PHOSPHOROUS ALLEVIATES OXIDATIVE DAMAGE OF RICE PLANTS UNDER IRON STRESS IN DIFFERENT IRRIGATION SYSTEMS

Júlia Gomes Farias[1], Felipe de Campos Carmona[2], Roberto Cristofari Saul[3], Leonardo Cocco Garlet[3], Gabriel Shaich[4], Victória Martini Sasso[5], Jover Alves[3], Roberta Farenzena[6], Heuri Guedes Temp[3], Janette Palma Fett[7], Fernando Teixeira Nicoloso[8]

Key words: ferrous, heavy metals, MDA, *Oryza sativa*, phosphate.

INTRODUCTION

Iron (Fe) toxicity is the major nutritional syndrome expressed in rice plants cultivated in paddy soils and can frequently result in formation of reactive aldehydes in plant tissues. Malondialdehyde (MDA) occurs as a result of oxidative degradation of polyunsaturated fatty acids and is one of the main thiobarbituric acid reactive substances (TBARS) present in plants. MDA is involved in deterioration of various biological functions through its attachment to biomolecules such as proteins and nucleic acids, which can result in agricultural production decline (CHATTERJEE et al., 2006; YAMAUCHI et al., 2008).

Fe-toxic soils share a significant amount of physical-chemical characteristics such as the content and type of the clay minerals, the amount of exchangeable Fe in the soil, the soil pH, and the presence of "stress factors" such as nutritional deficiencies (BECKER and ASCH 2005; RAJKUMAR et al., 2008). Phosphorus (P), in the form of phosphate (Pi), is an essential element for all living organisms, including plants. Interestingly, increased P availability can mitigate heavy metal stress through increases in biomass production and consequently metal dilution in tissue (MARSCHNER, 1995). Moreover, in cases of nutritional disorders resulting from nutrient excess, uptake of other nutrients can be affected, resulting in several secondary nutritional deficiencies.

A systematic approach, classification of Fe-toxicity sensitive species of rice plants, and knowledge of the required adaptation mechanisms in rice genotypes are all needed, in combination with agronomic management interventions to efficiently address the Fe toxicity problem. The aim of this study was to evaluate the effects of P levels, irrigation systems (which induce different Fe levels) and different genotypes on flag leaf TBARS levels as well as to evaluate differences between the TBARS levels in the main culm and in tillers.

MATERIALS AND METHODS

A field experiment was established at Restinga Seca, Rio Grande do Sul. The area has a history of iron toxicity in rice. There were 18 treatments consisting of three doses of P_2O_5 (0, 75 and 1200 kg ha^{-1}), three irrigation systems (I-continuous irrigation, with a water layer of 5 cm since V3-V4 stage; II-with one drainage between V6-V8 covering the whole phenological period between V6-V8 stages; and III-irrigation with a drainage covering the whole phenological period between V6-V8 stages and another between V8-V10 stages); and two rice genotypes, one susceptible and one tolerant to Fe toxicity (BR/IRGA 409 and IRGA 425 respectively). The reason to use the dose of 1200 kg $P_2O_5ha^{-1}$ was aimed for the observation of possible interactions with other treatments, as well as to evaluate differences in comparison to recommended agricultural doses (as 75 kg P_2O_5 ha^{-1}). It must be clear that

[1] Bióloga, doutoranda do programa de pós graduação em Agronomia, UFSM, R: Rodolfo Bher número 1795 CEP 97105440, fariasjuliag@hotmail.com.
[2] Eng. Agr., Dr., pesquisador do Instituto Rio Grandense do Arroz (IRGA), Divisão de pesquisa – Seção de Agronomia, Cachoeirinha – RS
[3] Graduando, Curso de Agronomia, UFSM.
[4] Eng. Agr., mestrando do programa de pós graduação em Agronomia, UFSM.
[5] Graduando, Curso de Engenharia Florestal, UFSM.
[6] Zootecnista, doutoranda do programa de pós graduação em Zootecnia, UFSM.
[7] Bióloga Ph.D., Professor Associado, Centro de Biotecnologia & Departamento de Botânica, UFRGS.
[8] Eng. Agr., Dr., Prof. Adjunto do Departamento de Biologia, CCNE,UFSM.

