PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Morphological and Biochemical Alterations of Paddy Rice in Response to Stress Caused by Herbicides and Total Plant Submersion

Alterações Morfológicas e Bioquímicas do Arroz Irrigado em Resposta ao Estresse Causado por Herbicidas e Submersão Total

MARCHEZAN, M.G.[1]
AVILA, L.A.[1]*
AGOSTINETTO, D.[1]
SCHAEDLER, C.E.[2]
LANGARO, A.C.[1]
OLIVEIRA, C.[1]
ZIMMER, M.[1]
SCHREIBER, F.[1]

*** Corresponding autor:** <laavilabr@gmail.com>

Received: August 27, 2015
Approved: June 20, 2016

Planta Daninha 2017; v35:e017139830

ABSTRACT - Herbicide selectivity in paddy rice varies in several aspects, among which are the environmental conditions. The aim of the study was to evaluate the effect of herbicide application and total plant submersion on morphological and biochemical changes in paddy rice. Total chlorophyll and carotenoids, catalase activity, ascorbate peroxidase and superoxide dismutase, total phenolic content, lipid peroxidation and hydrogen peroxide levels were assessed. Leaf samples were collected 24 hours and 7 days after the application of water regimes. The results observed in the first experiment show that cultivars Puitá INTA CL, IRGA 417 and IRGA 422 CL are more tolerant to total submersion. The most sensitive cultivars are IRGA 424, BRS Querência, EPAGRI 108 and BRS Taim. In general, cultivar Puitá INTA CL had lower oxidative damage than BRS Querência when under submersion. To eliminate excess free radicals, BRS Querência had increased activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) than Puitá INTA CL under submersion. Formulations with imazethapyr + imazapic and imazapyr + imazapic caused greater reduction in the total chlorophyll and carotenoid contents at 7 days after the establisment of water regimes (DAT). Therefore, the data show that exposing cultivars to total submersion and herbicides increased oxidative stress as well as induced changes in the activities of antioxidant enzymes.

Keywords: Oxidative stress, hypoxia, *Oryza sativa*, herbicide selectivity.

RESUMO - A seletividade de herbicidas em arroz irrigado varia em diversos aspectos, entre os quais estão as condições ambientais. O objetivo deste estudo foi avaliar a tolerância e as alterações morfológicas e bioquímicas do arroz irrigado quando submetidos à aplicação de herbicidas e a períodos de submersão. Foram avaliados teores de clorofila total e carotenoides, atividade da catalase, ascorbato peroxidase e superóxido dismutase, conteúdo fenólico total, peroxidação lipídica e teores de peróxido de hidrogênio. Amostras foliares foram coletadas 24 horas e sete dias após a aplicação dos regimes hídricos. Os resultados demonstraram que os cultivares Puitá INTA CL, IRGA 417 e IRGA 422 CL são mais tolerantes à submersão. Os cultivares mais sensíveis foram IRGA 424, BRS Querência, EPAGRI 108 e BRS Taim. Em geral, o cultivar Puitá INTA CL apresentou menores danos oxidativos que o cultivar BRS Querência quando submetidos à submersão. Para eliminar o excesso de radicais livres, o cultivar BRS Querência mostrou maior atividade das enzimas superóxido dismutase (SOD), catalase (CAT) e ascorbato peroxidase (APX) quando comparado ao cultivar Puitá INTA CL no regime hídrico com submersão. As misturas formuladas dos herbicidas imazethapyr + imazapic e imazapyr + imazapic causaram maior redução no teor de clorofila total e de

[1] Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brasil; [2] Universidade Federal do Pampa (Unipampa), Itaqui-RS, Brasil.

Doi: 10.1590/S0100-83582017350100010

carotenoides aos sete dias após o estabelecimento dos regimes hídricos (DAT). Portanto, os dados evidenciam que a exposição dos cultivares à submersão e aos herbicidas induzem maior estresse oxidativo, bem como alterações nas atividades das enzimas antioxidantes.

Palavras-chave: estresse oxidativo, hipóxia, *Oryza sativa*, seletividade de herbicida.

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important cereals produced worldwide. It is grown mainly in paddy fields (Kim et al., 2014), by establishing a continuous flood 30 days after seedling emergence and maintained throughout the growing season. The flood depth should range between 7.5 and 10 cm (IRGA, 2015).

The crop yield may vary depending on several conditions, which can be biotic or abiotic. Among them the complete submersion of the plants, which is observed especially in *El Niño* years (the warm phase of the El Niño Southern Oscillation, commonly called ENSO) and in areas near rivers and streams, is of utmost relevance. Higher flood depths (>10 cm) increase water consumption, reduce the number of tillers, elongate stems, increase the ethylene synthesis and stimulate the production of aerenchyma (Bressan et al., 2004), making rice plants higher and hence prone to lodging (IRGA, 2015). Furthermore, plants of both crops and weeds, submitted to stress by submersion may have greater sensitivity to some herbicides, which can cause physiological and biochemical changes and metabolic stress (Rouchaud et al., 2000), consequently may lead the plants to death or reduced yield potential.

Plants exposed to adverse conditions or undergoing significant changes in optimal conditions for development experience changes in all functional metabolism levels, which are often reversible, but in some cases become permanent (Larcher, 2000). Among the main changes, there is the increased peroxidation of membranes, which affects the structure and functionality of the photosynthetic apparatus, inactivating the reaction centers of the photosystems (Tripathy et al., 2007). Thus, crop selectivity to the herbicide, mainly in adverse conditions, is essential to the success of chemical control in post-emergence, without damage to crops.

One way of evaluating the selectivity of herbicides in plants is by analysing biochemical parameters in their tissues. These altered parameters may indicate oxidative stress, which is characterized by increased production of reactive oxygen species (ROS), alteration of the antioxidant system, or even an imbalance among them (Drew, 1997). The degree of oxidative stress in a cell is determined by the amount of superoxide, hydrogen peroxide and hydroxyl radicals. To reduce the damage caused due to oxidative stress, plants have defense systems that can be enzymatic or not, which allow the elimination of ROS and protection from oxidative damage.

The enzymatic antioxidant system includes several enzymes in different cellular compartments. Among the major enzymes are superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), which together promote the elimination of reactive oxygen species. In the non-enzymatic system, which consists of a low molecular weight mediator, the highlights are ascorbate, reduced glutathione, carotenoids and phenolic compounds, which are synthesized by plants in response to stress (Michalak, 2006). This mechanism protects the integrity of the membranes against the effects of ROS, enabling better performance of certain species in adverse environmental conditions (Noctor and Foyer, 1998).

