLL, and RDM at the dose 80, SL and SLL at 320, and SL and RL at 640  $\text{mg L}^{-1}$  of  $\text{Fe}^{2+}$ . The high correlation values between SL and SLL are noteworthy, in agreement with the large contribution of SLL to rice plantlet stature, even under iron stress conditions. Significance was observed for trait RL at the doses 80, 160 and 640  $\text{mg L}^{-1}$   $\text{Fe}^{2+}$ ; medium to high positive correlation values were detected between RL and the traits SL, NR, SLL and RDM at dose 160. A medium correlation between RL and DIFSL at the dose 160, and a high and positive correlation between RL and SL at a dose of 320  $\text{mg L}^{-1}$  of  $\text{Fe}^{2+}$ . Also, medium negative correlation values with the traits FLL and SLL were observed at this rate.

The correlated genetic gain for trait RL was 23.31% when selected based on trait SL at 640  $\text{mg L}^{-1}$  of  $\text{Fe}^{2+}$ , a dose closer to the ideal dose for evaluation of each genotype regarding  $\text{Fe}^{2+}$  (critical dose). On the other hand, the selection based on RL allows a correlated genetic gain of 29.04% in SL, and a

**Table 2.** Summary of analysis of variance for plantlet traits (RL, SL, NR, FLL, FLI, CL, DIFSL, SLL, RDM and SDM) of irrigated rice genotypes exposed to different iron doses for nine days

Source of variation	DF	MS									
		RL	SL	NR	FLL	FLI	CL	DIFSL	SLL	RDM	SDM
Genotype(G)	5	0.60*	0.11*	0.10*	0.06*	0.51*	0.17*	2.00*	2.90*	170.31*	8.23 <sup>ns</sup>
Dose(D)	5	18.30*	24.42*	8.85*	2.92*	51.63*	3.17*	11.51*	311.97*	10018.15*	1362.22*
G x D	25	0.05*	0.17*	0.03*	0.06*	0.28 <sup>ns</sup>	0.05*	1.10*	2.22*	105.04*	6.76 <sup>ns</sup>
Error	72	0.01	0.03	0.01	0.02	0.18	0.02	0.05	0.57	25.80	5.05
General mean	-	5.25	10.72	3.25	2.11	2.93	1.20	0.96	6.79	102.90	407.92
CV%	-	5.33	5.84	6.56	10.07	15.74	12.46	29.96	12.13	18.83	12.69

DF= Degrees of freedom; MS= Mean square; CV= Coefficient of variation; RL= Root length (cm); SL= Shoot length (cm); NR= Number of roots (units); FLL= First leaf length (cm); FLI= First leaf insertion (cm); CL= Coleoptile length (cm); DIFSL= Difference of insertion between the first and second leaf (cm); SLL= Second leaf length (cm); RDM= Root dry matter (mg); SDM= Shoot dry matter (mg); \*Significant at 5% probability; ns= non significant

Irrigated rice genotype performance under excess iron stress in hydroponic culture

**Table 3.** Estimate of genetic and phenotypic correlation between the traits RL and SL with the remaining plantlet traits (NR, FLL, CL, DIFSL, SLL, and RDM) and correlated genetic gain (% regarding mean) in irrigated rice genotypes exposed to different iron doses

