Oxidative stress caused by the use of preemergent herbicides in rice crops[1]

Estresse oxidativo causado pelo uso de herbicidas pré-emergentes na cultura do arroz

Ana Claudia Langaro[2], Dirceu Agostinetto[2]*, Queli Ruchel[2], Jessica Rodrigues Garcia[2] and Lais Tessari Perboni[2]

ABSTRACT - Among the methods of weed control, stands out chemical control. However, even selective, herbicides can trigger the production of reactive species of oxygen and cause oxidative stress. The aim of the study was to evaluate changes in photosynthetic parameters, oxidative damage, antioxidant enzyme activity and altered metabolism of rice plants after applying pre-emergent herbicides. The experiment was conducted in a greenhouse and herbicides used were oxadiazon, pendimethalin and oxyfluorfen, beyond the control without herbicide. There was a reduction of photosynthetic rate and efficiency of carboxylation, compared to the control, when applied herbicides oxyfluorfen and pendimethalin. The major lipid peroxidation and proline accumulation was observed for the herbicide oxyfluorfen. The oxyfluorfen and oxadiazon herbicides also resulted in increased activity of superoxide dismutase, compared to control. When evaluated ascorbate peroxidase activity, there was a higher enzyme activity in plants treated with oxadiazon and pendimethalin. Even selective herbicides registered for weed control in rice crops cause phytotoxicity, reduce height and alter the metabolism of plants, generating reactive oxygen species, which activate enzymatic and non-enzymatic defense systems and result in the degradation of photosynthetic pigments and in reduced protein content.

Key words: Antioxidant system. Chemical control. Reactive oxygen species. Selectivity.

RESUMO - Dentre os métodos de controle de plantas daninhas, destaca-se o controle químico. No entanto, mesmo seletivos a cultura, os herbicidas podem desencadear a produção de espécies reativas de oxigênio e causar estresse oxidativo. O objetivo do estudo foi avaliar alterações nos parâmetros fotossintéticos, danos oxidativos, atividade das enzimas antioxidantes e alterações do metabolismo de plantas de arroz após a aplicação de herbicidas pré-emergentes. O experimento foi conduzido em casa de vegetação e os herbicidas utilizados foram: oxadiazon, oxyfluorfen e pendimethalin, além do controle sem herbicida. Observou-se redução da taxa fotossintética e na eficiência da carboxilação, em relação à testemunha, quando aplicado os herbicidas oxyfluorfen e pendimethalin. A maior peroxidação lipídica e acúmulo de prolina foi observado para o herbicida oxyfluorfen. Os herbicidas oxyfluorfen e oxadiazon também resultaram em maior atividade da superóxido dismutase, comparado à testemunha. Quando avaliada a atividade da ascobato peroxidase, verificou-se maior atividade da enzima em plantas tratadas com oxadiazon e pendimethalin. Mesmo seletivos e registrados para controle de plantas daninhas na cultura do arroz, os herbicidas causam fitotoxicidade, reduzem a estatura e alteram o metabolismo das plantas, gerando espécies reativas de oxigênio, as quais ativam o sistema de defesa enzimático e não enzimático e resultam em degradação dos pigmentos fotossintéticos e redução no teor de proteínas.

Palavras-chave: Sistema antioxidante. Controle químico. Espécies reativas de oxigênio. Seletividade.

INTRODUCTION

The interference of weeds may cause a reduction of 80-90% in grain yield of irrigated rice (FLECK *et al*., 2008). In order to avoid losses arising from competition with weeds, several phytosanitary managements are used. The chemical control within the integrated management program is the main weed control tool for rice fields. Even if a particular active ingredient (a.i.) is culture-selective and does not cause a severe injury to the plants, biochemical and physiological changes may occur (SONG *et al*., 2007). Especially if they are not used according to the recommended dosages.