Knowing how plants defend themselves and behave in response to various adverse conditions and stressful situations is the first step to develop more resistant crop varieties, thereby increasing the quality and consequently plants yield. There are several substances involved in the induction of plant defense response against these negative effects that deserve to be studied and better understood.

In view of the above, this study was carried out with the objective of evaluate the effect of herbicide application and total plant submersion on morphological and biochemical changes in paddy rice.

MATERIALS AND METHODS

Experiment 1 – Paddy rice cultivars tolerance to submersion

The first experiment was conducted from October to November 2012 in a greenhouse and consisted of an exploratory study to evaluate the tolerance of paddy rice cultivars to periods of submersion. The experiment was arranged in a completely randomized design in a 10 x 6 factorial with four replications. Factor A consisted of ten paddy rice cultivars (BR IRGA 409, BR IRGA 410, IRGA 417, IRGA 422 CL, IRGA 424, Puitá INTA CL, BRS Querência, BRS Taim, BRS Atalanta and EPAGRI 108) and Factor B consisted of five periods of submersion (1, 5, 7, 15 and 20 days of submersion), and the control without submersion conducted with a regular 10 cm-looding.

The experimental units consisted of 800 mL-plastic pots filled with homogenate, sieved and fertilized soil, according to the recommendation for the crop (Sosbai, 2012). The soil used in the experiment is classified as Albaquaf, which had no herbicide application history in the last five years and, was collected from the top soil layer (10 cm).

The seeds were previously treated with fungicide carboxin + thiram (250 mL 100 kg^{-1} of seeds) and with insecticide fipronil (150 mL 100 kg^{-1} of seeds). Sowing was carried out on 10/5/2012 and then daily irrigations were performed in order to keep the soil at field capacity (10 kPa) until the water regimes were imposed. Ten days after seedling emergence, thinning was performed manually, setting the population level equivalent to 300 plants m^{-2}.

To simulate submersion the pots were placed in 73 L plastic-trays measuring 60 x 35 x 35 cm of length, width and depth, respectively. These trays were filled with water to the maximum level in the complete submersion treatments and a flood depth of 10 cm was kept in the treatments simulating optimal conditions for crop development. At the end of submersion periods the water excess was removed, keeping a 10 cm water layer until plants sampling, at 20 days after the start of the submersion (DAS).

It was measured the shoot dry matter of rice plant (SDM). For this, rice plants were cut near the soil at 20 DAS and placed for drying in a forced air circulation oven at 60 °C until constant weight was obtained.

The data were previously analyzed to meet the assumptions of analyses of variance (normality and homoscedasticity) and subsequently submitted to ANOVA ($P \leq 0.05$). When statistical significance was observed regression analysis was thusperformed, and comparisons among GR_{50}-values for periods of submersion and cultivars studied were done by confidence intervals at 95% of probability of error estimated for the parameters. Regression analysis was performed by adjusting the data to the logistic-type sigmoidal regression equation, according to equation 1:

$$y = \frac{a}{\left[1 + \left(\frac{x}{x_0}\right)^b\right]}$$

where: y = reduction of SDM; x = period of submersion; and a, x_0 and b = parameters of the equation, where a is the difference between the curve maximal and minimal points, x_0, is the period of submersion that provides 50% of response of the variable and b is the slope.

GR_{50} is the period of submersion that provides a reduction of 50% of the SDM of rice cultivars. In this analysis, the SDM was corrected for percentage values by comparing the mass obtained in increasing periods of submersion with the control mass (without submersion) considered 0%.

Experiment 2 – Oxidative stress in paddy rice cultivars due to submersion and herbicides

The experiment was conducted in a completely randomized design with a factorial arrangement (2x2x6) and three replications. Factor A consisted of two paddy rice cultivars, Puitá INTA CL and BRS Querência, tolerant and sensitive to flooding, respectively, selected based on the results obtained in Experiment 1. Factor B consisted of two water regimes (10 cm flood and total submersion) and Factor C consisted of herbicides treatments: clomazone (500 g a.i. ha^{-1}), bispyribac sodium (50 g a.i. ha^{-1}), penoxsulam (60 g a.i. ha^{-1}), imazethapyr + imazapic (75 + 25 g a.i. ha^{-1}) and imazapyr + imazapic (73.5 + 24.5 g a.i. ha^{-1}), and a control treatment without herbicides. Herbicides of imidazolinone group were applied in cultivar BRS Querência to observe their effect on plants susceptible to herbicides used in the Clearfield system.

The rice cultivars were sown at 12/5/2012. The experimental units, the soil used, the seed treatments, the basic fertilization, thinning and daily irrigations were arranged and carried similarly to Experiment 1.

Herbicides were applied in post-emergence (one day before submersion and the establishment of water regimes) at V_3-V_4 for rice (Counce et al., 2000), except for clomazone, which was applied in pre-emergence. The herbicide applications were made using a CO_2-pressurized backpack sprayer using TeeJet XR 110015 flat fan nozzles at 140 kPa pressure and spray volume of 150 L ha^{-1}.

Submersion was imposed on the same plastic trays used for Experiment 1, filled with water to the maximum level and with a water layer of 10 cm depth in treatments simulating submersion and the optimum conditions for crop development, respectively. The water treatments were applied when rice plants had three to four fully expanded leaves (V_3-V_4) (Counce et al., 2000), along with the nitrogen topdressing fertilization carried out in the form of urea, using the equivalent of 90 kg ha^{-1} of N.

Leaf samples were collected 24 HAT and at seven DAT establishing the water regimes for all herbicides, i.e., at 20 and 27 days after clomazone application, and at 48 hours and eight days after the other herbicides applications.

To evaluate the effect of herbicides and water regimes on biochemical parameters, collection of the last three leaves of rice was performed separately. The sampling consisted of all plants of the experimental unit, which were stored at -80 °C until the time of variables measuring: content of hydrogen peroxide, lipid peroxidation, activity of antioxidant enzymes, and total chlorophyll, carotenoids and phenolic compounds contents.

Hydrogen peroxide (H_2O_2) and lipid peroxidation contents

Cellular damage in tissues was determined using the hydrogen peroxide content (H_2O_2), as described by Loreto and Velikova (2001), and cellular damage in the species reacting to thiobarbituric acid (TBARS) via malondialdehyde accumulation (MDA), as described by Heath and Packer (1968). To carry out these analyses, 0.2 g of leaves were macerated with liquid nitrogen, homogenized in 2 mL of trichloroacetic acid (TCA) 0.1% (m/v) and centrifuged at 14,000 rpm for 20 minutes. To quantify H_2O_2, aliquots of 0.2 mL of the supernatant were added in 0.8 mL of phosphate buffer 10 mM (pH 7.0) and 1 mL of potassium iodide 1M. The solution was allowed to stand for 10 minutes at room temperature and the absorbance was read at 390 nm. The concentration of H_2O_2 was determined by standard curve and expressed in mM g^{-1}.