it is not the intention of this work to suggest exaggerative using of P in rice fields or even suggest this activity as viable. But this work aim to assess whether or not occur benefits in terms of production of rice plants under extremely high P2O5 doses. The rice was cultivated in a conventional system. The experiment was conducted in a three factorial randomized block design with three replications. Rice seeds, *indica* varieties, were obtained from IRGA **(**Instituto Rio Grandense do Arroz**)**, RS, Brazil. The potassium and nitrogen fertilizations were 200Kg ha^{-1} KCl and 330 Kg ha^{-1} $(NH_2)_2CO$ respectively. The soil P concentration in the experimental area, determined previously to the experiment, was 2.9 mg dm^3.

During the end of the vegetative stage, rice plants were harvested and separated into main culm and tillers. Flag leaves were than collected and immediately placed in liquid nitrogen, where they remained until pulverized to a fine powder using a porcelain mortar.

TBARS were quantified using the method described by El-Monshaty et al. (1995). A sample was mixed with the same volume of a 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The mixture was heated at 95 °C for 30 min and the reaction was stopped by abrupt placement in an ice-bath. The cooled mixture was centrifuged at 10,000g for 10 min, and the absorbance of the supernatant at 532nm and 600nm was measured. The data was subjected to a variance analysis using the averages for comparison by Tukey test at 5% probability of error, with the aid of the SAS program.

RESULTS

In this study, we observed a three-way interaction between the factors, irrigation systems, genotypes and phosphorus (P) levels in both tissues of the main culm and tillers tested. In general, the lipid peroxidation evaluated in terms of thiobarbituric acid reactive substances (TBARS) in flag leaves had distinct responses when quantified in flag leaves from main culm or from tillers. In tillers flag leaves, the overall TBARS concentration was significantly higher under low P levels (0 P_2O_5 Kg ha^{-1}) in comparison to the other treatments in all irrigation systems. However, for the BR/IRGA genotype, no difference occurred between 75 and 1200 Kg P_2O_5 ha^{-1} treatments in tillers tissues regardless of irrigation type. Moreover, the IRGA 425 genotype had a marked response with increasing addition of P, resulting in a significant reduction in TBARS levels from 75 to 1200 Kg P_2O_5 ha^{-1} treatment under one and two drainages (Tables 1, 2). Field symptoms of Fe toxic such as bronzing of leaves and plants undeveloped were observed in both genotypes, being however the bronzing more pronounced in the BR/IRGA 409 under treatments without addition of P_2O_5 (Data not shown). Interestingly for this genotype, the treatment with one drainage promoted a visual reduction of leaves bronzing as compared to the treatment with continuous irrigation, but this result was not confirmed in terms of TBARS concentration. Additionally this genotype had an increase in grain production with one and two drainages in relation to the treatment with continuous irrigation, and responded positively to the increase of P_2O_5 (Data not shown).

Interestingly, in TBARS terms, in flag leaves from the main culm of the BR/IRGA 409 genotype, the addition of P_2O_5 was effective in leading to lower TBARS levels in plants under continuous irrigation. Conversely, in the irrigation systems with drainages, the treatment with 1200 Kg P_2O_5 ha^{-1} had the same TBARS concentration as that observed in 0 Kg P_2O_5 ha^{-1} treatment. The IRGA 425 genotype was less responsive to P addition in terms of reduction of TBARS levels in the main culm tissue. Interestingly, when tillers were evaluated, the genotype susceptible to Fe toxicity (BR/IRGA 409) only showed a decrease in TBARS levels from 0 to 75 Kg P_2O_5 ha^{-1}, with no significant reduction from the 75 to 1200 Kg P_2O_5 ha^{-1}, regardless of the irrigation type.

On the other hand, the IRGA 425 genotype showed a continuous reduction of TBARS levels in tillers flag leaves as P_2O_5 increased. Overall, this genotype showed lower TBARS levels under low and high P conditions than BR/IRGA 409 in tillers tissues.