Dose (mg L <sup>-1</sup> Fe <sup>2+</sup> )	Trait	SL	RL	FLL	NR	CL	DIFSL	SLL	RDM
0	SL (rP)	1	-	-0.81*	0.29 <sup>ns</sup>	-	0.49*	-	0.04 <sup>ns</sup>
	SL (rG)	1	-	-1.12*	0.37 <sup>ns</sup>	-	0.53*	-	0.15 <sup>ns</sup>
	CGG (%)	-	-	-15.80	-	-	43.00	-	-
	RL (rP)	-	1	-	-	-	-	-	-
	RL (rG)	-	1	-	-	-	-	-	-
	CGG (%)	-	-	-	-	-	-	-	-
80	SL (rP)	1	0.79*	-	0.62*	-	0.77*	0.91*	0.62*
	SL (rG)	1	0.81*	-	0.67*	-	0.80*	0.97*	0.73*
	CGG (%)	-	22.00	-	1.00	-	65.00	14.67	13.89
	RL (rP)	0.79*	1	-	0.87*	-	0.29 <sup>ns</sup>	0.83*	0.84*
	RL (rG)	0.81*	1	-	0.94*	-	0.29 <sup>ns</sup>	0.95*	0.96*
	CGG (%)	27.78	-	-	12.22	-	-	14.43	18.34
160	SL (rP)	1	-	-	-	-	-	-	-
	SL (rG)	1	-	-	-	-	-	-	-
	CGG (%)	-	-	-	-	-	-	-	-
	RL (rP)	-	1	0.16 <sup>ns</sup>	-	-	0.59*	-	-
	RL (rG)	-	1	0.13 <sup>ns</sup>	-	-	0.66*	-	-
	CGG (%)	-	-	-	-	-	62.46	-	-
320	SL (rP)	1	-	-	-	-	-	0.97*	-
	SL (rG)	1	-	-	-	-	-	1.28*	-
	GGC (%)	-	-	-	-	-	-	32.34	-
	RL (rP)	-	1	-	-	-	-	-	-
	RL (rG)	-	1	-	-	-	-	-	-
	CGG (%)	-	-	-	-	-	-	-	-
640	SL (rP)	1	0.90*	-0.33 <sup>ns</sup>	-	-0.05 <sup>ns</sup>	-	-0.39 <sup>ns</sup>	-
	SL (rG)	1	1.00*	-0.44 <sup>ns</sup>	-	-0.13 <sup>ns</sup>	-	-0.44 <sup>ns</sup>	-
	GGC (%)	-	23.31	-	-	-	-	-	-
	RL (rP)	0.90*	1	-0.40 <sup>ns</sup>	-	-0.11 <sup>ns</sup>	-	-0.38 <sup>ns</sup>	-
	RL (rG)	1.00*	1	-0.60*	-	-0.13 <sup>ns</sup>	-	-0.48*	-
	CGG (%)	29.04	-	-21.49	-	-	-	-59.11	-

RL= Root length (cm); SL= Shoot length (cm); NR= Number of roots (units); FLL= First leaf length (cm); CL= Coleoptile length (cm); DIFSL= Difference of insertion between the first and second leaf (cm); SLL= Second leaf length (cm); RDM= Root dry matter (mg); CGG= correlated genetic gain; rP = phenotypic correlation coefficient; rG = genotypic correlation coefficient; DF= n-2 ? 16; \*Significant at 5% probability; ns= non significant

decrease of 21.49 and 59.11% in the traits FLL and SLL, respectively.

The genetic were higher than the phenotypic correlations, indicating that the environment negatively affected these associations, which can be misleading when working with indirect selection for excess iron tolerance. Therefore, the relationship between the performance of a trait as a function of the other could have been maximized in conditions favorable to the expression of both traits. These results allow the establishment of a selection strategy, as well as to

understand the expression of these traits in selecting populations, since the presence of correlations is related to pleiotropic effects, i.e., one gene controlling two or more traits or linkage, where genes are located in the same chromosome and do not segregate independently (Ramalho et al. 2004).

In general, doses above 160 mg L<sup>-1</sup> of Fe<sup>2+</sup> in the nutrient solution are considered severe for the rice genotypes used in the present experimental conditions, hampering the effective expression of relationships between traits at the plantlet level.



Therefore, the tested Fe doses interfere with the expression of the traits that could potentially be used for indirect selection. According to Benin et al. (2003), the GxE interaction interferes with the correlation between traits and affects the genetic gains directly. The GxE interaction is also a very important factor in the search for new selection strategies, particularly in unstable environmental conditions (Carvalho et al. 2004).

The multivariate analysis using all measured traits (RL, SL, NR, FLL, SLL, CL, FLI, DIFSL, RDM, and SDM) indicated the associations among genotypes regarding sensitivity to Fe<sup>2+</sup> (Figure 2), considering all doses and periods of exposure. In the dendrogram (Figure 2), two groups can be detected, based on the mean dissimilarity. The first group is formed by genotype BR IRGA 409, and the second comprises the cultivars IRGA 410 and IRGA 417, BR IRGA 414, IRGA 419 and BRS AGRISUL. These conclusions have a high degree of confidence, since the fitting between the dissimilarity matrix and the dendrogram showed a cophenetic correlation coefficient of 0.81.