To determine TBARS, aliquots of 0.5 mL of the supernatant, as described previously, were added to 1.5 mL of thiobarbituric acid (TBA) 0.5% (m/v) and trichloroacetic acid 10% (m/v) and incubated at 90 °C for 20 minutes. Then the reaction was stopped with an ice bath for 10 minutes. The absorbance was read at 532 nm, discounting the unspecific absorbance at 600 nm. The MDA concentration was calculated using the absorptivity coefficient of 155 mM cm^{-1} and the results were expressed in nM MDA g^{-1} of FM.

Total phenolic compounds

The total phenolic compounds were determined according to the method described by Rossi Jr and Singleton (1965), with modifications. The total carotenoid and total chlorophyll contents were calculated according to equations proposed by Lichtenthaler (1987), from the absorbance of the solution obtained by spectrophotometry at 647, 663 and 470 nm, and the results were expressed in mg g^{-1} of FM.

To determine the activity of antioxidant enzymes, superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11) and catalases (CAT; EC 1.11.1.6), first one proceeded to extraction, in which 0.2 g of sample was macerated in a porcelain mortar in the presence of liquid nitrogen and 0.02 g of polyvinylpyrrolidone (PVPP). Then, 900 µL of phosphate buffer 200 mM (pH 7.8), 18 µL of EDTA (ethylenediaminetetraacetic acid) 10 mM, 180 µL of ascorbic acid 200 mM and 702 µL of ultrapure water and centrifuged at 14,000 rpm were added at 4 ^{o}C for 20 minutes.

Catalase (CAT; EC 1.11.1.6) activity was determined by consumption of H_2O_2 with a molar extinction coefficient of 39.4 mM cm^{-1} (Havir andMchale, 1987). The ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined according to Azevedo et al. (1998), with modifications, by the consumption of H_2O_2 (extinction coefficient 2.9 mM cm^{-1}), whereas the superoxide dismutase (SOD; EC 1.15.1.1) activity was determined according to a methodology adapted from Peixoto et al. (1999) by the calculation of the amount of extract that inhibited 50% of the reaction of NBT and expressed in UA mg^{-1} protein $minute^{-1}$.

The data were analyzed for normality by the Shapiro-Wilk test, and homoscedasticity by the Hartley's test and subsequently submitted to an analysis of variance ($p \leq 0.05$). After the analysis of variance, the t test was used to compare the means in case of a significant difference among cultivars and water regimes or Duncan's test ($p \leq 0.05$) for comparison among the herbicide treatments.

RESULTS AND DISCUSSION

Experiment 1 – Paddy rice cultivars tolerance due to submersion

The periods of submersion necessary to promote a 50% reduction (GR_{50}) of shoot dry weight were 5.45, 5.72, 4.31, 6.11, and 12.2 days for cultivars IRGA 424, EPAGRI 108, BRS Querência, BR-IRGA 410 and BRS Atalanta, respectively (Table 1). For the other cultivars, the values of GR_{50} were higher than 20 days of submersion.

The two groups of cultivars response to flooding (tolerants and susceptibles) is probably due to their similar genetic background. Cultivar BR-IRGA 409, together with New Rex and IR 19743-25-2-2, has originated cultivar IRGA 417 which in turn has originated cultivars IRGA 422 CL and Puitá INTA CL. In Brazil, the rice cultivar resistant to imidazolinone herbicides, IRGA 422 CL, was developed by backcrossing using strain 93AS3510 as a donor source of the gene that gives tolerance to the herbicide. As for Argentina, the mutation that gave resistance to cultivar Puitá INTA CL was Ala_{122} for Thr_{122} ($A_{122}T$) (Livore, 2003). Both Puitá INTA CL and IRGA 422 CL were selected by their resistance to imidazolinones in populations generated by inducing mutation, using IRGA 417 as a recurring cultivar (Lopes et al., 2002).

Table 1 - Log-logistic-equations fitted for SDM(1) of rice cultivars at 35 DAE(2), their values of coefficient of determination (R^2), GR_{50} (days of submersion which provide 50% of the reduction of SDM) and confidence intervals (CI) in response to different periods of submersion (1, 5, 7, 15 and 20 days of submersion)

Cultivar	Parameters estimated			GR_{50}(3)	
	a	b	R^2	Days	CI(4)
IRGA 424	70.9	-6.93	0.98	5.4	5.0-5.8
EPAGRI 108	62.5	-6.43	0.95	5.7	5.1-6.4
BRS Querência	62.9	-3.26	0.99	4.3	3.7-5.0
BR-IRGA 410	57.5	-5.94	0.97	6.1	5.5-6.7
BRS Atalanta	51.00	-3.23	0.99	12.2	11.8-12.6
IRGA 417	34.71	-2.30	0.93	>20	-----
IRGA 422 CL	49.70	-1.24	0.98	>20	-----
BR-IRGA 409	47.39	-1.59	0.99	>20	-----
BRS Taim	66.75	-1.10	0.86	>20	-----
Puitá INTA CL	46.68	-0.792	0.98	>20	-----

(1) SDM = shoot dry matter. (2) DAE = days after emergence. (3) GR_{50} = period of submersion necessary to obtain 50% of reduction of shoot dry matter. (4) CI = confidence interval of the average at the level of 95% of probability of error. Equation used = f = a/(1+exp(-(x-x0)/b)).

The cultivars BR-IRGA 410, IRGA 424, BRS Querência and EPAGRI 108 presented major SDM reductions (Table 1). BRS Querência has also originated from cultivar BR-IRGA 409. However, it receives genetic material from several other cultivars and strains such as Bellemont and New Rex. Also cultivar Te-Tep and the intersection between strain CL 246 and cultivar Zho Fee N 10 were used. These genetic materials may have given BRS Querência a distinct behavior regarding submersion compared to the other cultivars derived from BR-IRGA 409. BRS Atalanta and IRGA 424 have originated from the same cultivar, BR-IRGA 410, which explains the similarity of the behavior of these cultivars in response to submersion. Thus, the cultivars genetics may be the key factor in the submersion process due to similarities in gene expression patterns.

According to Nagai et al. (2010), rice plants have two survival strategies during prolonged submersion periods adaptation and escape from stress. Thus, flood depth is critical to define the survival of submerged plants, demonstrating that cultivars having the feature of etiolate may protrude from those without this potential, due to rapid stretching of the internodes and petioles, allowing top leaves to remain above the water surface. This fact is a result of higher ethylene concentrations, which ultimately affect the concentration of gibberellic acid, increasing up to four times its normal concentration (Hoffmann-Benning and Kende, 1992).

