Original Research Article

Tolerance and Phenotypic Analysis of Irrigated Rice Genotypes under Iron Toxicity

ABSTRACT

Aims: The study aims to evaluate the response of genotypes of the Embrapa breeding program of irrigated rice against iron stress, as well as to envision the relationship of the effect of this disorder on leaf mass production in different phenological phases of the plant.
Study design: The trial was established in a randomized block design with additional controls, with the plots consisting of four rows 3 m long with 0.20 m spacing between rows.
Place and Duration of Study: The experiment was conducted in the experimental field of Embrapa Temperate Climate Lowlands Station, in a period of nine years, consisting of the harvest of 2006/2007 until 2014/2015.
Methodology: It were evaluated 255 lines along with 4 additional control cultivars with known tolerance levels. The symptoms were evaluated at 40, 70 and 100 days after plant emergence through a visual assessment in the field, based on the standard evaluation system for rice. In addition to the determination of the average toxicity levels of genotypes, the mass was collected, constituted by the dry matter of shoot, for the detection of interrelationships through their correlations.
Results: 58.82% of the developed irrigated rice lines show good tolerance to toxicity by excess iron. The association of the content of dry matter of shoots and the levels of indirect toxicity of iron showed a significant negative correlation (-0.6848), being that the highest magnitude of negative correlation was at 70 DAE (-0.6161).
Conclusion: There is variability for tolerance to indirect iron toxicity between the irrigated rice genotypes assessed. The breeding program of irrigated rice of Embrapa has been effective in developing genotypes with tolerance by excess iron in the soil over nine years. There is a negative association between the content of dry matter of shoots and the levels of indirect iron toxicity.

Keywords: Oryza sativa, abiotic stress, plant breeding, genetic variability, tolerant lines.

1. INTRODUCTION

Plants are often exposed to adverse environmental conditions that negatively affect cell homeostasis and ultimately harm the growth and development of the plant [1]. Hence, there is a need to understand how plants perceive, respond and adapt to such stresses [2].

In recent decades have been large increases in the potential of irrigated rice productivity due to the plant breeding contribution, through the advent of new cultivars of the modern type. However, the toxicity of iron has been a serious problem at Rio Grande do Sul, which accounts for over 65% of the Brazilian production of rice. Mainly due to the indirect toxicity of the plants associated with widespread nutritional deficiency, derived from excess iron in the soil solution, which precipitates on rice roots preventing the absorption of other essential nutrients for plant development [3].

Iron is a highly important nutrient for plants and its enrichment in the grain is highly desirable from the point of view of human nutrition [4]. Nonetheless, excessive conditions of this nutrient can result in considerable damage to the crop and can cause losses of up to 100% productivity [5]. Iron toxicity results from the most frequent nutritional disorder in the areas of irrigated rice in wetlands and can be expressed in two ways: directly and indirectly. The main symptoms of indirect toxicity are first evidenced in the roots of plants, which occurs due to the formation of a ferric layer (accumulation of iron oxide on the surface of roots), resulting in reduced absorption, transport and/or use of other nutrients by plants such as P, K, Ca, Mg and Zn, this being the most impactful in reducing the productivity of irrigated rice. Thus, the leaves develop a yellowish tinge (yellowing) that evolve from the base to the apex [6].

The major symptoms of iron toxicity are first evidenced in the plant roots, which tend to halt their growth and to increase their thickness. It can arise at any stage of development, being most commonly observed in the period of tillering and early flowering [7].

The iron metabolism is a complex mechanism under a homeostatic balance and may cause two major problems for plants: deficiency as a consequence of solubility problems under aerobic conditions and toxicity due to excessive solubility in anaerobic conditions [8].

The development of tolerant rice genotypes is the main tool to minimize the problems caused by excess iron [9]. Thus, it has been portrayed the existence of variation regarding the iron toxicity tolerance levels between rice genotypes, either through conventional breeding [10, 11, 12, 13, 14, 15] or with the aid of biotechnology [16, 17, 18]. Thus, this is a promising approach to meet the increasing demands for food, with excellent results in the association of productivity and sustainability in paddy crops.

Considering that the productive potential of crops is determined by the phenotype and expressed by the interaction of the genotype with the environment, the rice breeding programs seek to select genotypes whit tolerance to the stress caused by excess iron in the soil solution. Therefore, the study aims to evaluate the response of genotypes of the Embrapa breeding program of irrigated rice against iron stress, as well as to envision the relationship of the effect of this disorder on leaf mass production in different phenological phases of the plant.

