ACID PHOSPHATASE ACTIVITY IN FLAG LEAVES UNDER FE STRESS, DIFFERENT P LEVELS AND IRRIGATION SYSTEMS

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

INTRODUCTION

Iron (Fe) is an essential element for the optimum growth and development of plants, and is an essential cofactor in redox reactions involved in various biological processes. With high Fe levels in the tissue, free iron (in particular either Fe^{3+} or Fe^{2+}) produces reactive oxygen species (CHATTERJEE et al., 2006). Under flooding conditions, Fe toxicity has attributed to production losses and is considered the major nutritional disorder in waterlogged and lowland rice. Strategies such as tolerant cultivars and irrigation management have been developed to overcome iron toxicity, (ETHAN et al., 2012; ONAGA et al., 2013). Despite this, scarce data is available concerning the relationship between different rice cultivars, fertilization and the environment.

Phosphorus (P) is essential for plant development and provides a structural element for nucleic acids and the cell membrane phospholipid bilayer. In addition, increased P availability may mitigate heavy metal stress through increases in biomass production and consequently, dilutes the amount of metal in the tissues (MARSCHNER, 1995).

Although the total P content of soil is generally high, P availability is frequently a limiting factor for plant growth and productivity. This paradox arises because the concentration of available Pi in the soil solution averages about 1 μM and seldom exceeds 10 μM (BIELESKI, 1973). Plants have development mechanisms that allow them to grow and prosper in the presence of such low levels of available Pi. These responses include induction of high-affinity and low-affinity Pi transporters, morphological changes in root structures and secretion of phosphatases and organic acids (AI et al., 2009).

Acid phosphatases (E.C. 3.1.3.2) are a group of enzymes that catalyze the hydrolysis of a variety of phosphate esters in acidic environments (PARK and VAN ETTEN, 1986). These enzymes are widely distributed in plants and are related to phosphate supply and metabolism from a vast array of phosphate esters. Acid phosphatases also have been well characterized in plant tissues as an important tool in P remobilization (PENHEITER et al., 1997). The aim of this study was to evaluate the effects of P levels, irrigation systems (which induce different Fe levels) and the activity of different genotypes of flag leaf acid phosphatase 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

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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 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 P_2O_5 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 into a fine powder using a porcelain mortar. The total activities of acid phosphatases were determined according to Tabaldi et al. (2007). 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 system, genotypes and phosphorus (P) levels in both tissues of the main culm and tillers tested. The activity of acid phosphatases (APase) in flag leaves of the main culm generally increased with increasing P levels in both genotypes (Table 1). Conversely, the APase activity in tillers had a different pattern of response in the IRGA 425 genotype (Table 2) in which the treatment without added P promoted similar or higher activity than treatments with added P, regardless of the irrigation system used. Moreover, with continuous irrigation, a higher APase activity was found in this genotype in the treatment without added P, as compared to the other treatments.

In rice plants, tillers are important to ensure good grain production by promoting a higher interceptation of solar light, increasing the number of spikelets and also as protection from toxic elements by diluting these elements in plant tissues as a result of larger biomass production, as seen with cases of heavy metal intoxication. With this view, rice plants could show two strategies related to tillering: I) use of tillers biomass as an escape for compartmentalization and/or accumulation of toxic substances that occur at high levels in the environment, in order to ensure proper development and seed production by the main culm; II) promote the accumulation of toxins in the main culm, thus ensuring lower translocation to the tillers. These hypotheses are supported by previous works that describe different patterns of responses regarding the uptake and translocation of arsenic (As) and the sterility of spikelets in different rice genotypes (Personal data).

It is also possible to suggest different response patterns to P fertilization, regarding the APases activity between the main culm and tillers of both genotypes tested. The genotype BR/IRGA 409 is known for its high sensitivity to iron toxicity (STEIN et al., 2009). In this genotype, independent of the organ tested (flag leaf of the main culm or tillers), a lower APase activity occurred under low P conditions than in treatments with added P. Additionally, in main culm, there was a constant increase in the APase activity with increasing P addition in soil. Moreover, in main culm only in the treatment with two drainages, the APases activity did not vary between treatments without added P_2O_5 and with addition of 1200 kg P_2O_5 ha^{-1} (Tables 1, 2).