Another survival strategy of rice plants is related to stress adaptation to submersion, resulted from a low metabolism of carbohydrates. Cultivars tolerant to submersion have the ability to store energy during the stress period by reduction of plant growth, resuming it after reoxygenation, consuming the energy saved during submersion (Nagai et al., 2010).

Thus, according to the results, two rice cultivars were selected, one being Puitá INTA CL, considered as the most tolerant to submersion, and BRS Querência, the most sensitive to such condition. The periods of submersion were selected (one and seven days) to be used in the following experiment. The choice of the periods was made by representativity in order to compare non-stressed plants with those submitted to non-letal submersion stress.

Experiment 2 – Oxidative stress in paddy rice cultivars due to submersion and herbicides

For the levels of TBARS and of H_2O_2 there was no interaction between water regimes and herbicides at 24 HAT. Significance was observed for variable H_2O_2 only between the two cultivars assessed. Cultivar Puitá INTA CL presented the lower level of H_2O_2 than BRS Querência, with values of 0.87 and 1.14 mM g^{-1}, respectively.

It was observed that the lipid peroxidation in rice cultivars showed no differences to submersion. It is possible that the oxidative stress caused by increased levels of H_2O_2 in the short period of submersion of 24 hours had not been enough to cause degradation of cell membranes, or also due to the antioxidant system efficiency of plants, both "nonenzymatic" and "enzymatic," which can prevent cell damage.

At seven days after establishing the water regimes there was no interaction among cultivars, water regimes and herbicides. However, there was an interaction among cultivars and water regimes for levels of H_2O_2 and TBARS (Table 2). The levels of H_2O_2 were higher in leaves of cultivar BRS Querência than in Puitá INTA CL. The levels of H_2O_2 increased in both cultivars when compared to plants that were not submitted to submersion. However, the cultivar Puitá INTA CL did not show increased levels of TBARS, which suggests that there was no damage to the membranes in this cultivar, even with high levels of H_2O_2 in submerged plants. In BRS Querência higher levels of TBARS were observed in response to submersion than those found for Puitá INTA CL. Moreover, for Puitá INTA CL there was no difference regarding the water regime for the variable TBARS.

In general, increased levels of H_2O_2 and of TBARS were found in BRS Querência under submersion. This indicates that in these situations a state of stress is induced, which is related to the damage of membranes, which would explain increases in the activities of antioxidant enzymes.

Table 2 - Levels of hydrogen peroxide (H_2O_2) and of TBARS at 7 DAT[1] in leaves of two rice cultivars in response to the water regimes established in stage V4

Cultivar	Level of H_2O_2 (mM g^{-1} of FM)		Level of TBARS (nmol MDA g^{-1} of FM)	
	Without submersion	Submersion	Without submersion	Submersion
Puitá INTA CL	0.45 B[2]a	0.87 Ab	25.55 Aa	30.04 Ab
BRS Querência	0.52 Ba	1.33 Aa	27.92 Ba	38.82 Aa

[1] Days after establishing the water regimes. [2] Uppercase letters compare water regimes while lowercase letters compare cultivars, both by t test ($p \leq 0.05$).

H_2O_2, as well as other reactive oxygen species (ROS), such as hydroxyl radical (OH^-), singlet oxygen (O_2*) and superoxide (O_2^-), are strong oxidants that can cause cellular oxidative stress, because the ROS affect many cellular functions, damaging the nucleic acids, oxidizing proteins and lipids and causing peroxidation (Gill and Tuteja, 2010). However, the accumulation of H_2O_2 in specific tissues and in appropriate amounts can also be beneficial to plants, by mediating acclimatization and cross-tolerance to biotic and abiotic stress (Bowler and Fluhr, 2000). Adding H_2O_2 to leaf tissues or their endogenous production acts as a signal for inducing the expression of genes related to antioxidant enzymes. Nevertheless, in high concentrations this can cause lipid peroxidation adversely affecting the plant metabolism.

There was an interaction among the water regimes and the herbicides for the variable H_2O_2 at 7 DAT (Table 3). In the treatment with submersion, all herbicides were different from the control, which presented lower levels of H_2O_2. The treatment with herbicide clomazone presented higher levels of H_2O_2 in the leaves, distinct from herbicide penoxsulam and the imazapyr + imazapic formulation.

In a study conducted by Ahsan et al. (2008), increased levels of TBARS were observed when the rice leaves were submitted to exposure to herbicides glyphosate and paraquat for 12 hours. As for Zabalza et al. (2007), when assessing pea leaves, they have observed a slight increase in lipid peroxidation when exposed to imazethapyr. However, these studies suggest that the effects observed may not be related to the mode of action of ALS inhibiting herbicides (acetolactate synthase enzyme). In the present study, comparing the two water systems, it was possible to observe differences among all herbicide treatments, even among the controls, without herbicide. Thus, the submersion of rice plants may have contributed to potentiate the effects of ALS inhibiting herbicides, increasing the levels of H_2O_2. This fact is an indication that the plant may have oxidative stress when under submersion, thus producing ROS, which can initiate oxidative damage and consequently lead to disturbances in the metabolic functions and losses in cellular integrity (Karuppanapandian et al., 2011).

Oxidative stress occurs when the production of ROS is accelerated or when the mechanisms involved in the protection against the ROS are in poor condition (Giasson et al., 2002). To try to minimize this situation, the cells and their organelles (chloroplasts, mitochondria and peroxisomes) have developed an antioxidant system consisting of enzymatic and nonenzymatic components. Among the antioxidant enzymes, SOD, found in almost all cellular compartments, is responsible for catalyzing the dismutation of superoxide radicals (O_2^-) in hydrogen peroxide (H_2O_2) and oxygen (O_2), preventing the formation of hydroxyl radical ($OH^·$), which is a more reactive and toxic radical than the others (Gill and Tuteja, 2010). Thus, to better understanding of the stress mechanism on different plant species regarding to the levels of ROS and the activity of antioxidant enzymes in plant tissues is needed.