The injuries are usually evaluated through of visual scores on a scale of zero to 100, where zero is the absence of symptoms and 100 is the plant death. However, the criterion used by each evaluator may generate different results. Thus, assessments in relation to the plant metabolism can assist in verifying the selectivity of herbicides.

The negative effect of stress is often mediated by the oxidative damage initiated by reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (GILL; TUTEJA, 2010). The formation of reactive oxygen species has been described as being a result of several abiotic stresses, including the application of herbicides (SONG *et al*., 2007). The elimination of reactive oxygen species is necessary for cells to survive in an oxygen-rich environment, especially under stress conditions.

The most important ROS detoxification mechanism is the activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) enzymes. In general, the antioxidant response varies according to the mode of action of the applied herbicide and according to the crop. Thus, the role of antioxidant enzymes in stress situations is to control the accumulation of ROS, thus limiting oxidative damage (SHARMA *et al*., 2012).

The metabolism of proline is also involved in regulating intracellular redox potential and storage, transferring energy and reducing power (GIBERTI; FUNCK; FORLANI, 2014). The biosynthesis of proline occurs in the cytoplasm of plant cells, but it is possible that the production moves to chloroplasts under stress conditions, thus being able to detoxify the hydroxyl radical (SIGNORELLI *et al*., 2014). According to its chemical properties, proline may be involved in stress caused by metals, by mechanisms of osmotic and redox regulation, complexation of metals and detoxification of ROS (SHARMA; DIETZ, 2006).

Thus, the aim of the study was to evaluate changes in photosynthetic parameters, oxidative damage, antioxidant enzyme activity and alterations in the metabolism of rice plants after applying preemergent herbicides.

MATERIAL AND METHODS

The experiment was conducted in the greenhouse in a completely randomized design with four replications. The cultivar used was Puitá INTA-CL in a population of six plants per plot. Plants were placed in pots with a volumetric capacity of three liters filled with soil from a rice crop. The treatments consisted of the application of different preemergence herbicides: oxyfluorfen (960 g ai ha^{-1}), oxadiazon (1,000 g ai ha^{-1}) and pendimethalin (1,600 g ai ha^{-1}), as well as a control without application of herbicides. Herbicide application was performed one (1) day after sowing using a pressurized backpack sprayer with CO_2 and a bar with four array Teejet 110015 nozzles spaced 0.5 m, with a spray volume 120 L ha^{-1}.

The variable phytotoxicity was evaluated at eight (8) days after emergence (DAE) through visual notes following a scale from zero to 100, where zero is the absence of symptoms and 100 is plant death. Height measurement was performed at 8 and 12 DAE with the aid of a graduated ruler. All plants of the experimental unit were measured following their length from the ground level to the apex with the leaf blade distended.

Leaf samples were collected at 8 and 12 DAE and stored at -80 °C until the analyses of enzymatic activity and oxidative damage. The variables analyzed were chlorophylls and carotenoids contents, hydrogen peroxide content, lipid peroxidation, total protein content, activity of catalase, ascorbate peroxidase and superoxide dismutase enzymes and proline content.

Physiological assessments related to net photosynthesis, transpiration rate, stomatal conductance and substomatal CO_2 concentration were performed at 12 DAE. For this, the middle third of the last fully expanded leaf was used, and the evaluation was performed using an infrared gas analyzer (IRGA) LI-COR, model LI-6400. The carboxylation efficiency was calculated by the net photosynthesis/concentration of substomatal CO_2 ratio, and the water use efficiency by net photosynthesis/transpiration rate ratio.

The chlorophyll and total carotenoids contents were measured according to the methodology described by Arnon (1949), with modifications. Chlorophyll *a*, *b*, total and carotenoids contents were calculated from the absorbance of the solution obtained by spectrophotometry

at 647, 663 and 470 nm, respectively. The results were expressed in mg g^{-1} of fresh weight (FW).