2. MATERIAL AND METHODS

The experiment was conducted in the experimental field of Embrapa Temperate Climate Lowlands Station in Capão do Leão, in the state of Rio Grande do Sul (31º48’49,54’’ S latitude and 52º28’20,45’’ W longitude), in a period of nine years, consisting of the harvest of 2006/2007, 2007/2008, 2008/2009, 2009/2010, 2010/2011, 2011/2012, 2012/2013, 2013/2014 and 2014/2015. The area of the experiment, consisting of an Albaqualf soil, was previously systematized and undergone the decapitation of the layer corresponding to the A horizon, exposing the B horizon to emphasize the conditions that favor the occurrence of the disorder. The soil analysis indicated an estimated exchangeable iron amount of 3.36 cmolc dm^{-3} and percentage of saturation of the CEC ($PSFE^{2+}$) equivalent to 54%, corresponding to high probability of risk of iron toxicity. Irrigation was maintained permanently after 10 days of emergence of seedlings to keep the soil reducing conditions.

The trial was established in a randomized block design with additional controls, with the plots consisting of four rows 3 m long with 0.20 m spacing between rows. Sowing took place at the cultivation times recommended for the location, with a corresponding density of 100 kg ha^{-1}, using a mechanical sower in plots.

It was evaluated 255 lines developed by the irrigated rice breeding program of Embrapa along with four additional control cultivars with known iron toxicity tolerance levels, as follows: BRS Querência (tolerant), BRS 7 “Taim” (moderately tolerant), IRGA 417 (moderately susceptible) and BR IRGA 409 (susceptible). The assessment of the symptoms of indirect toxicity was performed at 40, 70 and 100 days after plant emergence (DAE) through a visual assessment of symptoms in the field using a scale of 1-9 (1- genotypes with normal development; 9- genotypes highly affected) based on the standard evaluation system for rice, developed by the International Rice Research Institute [19]. The evaluations were performed in all periods by 4 trained persons, for later achievement of joint averages, in order to get ratings with high accuracy. Subsequently, it was obtained the average levels of toxicity represented from the weighted average, assigning weight 2 for evaluation at 40 DAE, weight 6 to 70 DAE and weight 2 to 100 DAE. This greater weight assigned to the assessment at 70 DAE stems from the coincidence with the most critical stage of development of the culture, where the disorder causes the greatest damage to plants. The level of tolerance or susceptibility of the genotypes were obtained from the variation ranges in the average levels of toxicity, being: 1 to 3.5- tolerant; 3.6 to 5.5- average tolerant; 5.6 to 7.5- average susceptible and 7.6 to 9- susceptible. Data were subjected to analysis of variance and the discrimination between genotypes through the adjusted means considered the Scott-Knott grouping test at 5% probability.

In the 2011/2012 and 2012/2013 harvests it was collected plants corresponding to the area of 0.50 m at a linear portion in each plot, and obtained the dry matter of the shoot. To detect the interrelationships of the toxicity levels at 40 DAE, 70 DAE, 100 DAE to the dry matter of the shoots were estimated the correlations between data sets using the Pearson and the Mantel correlation tests, with 10,000 simulations [20].

Statistical procedures were processed through the computer application on quantitative genetics and experimental statistics, GENES [21].

3. RESULTS AND DISCUSSION

The analysis of the statistical parameters relating to the evaluations of tolerance of irrigated rice genotypes to the toxicity by excess iron in the soil (Table 1) indicated performance differences between the genotypes studied for most agricultural crops analyzed, except for the 14/15 crop, which can be explained by the low heritability of the trait observed in that crop. The coefficient of variation (CV) ranged between 12.14 and 30.19%.