At 24 hours after establishing the water regimes there was no interaction among cultivars, water regimes and herbicides. However, there was an interaction among cultivars and water regimes in relation to the activity of SOD enzyme (Table 4). BRS Querência presented increased activity of SOD enzyme in relation to Puitá INTA CL for both water regimes. Besides, increased activity of this enzyme under complete submertion was observed for BRS Querência compared to the treatment with 10 cm flood. However, Puitá INTA CL did not show any difference among the water regimes. The fact that BRS Querência presents an increased activity of enzyme SOD may be associated to the increase in levels of H_2O_2, which demonstrates that the SOD activity probably stimulated the production of reactive oxygen species (ROS) – in this case, represented

Table 3 - Levels of hydrogen peroxide (H_2O_2) at 7 DAT[(1)] in leaves of paddy rice plants submitted to different herbicides application in two water regimes established in stage V4

Herbicide	Level of H_2O_2 (mM g^{-1} of FM)		
	Dose (g a.i. ha^{-1})	Without submersion[(2)]	Submersion
Control	----------	0.30 B[(3)]a	0.80 Ac
Clomazone[(4)]	500	0.44 Ba	1.69 Aa
Bispyribac-sodium	50	0.38 Ba	1.51 Aab
Penoxsulam	60	0.50 Ba	1.29 Ab
Imazethapyr + imazapic	75 + 25	0.49 Ba	1.36 Aab
Imazapyr + imazapic	73.5 + 24.5	0.54 Ba	1.28 Ab

[(1)] Days after establishing the water regimes. [(2)] The values are averages among the cultivars. [(3)] Uppercase letters compare water regimes by t test (p≤0.05), while lowercase letters compare herbicides by Duncan's test (p≤0.05). [(4)] Seeds were treated with the safener diethولate.

Table 4 **-** Activity of the superoxide dismutase (SOD) enzyme at 24 HAT in leaves of two paddy rice cultivars in response to the water regimes established in stage V4

Cultivar	SOD (UA mg^{-1} prot. min^{-1}) 24 HAT[1]	
	Without submersion[2]	Submersion
Puitá Inta CL	16.50 A[3]b	16.97 Ab
BRS Querência	18.42 Ba	21.48 Aa

[1] Hours after establishing the water regimes. [2] The values are averages among the herbicides. [3] Uppercase letters compare the water regimes for each cultivar while lowercase letters compare the paddy rice cultivars for each water regime, both by t test ($p \leq 0.05$).

by H_2O_2. However, the increased activity of SOD was not enough to reduce the lipid peroxidation (Table 2).

SOD has been extensively investigated, mainly by presenting an activity induced under oxidative stress conditions, thereby metabolizing the superoxide radicals produced (Cataneo et al., 2005). In *Arabidopsis thaliana*, the increased content of H_2O_2 was observed, as well as all antioxidant enzymes (SOD, CAT, APX) tested and exposed to stress by cadmium (Cho and Seo, 2005). These data suggest that H_2O_2 produced in a particular cell site can coordinate the activity of antioxidant enzymes in different subcellular compartments.

Regarding the activity of enzyme SOD at 24 HAT, an interaction among the cultivars and the herbicide treatments was also observed (Table 5). In BRS Querência, the activity of this enzyme was different among the herbicide treatments. For the herbicide treatment with the mixture of imazapyr + imazapic there was an increased SOD activity, different from the control. Furthermore, penoxsulam caused a decreased activity of the enzyme in BRS Querência

As for Puitá INTA CL, no effect of the SOD activity on most of the herbicide treatments was observed. However, there was a decreased activity of this enzyme in the treatment with the formulation of herbicides imazapyr + imazapic, different from the control. Comparing both cultivars, only for imazapyr + imazapic a difference was observed, which caused an increased activity of this enzyme in BRS Querência leaves. This fact may be explained by the cultivar sensitivity to the herbicide, once this formulation is not recommended for cultivars that do not belong to the Clearfield system. Probably this behavior has occurred due to the increased amount of superoxide radicals (O_2^-) and the attempt to promote the dismutation of this radical to hydrogen peroxide (Sinha and Saxena, 2006).

At 7 DAT, a difference was observed in the activity of SOD enzyme among the cultivars. BRS Querência showed an increased activity of SOD (26.08 UA mg^{-1} prot. min^{-1}) in relation to Puitá INTA CL (20.72 UA mg^{-1} prot. min^{-1}). In this assessment was also noticed a significant difference among the water regimes. Under submersion SOD activity has shown to be increased in relation to the regime with flood conditions, with the following values: 30.32 and 17.75 UA mg^{-1} prot. min^{-1}, respectively.

The activity of APX enzyme at 24 HAT has not shown interaction among the treatments, however presented differences among herbicides and cultivars.At 24 HAT the activity of APX was increased in BRS Querência than Puitá INTA CL: 7.25 and 5.53 UA mg^{-1} prot. min^{-1}, respectively. In the case of the herbicides, the control differed from the formulations imazethapyr + imazapic and imazapyr + imazapic, which presented increased activities of this enzyme (Table 6).

Table 5 **-** Activity of the superoxide dismutase (SOD) enzyme at 24 HAT in leaves of two paddy rice cultivars submitted to the application of herbicides in stage V4

Herbicide	SOD (UA mg^{-1} prot. min^{-1}) 24 HAT[1]		
	Dose (g a.i. ha^{-1})	Puitá INTA CL	BRS Querência
Control	----------	17.81 A[2]a	20.82 Ab
Clomazone[3]	500	17.24 Aab	19.76 A bc
Bispyribac-sodium	50	17.74 Aa	18.14 A bc
Penoxsulam	60	16.69 Aab	16.70 A c
Imazethapyr + imazapic	75 + 25	17.67 Aa	21.23 Aab
Imazapyr + imazapic	73.5 + 24.5	14.18 Bb	23.29 Aa

[1] Hours after establishing the water regimes. [2] Uppercase letters compare cultivars by t test ($p \leq 0.05$) and lowercase letters compare herbicides by Duncan's test ($p \leq 0.05$). [3] Seeds treated with the safener dietholate.

At 7 DAT, the activity of APX presented the same response than at 24 HAT, where BRS Querência showed increased activity of APX, 32.30 UA mg^{-1} prot. min^{-1}, while for Puitá INTA CL it was 23.07 UA mg^{-1} prot. min^{-1}. Among the water regimes established, the activity of APX presented lower value for the water regime with flood conditions (19.35 UA mg^{-1} prot. min^{-1}) than for submersion (35.51 UA mg^{-1} prot. min^{-1}). This enzyme has an important role in eliminating H_2O_2, but APX

Table 6 **-** Activity of the ascorbate peroxidase (APX) enzyme at 24 HAT in leaves of paddy rice cultivars

Herbicide	Dose (g a.i. ha^{-1})	APX (UA mg^{-1} prot. min^{-1}) 24 HAT(1)
Control	----------	6.0 ab(2)
Clomazone(3)	500	5.8 b
Bispyribac-sodium	50	6.4 ab
Penoxsulam	60	5.6 b
Imazethapyr + imazapic	75 + 25	7.8 a
Imazapyr + imazapic	73.5 + 24.5	6.7 a

(1) Hours after establishing the water regimes. (2) Lowercase letters compare herbicides by Duncan's test ($p \leq 0.05$). (3) Seeds treated with the safener dietholate crops.

could not reduce the levels of H_2O_2 for BRS Querência. At 7 DAT, differences among herbicides were not observed.