Cellular tissue damage was determined according to hydrogen peroxide levels (H_2O_2), as described by Sergiev, Alexieva and Karanov (1997), and species reactive to thiobarbituric acid (TBARS) by accumulation of malondialdehyde (MDA), as described by Heath and Packer (1968). The concentration of H_2O_2 was determined using a standard curve with known concentrations of H_2O_2 and expressed in mM g^{-1} FW. For the determination of TBARS, the MDA concentration was calculated using the absorption coefficient of 155 mM cm^{-1} and the results were expressed as nM MDA g^{-1} FW.

To determine the activity of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), an extraction was performed and from this extract, the protein of samples was quantified by the method of Bradford (1976). Results were calculated in function of the casein standard curve and expressed in milligrams of protein per mL (mg of casein mL^{-1}).

The SOD activity was determined according to the methodology adapted by Peixoto *et al*. (1999). Using this method, the inhibition of the reduction of NBT (ρ-nitro blue tetrazolium) by the enzymatic extract was thus determined, avoiding the formation of the chromophore. In this assay, one enzyme activity unit (AU) of SOD was considered as the amount of enzyme required to obtain a 50% inhibition of NBT reduction by SOD contained in the enzyme extract. The activity was determined by calculating the amount of extract that inhibits 50% of the NBT reaction expressed in AU mg^{-1} of protein min^{-1}.

CAT and APX activity were determined, according to the methodology described by Azevedo *et al*. (1998), by the consumption of H_2O_2 (CAT extinction coefficient 39.4 mM cm^{-1} and APX extinction coefficient 2.9 mM cm^{-1}). Both for CAT activity and APX activity, for calculation purposes, it was considered that the decrease of one absorbance unit is equivalent to

one active unit (AU). The activities of total extract were determined by calculating the amount of extract that reduced the absorbance reading by one AU expressed in AU mg^{-1} of protein min^{-1}.

The proline content was determined according to the methodology described by Bates, Waldren and Teare (1973), with modifications. The results are expressed in micromoles of proline g^{-1} FM by designing a proline standard curve with known concentrations.

Data were analyzed for normality and homoscedasticity and then subjected to analysis of variance ($p \leq 0.05$). If statistically significant, the effects of the herbicides were separately evaluated by a Duncan test ($p \leq 0.05$) for each experiment.

RESULTS AND DISCUSSION

The higher phytotoxicity and height reduction was observed when plants were subjected to treatment with oxyfluorfen followed by oxadiazon and pendimethalin (Table 1).

A reduction in the photosynthetic rate and in the carboxylation efficiency, compared to the control, was observed when the herbicides oxyfluorfen and pendimethalin were applied (Table 2). The herbicide pendimethalin also affected the concentration of substomatal CO_2, transpiration rate and water use efficiency. Yet the herbicide oxadioazone only differed from the control regarding the variable water use efficiency.

The oxyfluorfen is an inhibitor of protoporphyrinogen oxidase (PROTOX), and because it has a direct effect on chlorophyll synthesis route, it may interfere with photosynthesis. Chlorophyll molecules are the main pigments responsible for the capture of light for photochemical reactions. Therefore, the decline of these compounds may compromise photosynthetic

Table 1 - Phytotoxicity (%) at eight days after emergence (DAE) and height (cm) at eight and 12 DAE of rice plants subjected to the application of pre-emergent herbicides

Herbicide	Phytotoxicity (%)	Height (cm)	
		8 DAE	12 DAE
Control	0 c[1]	16,9 a	19,2 a
Oxyfluorfen	35,5 a	8,4 c	12,9 b
Oxadiazon	16,7 b	12,4 b	16,0 ab
Pendimethalin	14,0 b	13,3 b	15,1 ab
CV (%)	27,4	14,8	16,9

[1]Means followed by different letters in the column differ by Duncan test ($p \leq 0.05$)