Table 1. Statistical parameters related to the evaluations of tolerance of the rice genotypes to the indirect toxicity caused by excess iron in the soil

Statistical parameters	Harvest 06/07	Harvest 07/08	Harvest 08/09	Harvest 09/10	Harvest 10/11	Harvest 11/12	Harvest 12/13	Harvest 13/14	Harvest 14/15
n	28	36	45	45	25	45	45	45	45
MS	6.988**	4.312**	5,548**	2.873**	5.493**	3.118**	2.144**	1.921**	1,226^{ns}
μ	5.39	5.14	6.02	5.14	5.41	5.09	5.44	5.23	5.41
CV (%)	30.19	22.18	20.05	23.24	14.58	19.08	14.32	12.14	18.28
σ_P	2.32	1.44	1.84	0.96	2.75	1.04	0.71	0.64	0.41
σ_G	1.45	1.00	1.36	0.48	2.43	0.73	0.51	0.51	0.08
σ_E	0.88	0.44	0.48	0.47	0.31	0.31	0.20	0.13	0.32

h^2	62.11	69.88	73.69	50.36	88.67	69.78	71.65	79.00	20.29

n: genotype number; MS: mean square; μ: average; CV: coefficient of variation; σP: phenotypic variance; σG: genotypic variance; σE: environmental variance; h2: heritability

The distribution chart of the frequencies of the genotypes regarding the iron toxicity levels (Figure 1) demonstrated the efficiency of the Embrapa breeding program to develop irrigated rice genotypes with tolerance to the character. 150 of the 255 evaluated lines presented themselves as tolerant or average tolerant, i.e. 58.82% of the developed lines show good tolerance to toxicity by excess iron. This demonstrates the appropriate strategy of the breeding program, being defined the "hot spot" and the methodology capable of discriminating the irrigated rice genotypes that present, as well as high productivity, tolerance to stress conditions that may prevent the expression of their full genetic potential.

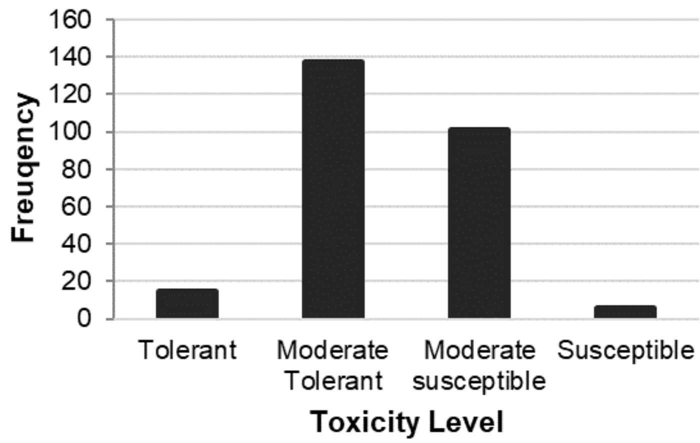

Fig. 1. Distribution of the frequency of the genotypes regarding the indirect toxicity caused by excess levels of iron in the soil

The irrigated rice genotypes are highly subject to environments with high iron content, therefore, variability analyses for tolerance to the character in field conditions are extremely important. Considering the grouping of the weighted averages of tolerance levels of the genotypes in Table 2, one can observe an magnitude variation with intensity between 1.6 (CNA0005014) and 8.3 (LTB 07016), 15 genotypes being grouped as tolerant according to the standard evaluation system for rice, of which 13 are elite lines of the program and 2 are cultivars, being BRS Querência the control and BRS AG "Gigante" launched in 2014 by Embrapa for use in animal feed and/or as raw material for the production of renewable energy, such as ethanol production [22]. In addition to these genotypes, 136 lines and the BRS 7 "Taim" cultivar also showed moderate tolerance to indirect iron toxicity, being considered average tolerant. This way, one can emphasize the high amount of sources of favorable alleles for this trait among the irrigated rice genotypes developed, both for releases of new cultivars through their agronomic characteristics and for use in future targeted crosses as sources of tolerance alleles.

It was evidenced that 107 genotypes showed susceptibility to indirect iron toxicity, as follows: 100 elite lines and the control IRGA 417, considered average susceptible; 5 elite lines and the control BR IRGA 409, classified as susceptible. Therefore, they should not be recommended for release on conditions that have high concentrations of iron in the soil solution.

Used as experimental correction factors, the control cultivars showed a good accuracy of the analysis, because the BRS Querência and BRS 7 "Taim" cultivars presented themselves as tolerant and moderate tolerant, respectively, and the IRGA 417 and BR IRGA 409 cultivars were considered moderate susceptible and susceptible, respectively. Hence, it was obtained an environmental condition ideal for the evaluations of the genotypes for the trait in question. According to Audebert and Fofana [23], the duration and intensity of the stress by iron are directly correlated to the environmental conditions and the availability of the element present in the soil solution. Thus, the effectiveness of a particular mechanism of tolerance is dependent on the intensity and duration of the stress [24].