The increased expression of APX in plants has been demonstrated during different stress situations (Gill and Tuteja, 2010). As with CAT, APX converts H_2O_2 into water and O_2 using ascorbate as an electron donor, but it presents different affinities for ROS: APX demonstrates affinity for H_2O_2 on the micromolar order and CAT of millimolar. Therefore, APX seems to be responsible by the fine regulation of the answer to the ROS (Mittler, 2002). However, it has high importance in the protection against oxidative damage in subcellular compartments where the CAT is not present.

There was a difference for the activity of CAT enzyme at 24 HAT only among cultivars of paddy rice. BRS Querência showed an increased activity of CAT in relation to Puitá INTA CL: 0.77 and 0.63 UA mg^{-1} prot. min^{-1}, respectively. At 7 DAT there was an interaction among the cultivars and the water regimes for the activity of CAT (Table 7). BRS Querência showed increased activity of CAT in relation to Puitá INTA CL under submersion. However, there was no difference among the cultivars under flood conditions. Besides, an increased activity of this enzyme was observed, comparing the water regime, in the condition of submersion for BRS Querência in relation to the flood treatment, while for Puitá INTA CL there was no difference among the water regimes.

The increased activity of CAT aims to minimize the first signs of stress caused by the submersion. This enzyme is responsible for removing the excess ROS during stress (Gratão et al., 2005). Besides, it has the role of protecting the inactivation of SOD by high levels of H_2O_2 (Fridovich, 1995).

Table 7 **-** Activity of catalase (CAT) enzyme at 7 DAT in leaves of two rice cultivars in response to the water regimes established in stage V4

Cultivar	CAT (UA mg^{-1} prot. min^{-1}) at 7 DAT(1)	
	Without submersion	Submersion
Puitá Inta CL	0.88 A(2)a	1.07 Ab
BRS Querência	0.96 Ba	1.57 Aa

(1) Days after establishing the water regimes. (2) Uppercase letters compare water regimes while lowercase letters compare cultivars by t test ($p \leq 0.05$).

For total chlorophyll at 24 HAT, an interaction among the water regimes and herbicides was observed (Table 8). In the regime without submersion, herbicides penoxsulam and the formulation of herbicides imazethapyr + imazapic had the major reductions of total chlorophyll contents, while herbicides clomazone, bispyribac sodium and imazapyr + imazapic did not differ from the control. In the regime with submersion there were no differences among the plants treated with herbicides and the control without application. Comparing the water regimes, it was possible to observe that herbicides bispyribac sodium and penoxsulam did not show differences among themselves. As for the others, the regime with submersion shows reduction in the chlorophyll content.

Table 8 **-** Concentration of total chlorophyll at 24 HAT in leaves of paddy rice plants submitted to the application of herbicides in two water regimes established in stage V4

Herbicide	Dose (g a.i. ha^{-1})	Total chlorophyll (mg g^{-1} FM) 24 HAT(1)	
		Without submersion	Submersion
Control	----------	1.84 A(2)a	1.34 Bab
Clomazone(3)	500	1.87 Aa	1.45 Ba
Bispyribac-sodium	50	1.50 Aab	1.41 Ab
Penoxsulam	60	1.41 Ab	1.31 Aab
Imazethapyr + imazapic	75 + 25	1.40 Ab	1.19 Bb
Imazapyr + imazapic	73.5 + 24.5	1.56 Aab	1.27 Bb

(1) Hours after establishing the water regimes. (2) Uppercase letters compare water regimes by t test ($p \leq 0.05$), while lowercase letters compare herbicides by Duncan's test ($p \leq 0.05$). (3) Seeds treated with the safener dietholate.

Taking into account the cultivars, Puitá INTA CL presented higher content of total chlorophyll in relation to BRS Querência at 24 HAT: 1.79 and 1.28 mg g^{-1} FM, respectively. At 7 DAT, cultivar Puitá INTA CL kept a higher content of total chlorophyll in relation to cultivar BRS Querência: 1.23 and 0.93 mg g^{-1} FM, respectively. The water regime with submersion provided plants with lower chlorophyll contents (1.36 mg g^{-1} FM), contrasting with plants conducted under flood conditions (1.69 mg g^{-1} FM).

At 7 DAT, there was a statistical significance for the treatments with herbicides regarding the concentration of total chlorophyll and carotenoids (Table 9). For both variables, the major reductions in concentration were for the treatments with imazethapyr + imazapic and imazapyr + imazapic. Although clomazone inhibits the synthesis of carotenoids, reduction in contents of total chlorophyll and carotenoids was not observed for this treatment. Probably this fact is justified due to the treatment of rice seeds with dietholate, which protects rice plants from the herbicide action (Sanchotene, 2009), and also due to the long period between the herbicide application (pre-emergence) and the material collection for the biochemical analysis (27 days), which provides enough time for plant recovering.

Photosynthesis is one of the primary processes that are affected by the imposition of stressful situations (Chaves et al., 2009). The electron transfer process between PSII and PSI results in the production of ROS and is part of the normal plant metabolism (Müller et al., 2001). The ROS production source probably increased in the submerged plants due to the restriction of gas exchanges (lower carbon sequestration) and the accumulation of reducing power.

An important role of carotenoids is the action as photoprotection therefore, preventing photooxidative damage (Cogdell, 1988). Carotenoids are able to prevent the reactive action of singlet oxygen produced by chlorophyll (Cogdell, 1988). In a study conducted by Piesanti et al. (2012), chlorophyll content and photosynthetic rate showed no change due to the use of penoxsulam, bispyribac-sodium and cyhalofop-butyl on cultivar BRS Querência. Inhibition of chlorophyll synthesis can compromise photosynthesis, resulting in the reduction of the plant's ability to take advantage of environmental resources such as water.

The increase in chlorophyll concentration in plants submitted to some herbicide treatments possibly result from chloroplast development (increase in the number of thylakoids) or an increase in the chloroplast number, suggesting the activation of a mechanism of protection to the photosynthetic apparatus (García et al., 2005). The differences in pigment content probably occurred because even biotypes belonging to the same species (*Oryza sativa*) may exhibit morphological differences, such as plant height and leaf width. These differences probably arise from the origin of the cultivars with for example different environmental conditions.