Table 2 - Net photosynthesis (A) (μmol CO_2 m^{-2} s^{-1}), CO_2 substomatal concentration (Ci) (μmol CO_2 mol^{-1}), transpiration rate (E) (mmol H_2O m^{-2} s^{-1}), water use efficiency (WUE) (mol CO_2 mol H_2O^{-1}) e efficiency of carboxylation (CE) (μmol m^{-2} s^{-1}) in rice plants subjected to the application of pre-emergent herbicides, evaluated at 12 days after emergence (DAE)

Herbicide	A	Ci	E	WUE	CE
Control	12,5 a[1]	327 b	6,7 b	1,86 a	0,038 a
Oxyfluorfen	7,9 b	333 ab	5,4 b	1,46 ab	0,024 b
Oxadiazon	10,5 ab	340 ab	6,9 b	1,52 bc	0,030 ab
Pendimethalin	8,2 b	345 a	9,1 a	0,90 c	0,024 b
CV (%)	19,7	2,8	18,1	20,8	31,1

[1]Means followed by different letters in the column differ by Duncan test (p$\leq$0.05)

activity, hindering the development of plants. The main reason is the stomatal closure, which leads to a decrease in the internal concentration of CO_2 and may lead to transfer of an electron to the O_2, thus being a major cause of ROS production (RODZIEWICZ *et al.*, 2014). The herbicide oxadiazon is also an inhibitor of PROTOX. However, plants have a differential selectivity even within the same group of herbicides and may be more susceptible or tolerant to a given product.

The high concentration of substomatal CO_2 verified for the herbicide pendimethalin (Table 2) may be linked to a lower consumption of CO_2 in plants treated with the herbicide. This variable may be influenced by the application of herbicides and by environmental factors such as water, light and energy availability. Yet, the response of rice plants after the application of the herbicide pendimethalin showed an increase in transpiration rates probably due to an increase in metabolic activity linked to a detoxification, inactivation or compartmentalization processes of the herbicide (AHSAN *et al.*, 2008).

Because of the use of pendimethalin, there was a reduction in water use efficiency and carboxylation (Table 2). The reduction in these variables is linked to a lower absorption of CO_2, resulting in a lower photosynthetic activity. It is suggested that under the stress caused by the herbicide, the available CO_2 is not converted efficiently into photosynthetic products, thereby increasing its concentration in the substomatal cavity.

A high lipid peroxidation, both at 8 and at 12 DAE, was observed for the herbicide oxyfluorfen (Tables 3 and 4). This herbicide inhibits protoporphyrinogen oxidase (PROTOX, EC 1.3.3.4), an enzyme located on the inner membrane of the chloroplast responsible for the oxidation of protoporphyrinogen IX into protoporphyrin IX by establishing a conjugated system of double bonds. With the inhibition of PROTOX, the protophyrinogen IX accumulates in the chloroplast and diffuses into the

cytosol, where it undergoes auto-oxidation, converting into protoporphyrin IX due to the action of the cytosolic enzyme, insensitive to the inhibitor. The protoporphyrin IX interacts with molecular oxygen and light, forming singlet oxygen, which in turn triggers oxidative processes such as the peroxidation of membrane lipids (TRIPATHY; MOHAPATRA; GUPTA, 2007), accelerating the oxidative stress process.

The lipid peroxidation generates malondialdehyde (MDA), a product of the decomposition of fatty acids of biomembranes. Lipid peroxidation is one of the most investigated consequences of the actions of ROS on membrane structures, being one of the first responses to damage induced by stress in plant tissues (AMRI; SHAHSAVAR, 2010). The lipid peroxidation is the last step and also a physical action of herbicides related directly or indirectly to photosynthesis.

In a similar study, it was observed that soybean plants treated with oxyfluorfen showed almost doubled malondialdehyde values in comparison to the control (CATANEO *et al.*, 2010). In addition, lipid peroxidation could have damaged the chloroplast by inhibiting the synthesis of chlorophyll and thus photosynthesis (ELLA; KAWANO; ITO, 2003). It was expected that the herbicide oxyfluorfen caused changes in the chlorophyll content. This hypothesis is based on the mechanism of action of the herbicide, which inhibits PROTOX enzyme, involved in the synthesis route of chlorophylls in plants. Thus, it is possible to infer that, in this experiment, because the herbicide application was performed at the preemergence of plants, it caused an adaptation of plants to the production of these light-receptor pigments.