The grouping based on the Scott-Knott test (Table 2) discriminated the genotypes in four groups, corroborating the classification of the evaluation system used for the character of tolerance to iron toxicity. However, there was little variation in the distribution of frequencies of genotypes in each group, a fact conditioned to difficulties in accurate inferences when working under field conditions.

Several authors also found that there is variability for the character of tolerance to iron through assessments under field conditions, providing new sources of use in the genetic breeding of rice aiming tolerance to iron toxicity. Onaga et al. [14], evaluating 19 genotypes, being 10 lowland rice cultivars obtained from the germplasm collections of the Rice Centre for Africa and 9 popular varieties of Uganda, identified the existence of four tolerant genotypes (PNA, IR73678-20-1-B, K98 and WITA4). Tests done by Lantin and Neue [25] in greenhouses in the Philippines showed that 479 of 6,140 rice cultivars were moderately tolerant to excess iron in the soil.

Table 2. Grouping of the weighted averages (WA) adjusted by the tolerance levels of the genotypes according to the standard evaluation system for rice and Scott-Knott grouping test.

Reaction**	Genotype	WA	*	Genotype	WA	*	Genotype	WA	*	Genotype	WA	*	Genotype	WA	*
Tolerant	CNA0005014	1.6	a	BRA 050106	2.8	a	BRS Querência	3.2	a	CNA0006961-A	3.3	a	BRA 050101	3.5	a
	CNA0003195	1.8	a	BRA 02103	2.8	a	AB08008	3.3	a	AB08127	3.3	a	BRS AG	3.5	a
	AB10004	2.5	a	BRA 050104	2.9	a	AB10571	3.3	a	AB08134	3.5	a	AB11004	3.5	a
Moderate Tolerant	AB08009	3.6	a	AB07005	4.5	b	AB13005	4.8	b	BRA 040081	5.0	b	BRA 01079	5.3	b
	AB12004	3.8	a	AB08055	4.5	b	BRA 040075	4.8	b	BRA 01100	5.0	b	AB13006	5.3	b
	CNA0008229	3.8	a	AB14003	4.5	b	AB11540	4.8	b	AB11039	5.1	b	AB11564	5.3	b
	BRA 01059	3.8	a	AB10595	4.5	b	AB07004	4.8	b	AB10572	5.1	b	AB13012	5.3	b
	BRA 050099	3.9	a	CNA0006961-B	4.5	b	AB07010	4.8	b	BRS Pampeira	5.1	b	BRA 040291	5.3	b
	AB08004	3.9	a	AB10574	4.5	b	AB08024	4.8	b	AB08002	5.1	b	AB12683	5.3	b
	AB08001	3.9	a	AB10602	4.5	b	AB08148	4.8	b	BRS 7 'Taim'	5.1	b	AB11547	5.3	b
	AB09044	3.9	a	AB12101	4.5	b	AB13708	4.8	b	BRA 01073	5.1	b	AB11548	5.3	b
	BRA 02498	4.0	a	BRA 02099	4.5	b	CNA0005853	4.8	b	AB08076	5.1	b	AB10578	5.3	b
	BRA 051279	4.0	a	AB11002	4.6	b	BRA 050145	4.8	b	AB08153	5.1	b	AB07070	5.3	b
	CNA0003490	4.0	a	AB06048	4.6	b	BRA 02665	4.8	b	AB10555	5.1	b	AB08066	5.3	b
	AB07181	4.1	a	AB07182	4.6	b	BRA 040082	4.8	b	AB13007	5.1	b	AB14005	5.3	b
	AB13713	4.1	a	AB10101	4.6	b	AB09043	4.9	b	AB13691	5.1	b	BRA 050054	5.4	b
	AB12588	4.1	a	AB13718	4.6	b	AB13705	4.9	b	BRA 030008	5.1	b	AB08147	5.4	b
	AB08057	4.1	a	AB14003	4.6	b	AB10589	4.9	b	BRA 050002	5.1	b	AB10009	5.4	b
	AB09028	4.2	b	BRA 051272	4.6	b	AB10597	4.9	b	BRA 051077	5.1	b	BRA 050141	5.4	b
	CNA0010476	4.2	b	AB101027	4.6	b	BRA 041049	4.9	b	AB13010	5.2	b	LTB07002	5.4	b
	CNA 10755	4.2	b	AB10528	4.6	b	CNA0006422	4.9	b	AB11551	5.2	b	AB08011	5.5	c
	AB08003	4.3	b	BRS 6 'Chui'	4.7	b	AB09023	4.9	b	AB12604	5.2	b	AB08123	5.5	c
	AB08108	4.3	b	AB08077	4.7	b	AB13724	4.9	b	AB08140	5.2	b	AB101026	5.5	c
	AB12574	4.3	b	AB10518	4.7	b	AB08020	5.0	b	AB11533	5.2	b	AB13706	5.5	c
	BRA 050069	4.3	b	BRA 051083	4.7	b	AB10591	5.0	b	AB12001	5.2	b	CNA0002672	5.5	c
	BRS Fronteira	4.3	b	AB12597	4.7	b	AB13712	5.0	b	AB12003	5.2	b	AB08141	5.5	c
	AB12546	4.3	b	AB09011	4.7	b	AB13720	5.0	b	BRA 050159	5.2	b	AB13715	5.5	c
	AB14001	4.3	b	AB10007	4.7	b	BRA 030040	5.0	b	BRA 051267	5.2	b	AB14004	5.5	c