The reported description of genotypes regarding Fe tolerance is apparently more efficient when based on shoot traits. This is in agreement with the fact that the SL trait in this hydroponic study was highly indicative of this response. In general, the majority of traits evaluated did not differ among the cultivars and expressed small correlations. This can be explained by the fact that the joint analysis for all traits has not revealed a consistency in order to give a fine discrimination of tolerance levels similarly to what is obtained in the field. However, the genotype BR IRGA 409 was very distantly positioned in the dendrogram when compared to the other cultivars, confirming its high sensitivity to the toxic Fe<sup>2+</sup> ion under hydroponic conditions even when one considers the result of the joint analysis of all variables.

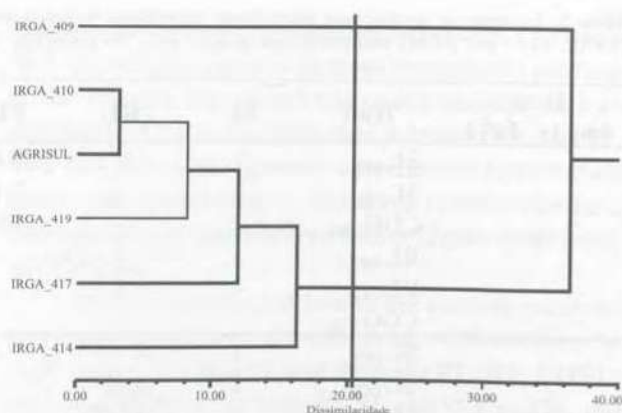


Figure 2. Dendrogram representing the genetic dissimilarity based on the generalized distance of Mahalanobis ( $D^2$ ) among irrigated rice genotypes, based on the plantlet traits RL, SL, NR, FLL, SLL, CL, FLI, DIFSL, RDM, and SDM evaluated under toxic iron stress, using the UPGMA clustering method

## CONCLUSIONS

The trait shoot length in V<sub>2</sub> stage plantlets is effective for the characterization of irrigated rice cultivars regarding Fe<sup>2+</sup> presence in the nutrient solution, considering the parameters critical dose and regression equation coefficient.

In the hydroponics system, high sensitivity to iron toxicity by Fe<sup>2+</sup> was observed in genotype BR IRGA 409 and higher tolerance in cultivar BRS AGRISUL, confirming the field performance.

Hydroponics can be recommended as a non-destructive technique of characterization of iron-sensitive and tolerant rice genotypes, making the advance of generations after selection possible.

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# Comportamento de genótipos de arroz irrigado submetidos ao estresse por excesso de ferro em condições de hidroponia

**RESUMO** - Cultivares de arroz irrigado (*Oryza sativa* L.) foram submetidas ao estresse por Fe<sup>2+</sup> em condições de hidroponia, com o objetivo de verificar o comportamento dos genótipos e a viabilidade de uso deste sistema como ferramenta auxiliar no incremento da eficiência de seleção em programas de melhoramento desta espécie. O delineamento experimental adotado foi o completamente casualizado, num esquema fatorial triplo 2 x 5 x 6



(tempo x dose x genótipo). O caráter comprimento de parte aérea e o tempo de exposição nove dias proporcionaram a melhor discriminação dos genótipos quanto à sensibilidade ao ferro. O genótipo BR IRGA 409 evidenciou a maior sensibilidade ao estresse pelo íon metálico, enquanto a cultivar BRS AGRISUL indicou a maior tolerância. De acordo com o desempenho dos genótipos, a hidroponia pode ser recomendada como uma técnica eficiente na seleção de constituições genéticas de arroz tolerantes ao estresse por ferro.

**Palavras-chave:** *Oryza sativa* L., Fe<sup>2+</sup>, condições controladas, caracteres de plântula.

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