Oxyfluorfen and oxadiazon herbicides also resulted in an increase in SOD activity compared to control (Table 3). In order to keep ROS under control, plants balance ROS and the antioxidant system. The increase in SOD activity may be one of the possible reasons for an increased lipid peroxidation in plants treated with

Table 3 - Lipid peroxidation in terms of thiobarbituric acid reactive species (TBARS) (nM MDA g^{-1} FW), activity of superoxide dismutase (SOD) (AU mg^{-1} protein min^{-1}), catalase activity (CAT) (AU mg^{-1} protein min^{-1}), protein (PROT) (mg casein g^{-1} FW) and proline content (PROL) (mg proline g^{-1} FW) in rice plants subjected to application of pre-emergentes herbicides, at eight days after emergence (DAE)

Herbicide	TBARS	SOD	CAT	PROT	PROL
Control	12,78 b[1]	1,93 c	0,157 a	20,97 a	0,194 ab
Oxyfluorfen	22,29 a	3,58 a	0,124 b	14,77 b	0,238 a
Oxadiazon	13,75 b	2,65 b	0,158 a	21,42 a	0,167 b
Pendimethalin	12,99 b	2,29 bc	0,144 ab	21,71 a	0,191 ab
CV (%)	15,7	15,7	11,8	8,5	14,8

[1]Means followed by different letters in the column differ by Duncan test (p≤0.05)

Table 4 - Lipid peroxidation in terms of thiobarbituric acid reactive species (TBARS) (nM MDA g^{-1} FW), hydrogen peroxide content (H$_2$O$_2$) (mM g^{-1} FW) and activity of ascorbate peroxidase (APX) (AU mg protein^{-1} min^{-1}) in rice plants subjected to the application of pre-emergent herbicides, evaluated at 12 days after emergence (DAE)

Herbicide	TBARS	H2O2	APX
Control	16,27 b[1]	2,93 ab	0,134 b
Oxyfluorfen	20,06 a	1,84 b	0,162 b
Oxadiazon	19,96 b	3,06 a	0,593 a
Pendimethalin	14,68 b	3,39 a	0,661 a
CV (%)	9,50	25,30	18,3

[1]Means followed by different letters in the column differ by Duncan test (p≤0.05)

oxyfluorfen, while, as the activity of SOD increases, hydrogen peroxide levels also increase. However, in this study, it is likely that lipid peroxidation may be caused due to other ROS, since there was no increased levels of H$_2$O$_2$. SOD, considered the first line of defense against the damage caused by ROS, catalyzes the conversion of superoxide anion (O$_2^{-*}$) into H$_2$O$_2$ and O$_2$ in chloroplasts, mitochondria, cytoplasm and peroxisomes (WANG *et al.*, 2012). Thus, it interferes with the concentration of reactive oxygen species involved in the Haber-Weiss reaction for the production of the radical hydroxyl (*OH).

The enzymes of the antioxidant system do not eliminate *OH directly. Thus, the regulation of its precursors, O$_2$* and H$_2$O$_2$, is the key step in preventing *OH risks, bringing together the action of SOD, APX and CAT enzymes. Thus, an efficient combination of SOD, CAT and APX would minimize the effects of oxidative stress, playing an important role in the regulation of ROS (DAMANIK *et al.*, 2012).