Table 1. Thiobarbituric acid reactive substances (TBARS) levels in the flag leaves of the main culm from two rice genotypes, BR/IRGA 409 and IRGA 425, under different irrigation systems and P levels. Restinga Seca, RS. In 2013.

		BR/IRGA 409	IRGA 425
added P	irrigation	main culm TBARS [mmol mg FW $^{-1}$]	
0 Kg P_2O_5 ha $^{-1}$	*continuous*	0.318 ± 0.012 A a (a)	0.310 ± 0.013 A a (a)
75 Kg P_2O_5 ha $^{-1}$		0.241 ± 0.017 B a (bc)	0.228 ± 0.017 B b (c)
1200 Kg P_2O_5 ha $^{-1}$		0.249 ± 0.021 B b (b)	0.310 ± 0.021 A a (a)
0 Kg P_2O_5 ha $^{-1}$	*one drainage*	0.318 ± 0.030 A a (a)	0.318 ± 0.030 A a (a)
75 Kg P_2O_5 ha $^{-1}$		0.219 ± 0.013 B b (c)	0.297 ± 0.013 A a (ab)
1200 Kg P_2O_5 ha $^{-1}$		0.275 ± 0.017 AB b (ab)	0.310 ± 0.017 A a (a)
0 Kg P_2O_5 ha $^{-1}$	*two drainages*	0.305 ± 0.017 AB a (a)	0.340 ± 0.017 A a (a)
75 Kg P_2O_5 ha $^{-1}$		0.271 ± 0.016 B a (abc)	0.310 ± 0.016 AB a (a)
1200 Kg P_2O_5 ha $^{-1}$		0.323 ± 0.016 AB a (a)	0.318 ± 0.018 AB a (a)

Means followed by capital letters indicate comparison between P doses within the same genotype and irrigation system, while lower case letters indicate comparisons between genotypes in the same P level and irrigation system. Means followed by the letters in parentheses indicate comparison of irrigation within the same genotype and dose of P. Tukey test at 5% probability of error.

It is important to point out that the tested genotypes had different phenotypic responses in relation to tillering. The IRGA 425 genotype was more effective than BR/IRGA 409 (Data not shown). In rice plants, tillers are important to ensure good grain production. They are also crucial for tolerance of heavy metals as well as maintenance of regular plant metabolism under increased reactive oxygen species, avoiding consequent cell damage. TBARS contain highly reactive aldehydes such as MDA, 2-alkenals and 2,4-alkadienals. High levels of theses substances in plant tissues are indicative of severe stress (YAMAUCHI et al., 2008).

Table 2. Thiobarbituric acid reactive substances (TBARS) levels in the flag leaves of tillers from two rice genotypes, BR/IRGA 409 and IRGA 425, under different irrigation systems and P levels. Restinga Seca, RS. In 2013.

		BR/IRGA 409	IRGA 425
added P	irrigation	tillers TBARS [mmol mg FW $^{-1}$]	
0 Kg P_2O_5 ha $^{-1}$	*continuous*	0.387 ± 0.022 A a (a)	0.310 ± 0.020 A b (ab)
75 Kg P_2O_5 ha $^{-1}$		0.297 ± 0.013 B a (a)	0.262 ± 0.013 AB a (a)
1200 Kg P_2O_5 ha $^{-1}$		0.275 ± 0.013 B a (a)	0.237 ± 0.014 B b (abc)
0 Kg P_2O_5 ha $^{-1}$	*one drainage*	0.344 ± 0.009 A a (ab)	0.323 ± 0.009 A a (ab)
75 Kg P_2O_5 ha $^{-1}$		0.254 ± 0.017 B a (a)	0.284 ± 0.016 AB a (a)
1200 Kg P_2O_5 ha $^{-1}$		0.266 ± 0.007 B a (ab)	0.215 ± 0.017 C b (bc)
0 Kg P_2O_5 ha $^{-1}$	*two drainages*	0.374 ± 0.030 A a (ab)	0.331 ± 0.028 A b (a)
75 Kg P_2O_5 ha $^{-1}$		0.275 ± 0.026 B a (a)	0.258 ± 0.026 B b (a)
1200 Kg P_2O_5 ha $^{-1}$		0.279 ± 0.017 B a (a)	0.202 ± 0.015 C b (c)