	AB10558	4.4	b	AB11041	4.7	b	BRA 050138	5.0	b	AB11502	5.2	b			
	CNA0010433	4.4	b	AB11001	4.8	b	H7 CL	5.0	b	AB11544	5.3	b			
	AB10526	4.4	b	AB11005	4.8	b	CNA0002442	5.0	b	AB10592	5.3	b			
Moderate Susceptible	AB13008	5.6	c	AB06046	5.7	c	CNA0005462	6.0	c	AB061137	6.3	c	CNA0002293	7.1	d
	AB08072	5.6	c	BRA 040076	5.7	c	AB13001	6.0	c	CNA 10759	6.3	c	CNA0005465	7.1	d
	AB11501	5.6	c	AB11503	5.8	c	AB11542	6.1	c	LTB07006	6.3	c	LTB07010	7.1	d
	AB13689	5.6	c	AB06039	5.8	c	AB11554	6.1	c	AB12660	6.3	c	AB09003	7.1	d
	AB13719	5.6	c	AB09025	5.8	c	CNA 10756	6.1	c	AB09009	6.4	c	CNA 10758	7.2	d
	CNA0002258	5.6	c	AB12614	5.9	c	BRA 040286	6.1	c	AB12623	6.4	c	CNA0002416	7.2	d
	CNA0010503	5.6	c	BRA 01024	5.9	c	AB 12101	6.1	c	BRA 040127	6.4	c	IRGA 417	7.2	d
	AB10501	5.6	c	AB06078	5.9	c	AB13704	6.1	c	AB11003	6.5	c	CNA0002480	7.3	d
	AB10594	5.7	c	AB09001	5.9	c	AB12625	6.2	c	LTB07014	6.6	d	CNA0003005	7.3	d
	AB10579	5.7	c	AB13687	5.9	c	AB08053	6.2	c	AB08058	6.7	d	AB11006	7.3	d
	AB10580	5.7	c	AB11514	5.9	c	AB10003	6.2	c	AB08063	6.7	d	LTB07001	7.3	d
	AB13707	5.7	c	BRA 040079	5.9	c	LTB07011	6.2	c	LTB07013	6.7	d	CNA 10757	7.3	d
	BRS 358	5.7	c	AB06088	5.9	c	BRA 01461	6.2	c	AB12677	6.7	d	CNA 10754	7.4	d
	LTB07008	5.7	c	AB07142	5.9	c	AB09006	6.3	c	AB08099	6.8	d	LTB07009	7.5	d
	AB13009	5.7	c	AB09021	5.9	c	AB13004	6.3	c	AB08101	6.8	d	LTB07017	7.5	d
	CNA0005287	5.7	c	BRA 050055	5.9	c	LTB07007	6.3	c	AB11549	6.8	d	LTB07003	7.5	d
	AB13002	5.7	c	AB06077	5.9	c	LTB07015	6.3	c	AB12676	6.9	d	LTB07012	7.5	d
	AB11565	5.7	c	AB13011	6.0	c	Tiba	6.3	c	CNA0005461	7.0	d			
	AB11575	5.7	c	AB06075	6.0	c	AB13003	6.3	c	CNA0004759	7.0	d			
	AB13692	5.7	c	AB07137	6.0	c	CNA0004243	6.3	c	AB09007	7.0	d			
	BRA 050142	5.7	c	AB14006	6.0	c	AB06081	6.3	c	CNA0004480	7.1	d			
Susceptible	LTB07004	7.6	d	BR IRGA 409	8.0	d									
	BRA 01455	7.6	d	LTB07005	8.1	d									
	AB06087	7.7	d	LTB07016	8.3	d									
Average							**5.4**								