Regarding the total phenolic content, there was a difference among cultivars at 24 HAT. Puitá INTA CL showed lower phenolic content than BRS Querência: 33.2 and 40.5 mg GAE g^{-1} FM, respectively. At 7 DAT, a difference among the water regimes was observed: in the regime with submersion the phenolic content was 14.41 mg GAE g^{-1} FM, lower than that for the treatment under flood, of 31.3 mg GAE g^{-1} FM. Also at 7 DAT there was an interaction among the herbicides and the cultivars (Table 10).

Puitá INTA CL presented a higher concentration of phenols in response to

Table 9 - Concentration of total chlorophyll and carotenoids (mg g^{-1} FM) at 7 DAT in leaves of paddy rice plants submitted to the application of herbicides in two water regimes established in stage V4

Herbicide	Dose (g a.i. ha^{-1})	Total chlorophyll	Carotenoids
Control	----------	1.29 a[(1)]	0.28 ab
Clomazone[(2)]	500	1.30 A	0.33 a
Bispyribac-sodium	50	1.22 Ab	0.30 ab
Penoxsulam	60	1.28 A	0.31 ab
Imazethapyr + imazapic	75 + 25	1.10 B	0.24 b
Imazapyr + imazapic	73.5 + 24.5	0.85 B	0.17 c

(1) Lowercase letters compare herbicides by Duncan's test ($p \leq 0.05$). (2) Seeds treated with the safener dietholate.

Table 10 - Total phenolic content at 7 DAT[(1)] in leaves of two rice cultivars in response to the herbicides applied

Herbicide	Dose (g a.i. ha^{-1})	Total phenolic content (mg GAE g^{-1} of FM)	
		Puitá INTA CL	BRS Querência
Control	----------	21.4 A[(2)]b	21.7 Aa
Clomazone[(3)]	500	26.9 Aa	15.9 Bb
Bispyribac-sodium	50	23.9 Aab	22.6 Aa
Penoxsulam	60	27.1 Aa	22.4 Ba
Imazethapyr + imazapic	75 + 25	25.9 Aab	18.7 Bb
Imazapyr + imazapic	73.5 + 24.5	22.5 Aab	17.8 Ab

(1) Days after establishing the water regimes. (2) Uppercase letters compare cultivars by t test ($p \leq 0.05$), while lowercase letters compare herbicides by Duncan's test ($p \leq 0.05$). (3) Seeds treated with the safener dietholate crops.

herbicide treatments penoxsulam and clomazone, differing from the control. As for cultivar BRS Querência, a reduction was observed in the treatments with penoxsulam and imazethapyr + imazapic and imazapyr + imazapic.

Phenols are a product of the plants secondary metabolism and serve as defense mechanisms. These results suggest that the cultivars have different responses in relation to the production of phenols when submitted to different herbicides as well as the water regimes interfering with the production of this compound. Puitá Inta CL has activated this defense mechanism when exposed to herbicides penoxsulam and clomazone, but for these herbicides there was no increase in enzyme activity or oxidative damage. Harir and Mitller (2009) have attributed the lower content of phenols to the increase in the peroxidase activity (POD), since this antioxidant uses mainly phenolic compounds as electron donors to reduce the hydrogen peroxide.

Plants have important non-enzymatic antioxidant defenses, such as for example ascorbic acid, reduced thiol groups, α-tocopherol, phenolic compounds, among others (Michalak, 2006). Phenolic compounds are among the most common and varied groups of secondary compounds. Many studies have shown that phenolic compounds present a great antioxidant potential because they have the ability to donate electrons or hydrogen, chelate metals and capture singlet oxygen (Rice-Evans et al., 1996).

Submersion increases the enzymatic activity in BRS Querência, which may be closely associated with higher levels of ROS, such as H_2O_2 found in both periods analyzed, which consequently provides higher levels of TBARS in this cultivar. However, Puitá INTA CL in general did not present significant variations of the enzyme activity when submerged, which may be explained by the low levels of H_2O_2, which does not cause relevant increase in the levels of TBARS.

Under stress conditions by submersion, damage caused depends on a number of factors. The stage of plant development, the submersion time, temperature, water turbidity and rice variety directly interfere in the plant recoverability ability (Vergara et al., 1976). These variables also require a better understanding, especially regarding to the secondary metabolism under stress by submersion.

Based on the foregoing, it can be inferred that the herbicide treatments induce different responses among cultivars, stimulating the secondary metabolism of rice plants by synthesis of antioxidant, enzymatic and nonenzymatic molecules. Submersion situations soon after herbicide application may impair the plant absorption due to greater herbicide dilution herbicide in water, with consequent outward movement of the leaf area, thus making it more selective.

Therefore, cultivars Puitá INTA CL, IRGA 417, BR-IRGA 409, BRS Taim and IRGA 422 CL show tolerance to submersion. Cultivar BRS Atalanta shows an intermediate behavior. And the most sensitive cultivars are IRGA 424, BRS Querência, EPAGRI 108 and BR-IRGA 410.

The cultivar tolerant to submersion, Puitá INTA CL, has a lower oxidative stress than the sensitive cultivar, i.e., BRS Querência. The activity of antioxidant enzymes is stimulated in BRS Querência when this is submerged, but not enough to prevent lipid peroxidation.

Submersion provides increased levels of H_2O_2 in rice leaves and the herbicides further increase these levels. The treatment with the herbicides imazethapyr + imazapic and imazapyr + imazapic cause major reductions in total chlorophyll and carotenoid content in rice plants.

ACKNOWLEDGEMENT

We thank CNPq (Edital MCT/CNPq N. 014/2010 – UNIVERSAL, N.Proc. 479167/2010-9) and FAPERGS (Edital PRONEM/FAPERGS n. 003/2011, N. Proc. 11/2207-0) for the financing support. CAPES and CNPq are also acknowledge for providing the students scholarships.

REFERENCES

Ahsan N. et al. Glyphosate-induced oxidative stress in rice leaves revealed by proteomic approach. **Plant Physiol Biochem.** 2008;46:1062-70.

Azevedo R.A. et al. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. **Physiol Plant.** 1998;104:280-92.

Bowler C., Fluhr, R. The role of calcium and activated oxygens as signals for controlling cross-tolerance. **Trends Plant Sci.** 2000;5**:**241-6.

Bressan R.A. et al. Fisiologia do estresse. In: Taiz L., Zeiger E., editors. **Fisiologia vegetal**. 3ª.ed. Porto Alegre: Artmed, 2004. p.613-43.