Table 1. Acid phosphatases activity 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 Apase activity [nmol Pi g FW^{-1} min^{-1}]	
0 Kg P_2O_5 ha $^{-1}$	*continue*	6.76 ± 0.80 C a (a)	6.26 ± 0.80 C a (a)
75 Kg P_2O_5 ha $^{-1}$		12.39 ± 2.10 B a (bc)	9.17 ± 2.10 BC b (c)
1200 Kg P_2O_5 ha $^{-1}$		23.15 ± 4.20 A a (a)	13.15 ± 1.70 A b (ab)
0 Kg P_2O_5 ha $^{-1}$	*one drainage*	6.24 ± 1.00 D a (a)	7.03 ± 1.10 CD a (a)
75 Kg P_2O_5 ha $^{-1}$		11.59 ± 1.70 BC a (bc)	15.69 ± 0.80 AB ab (ab)
1200 Kg P_2O_5 ha $^{-1}$		19.65 ± 1.40 A a (ab)	13.00 ± 1.40 B b (ab)
0 Kg P_2O_5 ha $^{-1}$	*two drainages*	6.87 ± 0.70 B a (a)	6.49 ± 0.90 B a (a)
75 Kg P_2O_5 ha $^{-1}$		16.18 ± 1.17 A a (ab)	19.02 ± 1.60 A a (a)
1200 Kg P_2O_5 ha $^{-1}$		8.12 ± 0.40 B b (c)	15.51 ± 0.40 A a (a)

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 for comparison of irrigation within the genotype and the dose of P. Tukey test at 5% probability of error.

On the other hand, the genotype IRGA 425 only showed exponential increase in APase activity in tissues of the main culm with increasing P_2O_5 levels when associated with continuous irrigation. In situations of one or two drainages, no significant difference between the treatments of 75 to 1200 kg P_2O_5 ha $^{-1}$ was observed in the main culm tissue. Moreover, in tillers, a higher activity of APases under low P conditions occurred as compared to treatments with P addition under continuous irrigation (Tables 1, 2).

Table 2. Acid phosphatases activity 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	main culm Apase activity [nmol Pi g FW^{-1} min^{-1}]	
0 Kg P_2O_5 ha $^{-1}$	*continue*	6.76 ± 0.80 C a (a)	6.26 ± 0.80 C a (a)
75 Kg P_2O_5 ha $^{-1}$		12.39 ± 2.10 B a (bc)	9.17 ± 2.10 BC b (c)
1200 Kg P_2O_5 ha $^{-1}$		23.15 ± 4.20 A a (a)	13.15 ± 1.70 A b (ab)
0 Kg P_2O_5 ha $^{-1}$	*one drainage*	6.24 ± 1.00 D a (a)	7.03 ± 1.10 CD a (a)
75 Kg P_2O_5 ha $^{-1}$		11.59 ± 1.70 BC a (bc)	15.69 ± 0.80 AB ab (ab)
1200 Kg P_2O_5 ha $^{-1}$		19.65 ± 1.40 A a (ab)	13.00 ± 1.40 B b (ab)
0 Kg P_2O_5 ha $^{-1}$	*two drainages*	6.87 ± 0.70 B a (a)	6.49 ± 0.90 B a (a)
75 Kg P_2O_5 ha $^{-1}$		16.18 ± 1.17 A a (ab)	19.02 ± 1.60 A a (a)
1200 Kg P_2O_5 ha $^{-1}$		8.12 ± 0.40 B b (c)	15.51 ± 0.40 A 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 for comparison of irrigation within the genotype and the dose of P. Tukey test at 5% probability of error.

In acidic soils, anoxia and pH reduction as a result of flooding may lead to solubilization of large amounts of iron chelate that under aerobic conditions are precipitated and fixed to organic matter present in soil (PONNAMPERUMA, 1972). For this specific study, two important aspects had to be considered in relation to APases activity responses. One was the effect of P deficiency, which as cited in literature, included symptoms as severe stunting; small stem diameter; reduced or no tillering and delayed plant development; or lack of vigorous growth after N fertilization and flooding. Another aspect was the effect of drainage on Fe toxicity alleviation, and the impact of this phenomenon in different genotypes.

More severe symptoms of Fe toxicity were observed in the BR/IRGS 409 genotype as compared to the IRGA 425 genotype (Data not shown). A marked deficiency of P was also observed in both genotypes, which resulted in stunted plants and could explain the decreased activity of APases in the treatment without added P in comparison to the others (Tables 1, 2), and was probably due to the combination of low metabolism and Fe toxicity. Alteration in the activity of APases in plants has been observed under toxic conditions due to various heavy metals (SHAH and DUBEY 1998; SHARMA and DUBEY 2005). For example, Zinc is a more potent inhibitor of acid phosphatases in cucumber than mercury (TABALDI et al. 2007).

CONCLUSIONS

There is a wide variation in tissue APase activity in response to P levels and irrigation systems between rice genotypes. The effect observed in acid phosphatases analyzed in flag leaf tissues of the main culm and tillers exhibited different behavior, which suggests variability of the effects on the nutritional quality of rice plants. Moreover, tissues of tillers from both genotypes seem to be more efficient as compared to the main culm tissues in terms of acid phosphatases activity, IRGA 425 being the genotype with higher activity under P deficient conditions.

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