*Means followed by the same letter do not differ by the Scott-Knott grouping test; ** Grouping according to the standard evaluation system for rice.*

Knowledge of the impact of excess iron on the physiology of rice plants is necessary to associate characteristics that may be related to this disorder. In this sense, the association of the content of dry matter of shoots and the levels of indirect toxicity of iron (Figure 2) showed a significant negative correlation (-0.6848) through the Pearson and Mantel correlation tests. Thus, as the susceptibility of genotypes to stress caused by iron increases, the content of dry matter of shoot decreases. Nonetheless, it is noteworthy that this association is very conditioned to the genetic constitution of the genotypes, as changes in the translocation parameters of photoassimilates are very evident between plants of different subspecies [26], as well as the intrinsic characteristics of each genotype such as the crop cycle and plant architecture. Onaga et al. [14] also found a negative correlation for these variables, notwithstanding, with lower magnitude, equivalent to -0.40, evaluating genotypes under field conditions and under controlled conditions in a greenhouse.

It is noteworthy that changes in management conditions, intensity and duration of the stress can lead to differences in the genotypes response patterns. Therefore, agronomic intervention strategies and a systematic approach of the adaptation mechanisms are needed in rice genotypes to solve the problem efficiently [27].

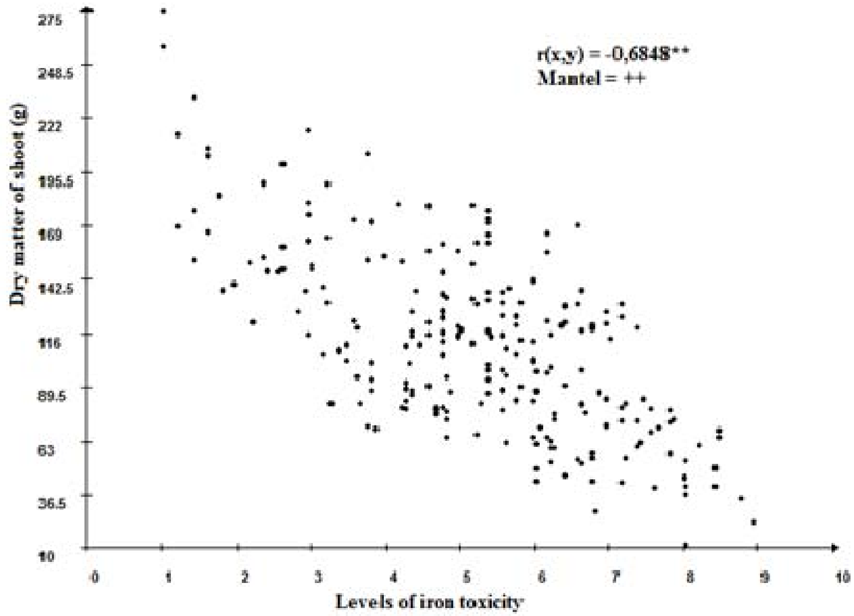

Fig. 2. Correlation of the content of dry matter of shoot and the levels of indirect iron toxicity

***: Significant at 1 % probability by the t test*

++: Significant at 1% probability by the Mantel test based on 10,000 simulations

The problems caused by iron toxicity in rice highly depend on the phenological stage and the mechanisms involved. For the culture, the most critical phenological stages are: early tillering, as the plants suffer severe growth retardation and show little germination, affecting crop establishment; flowering, which is the most sensitive stage to physiological functional disorders, as it can cause irreversible damage; and grain formation, for photosynthetic drastic reductions can interfere with the production of constituent carbohydrates of the grains.

The relationship between the toxicity caused by iron and the dry matter production of shoots in the critical phenological phases of the culture (Figure 3) showed that there was a significant negative correlation for the 40 DAE, 70 DAE and 100 DAE, i.e. all these phases contribute significantly to the reduction of the dry matter of shoots. The highest magnitude of negative correlation was at 70 DAE (-0.6161), proving that this period corresponds to the beginning of the breeding season, this phase being the most susceptible to the stress caused by excess iron in the soil solution. The lower magnitude of correlation found was for 100 DAE (-0.4564), since the grain filling stage most directly affects the productivity of crop grains.