Cataneo A.C. et al. Atividade de superóxido dismutase em plantas de soja (*Glycine max* L.) cultivadas sob estresse oxidativo causado por herbicida. **R Bras Herbic.** 2005;4:23-31.

Chaves M.M., Flexas J., Pinheiro C. Photosynthesis and salt stress: Regulation mechanisms from whole plant to cell. **Ann Bot.** 2009;103:551-60.

Cho U.H., Seo N.H. Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation. **Plant Sci.** 2005;168:113-20.

Cogdell R. The functions of pigments in chloroplasts. In: Goodwin T.W., editor. **Plant pigments**. London: Academic Press, 1988. p.183-230.

Counce P. et al. A uniform, objective, and the adaptive system for expressing rice development. **Crop Sci.** 2000;40:436-43.

Drew M.C. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. **Ann Rev Plant Physiol.** 1997;48:223-50.

Fridovich I. Superoxide radical and superoxide dismutase. **Ann Rev Biochem.** 1995;64:97-112.

García X.V. et al. Chlorophyll accumulation is enhanced by osmotic stress in graminaceous chlorophyllic cells. **J Plant Physiol.** 2005;162:650-61.

Giasson B.I. et al. The relationship between oxidative/nitrative stress and pathological Alzheimer's and Parkinson's diseases. **Free Rad Biol Med.** 2002;32:1264-75.

Gill S.S., Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiol Biochem.** 2010;48:909-30.

Gratão P.L. et al. Making the life of heavy metal-stressed plants a little easier. **Func Plant Biol.** 2005;32:481-94.

Harir Y., Mittler R. The ROS signaling network of cells. In: Del Rio L.A., Puppo A., editor. **Reactive oxygen species in plants signaling.** Berlin: Sringer Verlag, 2009. p.165-74.

Havir E.A., Mchale N.A. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. **Plant Physiol.** 1987;84:450-5.

Heath R.L., Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and Stoichiometry of fatty acid peroxidation. **Arch Biochem Biophys.** 1968;125:189-98.

Hoffmann-Benning S., Kende H. On the role of abscisic acid and gibberellin in the regulation of growth in rice. **Plant Physiol.** 1992;99:1156-61.

Instituto Riograndense do Arroz – IRGA. (Rio Grande do Sul State Rice Research Institute). [Online]. (2019). Available: http://www.irga.rs.gov.br/index.php?action=dados_safra_detalhesandcod_dica=173 [19 Sept. 2015].

Karuppanapandian T. et al. Reactive oxygen species in plant: their generation, signal transduction, and scavenging mechanisms. **Austr J Crop Sci.** 2011;5:709-25.

Kim G.Y., Gutierrez J., Jeong H.C., Lee J.S., Haque M.M., Kim P.J. Effect of intermittent drainage on methane and nitrous oxide emissions under different fertilization in a temperate paddy soil during rice cultivation. **J Korean Soc Appl Biol Chem**. 2014;57:229-36.

Larcher W. **Ecofisiologia vegetal**. São Carlos: RiMa, 2000. 531p.

Lichtenthaler H.K. Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. In: Colowick S.P., Kaplan N.O. **Methods in enzymology.** New York: Academic Press, 1987. p.350-82.

Livore A.B. **Rice plants having increased tolerance to imidazolinone herbicides.** International Application Published Under the Patent Cooperation Treaty (PCT), n.WO2005/020673A1. 29 ago. de 2003, 10 fev. de 2014.

Loreto F., Velikova V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. **Plant Physiol.** 2001;127:1781-7.

Lopes M.C.B. et al. **IRGA 422 CL a cultivar desenvolvida para o sistema de produção clearûeld arroz (2002).** [acesso em: 28 mar. 2014] Disponível em: http://www.irga.rs.gov.br/arquivos/20071107163254.pdf.

Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. **Polish J Environ Stud.** 2006;15:523-30.

Mittler R. Oxidative stress, antioxidants and stress tolerance. **Trends Plant Sci.** 2002;7:405-10.

Müller P., Li X.P., Niyogi K.K. Non-photochemical quenching. A response to excess light energy. **Plant Physiol.** 2001;125:1558-66.

Nagai K., Hattori Y., Ashikari M. Stunt or elongate? Two opposite strategies by which rice adapts to floods. **J Plant Res.** 2010;123:303-9.

Noctor G., Foyer C.H. Ascorbate and glutathione: keeping active oxygen under control. **Ann Rev Plant Physiol Plant Mol Biol.** 1998;49:249-79.

Peixoto P.H.P. et al. Aluminum effects on lipid peroxidation and on activities of enzymes of oxidative metabolism in sorghum. **Rev Bras Fisiol Veg.** 1999;11:137-43.

Piesanti S.R. et al. Fisiologia de plantas de arroz submetidas à aplicação de herbicidas. In: Anais do 28º. Congresso Brasileiro da Ciência das Plantas Daninhas. Campo Grande: 2012. p.133-7.

Rice-Evans C., Miller N., Paganda G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. **Emerg Med Australas.** 1996;23:532-40.

Rossi Jr J.A., Singleton V.L. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **Am J Enol Vitic.** 1965;16:144-58.

Rouchaud J. et al. Soil dissipation of diuron, chlorotoluron, simazine, propyzamide, and diflufenican herbicides after repeated applications in fruit tree orchards. **Arch Environ Contam Toxicol.** 2000;39:60-5.

Sanchotene D.M. **Tolerância do arroz irrigado ao herbicida clomazone pela ação de protetores de plântulas** [dissertação] Santa Maria: Universidade Federal de Santa Maria, 2009.

Sinha S., Saxena R. Effect of iron on lipid peroxidation, and enzymatic and nonenzymatic antioxidants and bacoside-A content in medicinal plant *Bacopa monnieri* L. **Chemosphere.** 2006;62:1340-50.

Sociedade Sul Brasileira de Arroz Irrigado – Sosbai. Arroz irrigado: recomendações técnicas da pesquisa para o sul do Brasil. In: Anais da 28ª Reunião Técnica da Cultura do Arroz Irrigado. Gravatal: 2012.

Tripathy B.C., Mohapatra A., Gupta I. Impairment of the photosynthetic apparatus by oxidase stress induced by photosensitization reaction of protoporphyrin IX. **Biochim Biophys Acta**. 2007;1767:860-8.

Vergara B.S., Jackson B., Datta S.K. Deep water and its response to deep water stress. In: International Rice Research Institute. **Climate and rice**. Los Baños: 1976. p.301-16.

Zabalza A. et al. Oxidative stress is not related to the mode of action of herbicides that inhibit acetolactate synthase. **Environ Exp Bot.** 2007;59:150-9.