Means followed by capital letters indicate comparison between P doses within the same genotype and irrigation system, while lower case letters indicate comparisons between genotypes in the same P level and irrigation system. Means followed by the letters in parentheses indicate comparison of irrigation within the same genotype and dose of P. Tukey test at 5% probability of error.

In previous works, our group described different patterns of response regarding the uptake and translocation of arsenic (As) and sterility of spikelets in different rice genotypes from the main culm to tillers (personal data). This response may be a survival strategy under high stress. Moreover, the capacity to produce tillers in this condition may dilute toxic substances and guarantee tolerance. In the IRGA 425 genotype, the difference between TBARS values from 0 to 1200 Kg P_2O_5 ha^{-1} treatment increased with the number of drainages, which suggests a mutual response to P fertilization and Fe oxidation, resulting in alleviation of Fe intoxication and lower oxidative damage when compared to BR/IRGA 409.

In flooded soils, the concentration of soluble Fe can reach up to about 600 mg L^{-1} (PONNAMPERUMA et al. 1978). Under aerobic conditions, the concentration of soluble Fe declines because part of the available Fe is oxidized with oxygen contact, which is further promoted by drainages. With this view, drainages could alleviate Fe toxicity to rice plants. For this specific study, two important aspects had to be considered in relation to recovery or reduction of TBARS levels with drainages. One was the speed/degree of damage already generated up to the moment of drainage. If the damage exceeded the capacity of the plant, it was not possible for the plant metabolism to recover in a short time. Another important question was that even with the soluble Fe decline, there can be a stress condition of P deficiency with the treatment of 0 kg P_2O_5 ha^{-1}. In this view, plants without an efficient amount of P acquisition and use will still have a limiting growth condition.

CONCLUSIONS

There is a wide variation in the levels of TBARS in response to P levels and irrigation systems between rice genotypes. The effect observed in flag leaf tissues of the main culm and tillers exhibited different behavior. Moreover, the differences in TBARS levels under low and high P conditions suggest distinct P use efficiency between the genotypes tested, with evidence of higher efficiency in the IRGA 425 genotype.

REFERENCES

BECKER, M.; ASCH, F. Iron toxicity in rice conditions and management concepts. **Journal of Plant Nutrition and Soil Science**, v. 168, p. 558–578, 2005.

CHATTERJEE C.; GOPAL R.; DUBE B.K. Impact of iron stress on biomass, yield, metabolism and quality of potato (*Solanum tuberosum L.).* **Scientia Horticulturae,** v.108, p. 1-6, 2006.

EL–MOSHATY, F.I.B.; PIKE, S.M.; NOVACKY, A.J. et al. Lipid peroxidation and superoxide production in cowpea (Vigna unguiculata) leaves infected with tobacco ringspot virus or southern bean mosaic virus. **Physiological and Molecular Plant Pathology**, v.43, p.109-119, 1993.

MARSCHNER, H. Mineral nutrition of higher plants. London, **Academic Press**. 889p. 1995.

PONNAMPERUMA, F.N. Chemical kinetics of wetland rice soils relative to soil fertlity. In: INTERNATIONAL RICE RESEARCH INSTITUTE**. Wetland Soils:** Characterization, Classification, and Utilization**.** Los Baños, 1978. p.71-90.

RAJKUMAR, M.; FREITAS, H. Influence of metal resistant-plant growth-promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. **Chemosphere**, v. 71, p. 834–842, 2008.

YAMAUCHI, T.; KEOUGH, R. A.; GONDA, T. J.; ISHII, S. Ribosomal stress induces processing of Mybbp1a and its translocation from the nucleolus to the nucleoplasm. **Genes Cells**, v.13, p. 27-39, 2008.