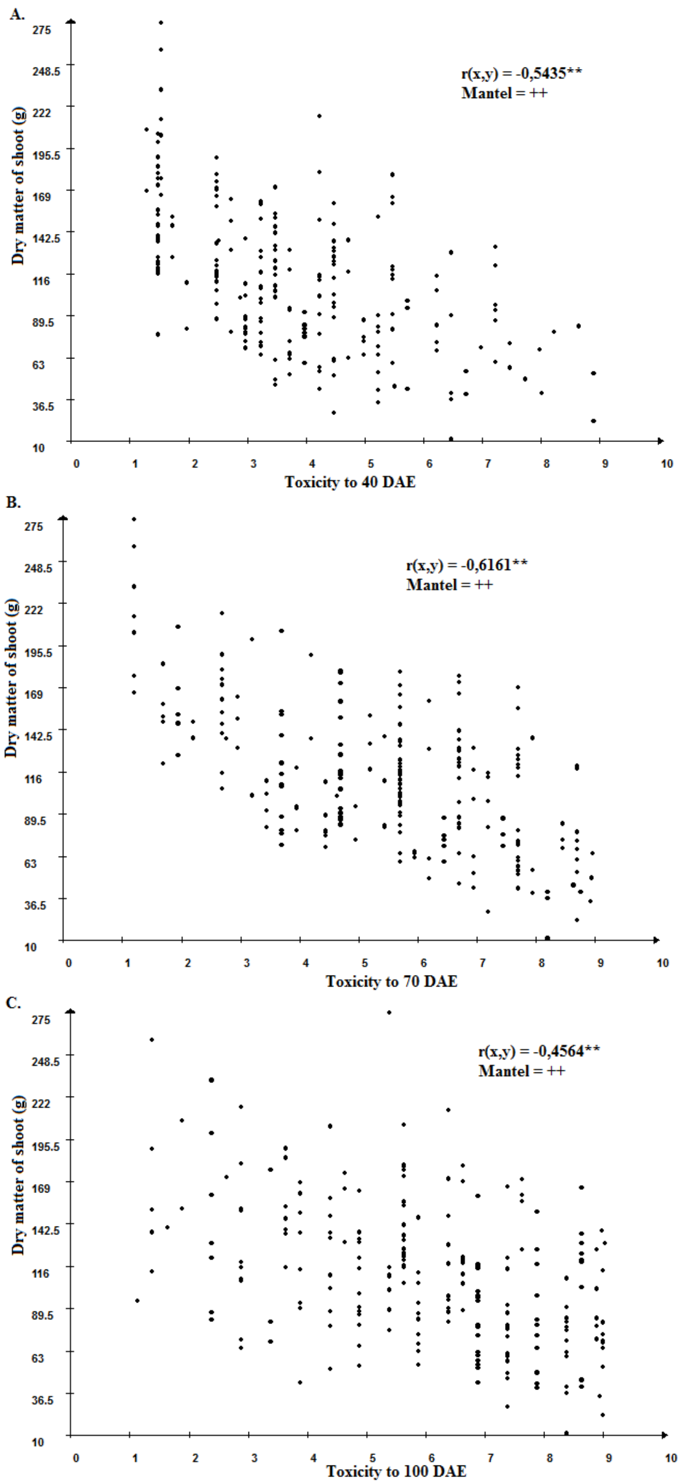

Fig. 3. Correlation between the toxicity caused by iron and the dry matter production of shoots at different critical phenological stages of the culture. (A) Correlation between the toxicity caused by iron and the dry matter production of shoots at 40 DAE; (B) Correlation between the toxicity caused by iron and the dry matter

production of shoots at 70 DAE; (C) Correlation between the toxicity caused by iron and the dry matter production of shoots at 100 DAE

***: Significant at 1 % probability by the t test*

++: Significant at 1% probability by the Mantel test based on 10,000 simulations

4. CONCLUSION

There is variability for tolerance to indirect iron toxicity between the irrigated rice genotypes assessed.

The breeding program of irrigated rice of Embrapa has been effective in developing genotypes with tolerance by excess iron in the soil over nine years.

There is a negative association between the content of dry matter of shoots and the levels of indirect iron toxicity.

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