The application of oxyfluorfen results in a lower activity of CAT (Table 3) and may be due to the low content of H$_2$O$_2$ produced at the time of evaluation. CAT, together with APX, acts by removing the hydrogen peroxide resulting from SOD activity by converting it to water. However, there are different affinities of these two enzymes to their substrate, so that APX, with its high affinity, acts when H$_2$O$_2$ is present in low concentrations. CAT, on the other hand, has an opposite behavior (GILL; TUTEJA, 2010). CAT enzyme inhibition was also observed in wheat plants subjected to high doses of the herbicide chlorotoluron (SONG *et al.*, 2007). However, this is not completely known because, even with a high SOD activity, less accumulation of H$_2$O$_2$ may occur. A possible explanation for this characteristic is the conversion of free radicals into *OH. *OH may react with all biological molecules such as DNA, proteins, lipids and nearly all of the cell constituents.

Another consequence of stress caused by herbicides is the reduction in the total protein content. This effect can be observed for the oxyfluorfen herbicide, which reduced protein levels compared to control and other herbicides (Table 3). Proteins are found in all parts of cells, as they are in all aspects fundamental to the cell structure and function. Changes in protein levels may cause a great damage to the growth and development of plants. Studies demonstrate that the application of herbicides may result in a decreased protein synthesis (SOOD *et al.*, 2012).

362

Rev. Ciênc. Agron., v. 48, n. 2, p. 358-364, abr-jun, 2017

It was observed that, in general, the proline content increased in plants subjected to the application of oxyfluorfen (Table 3). It is believed that this increase, because of the application of oxyfluorfen, is due to the role played by proline against oxidative damage due to the ability to eliminate ROS from the cell or activate an antioxidant defense mechanism (MOLINARI *et al.*, 2007). Under stress, the protein synthesis is inhibited and protein degradation is accelerated, which leads to the accumulation of amino acids and free amines.

The proline accumulation occurs due to the increase of protein hydrolysis under stress or because of the conversion of sugars in the glutamate pathway. This is because proline acts as a mediator of osmotic adjustment and on integrity and protection of the plasma membrane as a source of carbon and nitrogen (HEMAPRABHA *et al.*, 2013) and as an antioxidant agent, removing reactive oxygen species during oxidative stress.

Upon evaluating the changes at 12 DAE, there was a reduction in the levels of H_2O_2 in plants treated with oxyfluorfen (Table 4). Hydrogen peroxide is usually derived from the SOD activity, which converts O_2^{-*} into H_2O_2. However, if the superoxide anion was not converted by SOD, it may form the radical hydroxyl through a Haber-Weiss reaction, which is the radical most harmful to the cell.

Based on other variables, it is possible to suggest that low levels of H_2O_2 in plants treated with oxyfluorfen is not directly linked to a lower selectivity of this herbicide. That is, it does not mean that the formation of H_2O_2 is not happening: it may have been converted to $^*OH$ or dismuted into water by the activity of SOD. Furthermore, it is known that in low concentrations, H_2O_2 may act as a signaling molecule for the activation of the antioxidant defense system. Evidence report the response of the variable in face herbicide application (WU *et al.*, 2010), which reinforces the relation between H_2O_2 and stress. When compared to other radicals, O_2^{-*} and H_2O_2 are little reactive. However, when in the presence of metal ions such as Fe, for example, they activate a sequence of reactions that lead to the formation of $^*OH$ by the Haber-Weiss reaction (BOWLER; MONTAGU; INZE, 1992). Thus, the activation of the defense system is crucial to ensure the survival of plants.

A higher activity of the enzyme ascorbate peroxidase in plants treated with oxadiazon and pendimethalin was verified (Table 4). These results may be due to a greater accumulation of hydrogen peroxide because of the application of these herbicides. APX is known to play the most important role in eliminating ROS, thus protecting the cells of higher plants, algae and other organisms. This enzyme is involved in the elimination of H_2O_2 and uses ascorbate as an electron donor (GILL; TUTEJA, 2010). Furthermore, APX has a higher affinity for H_2O_2 when compared to CAT, that is, even in a lesser amount, H_2O_2 may activate APX in order to convert this radical into water. In a similar study, it was found that the activity of ascorbate peroxidase increased in both leaves and roots of wheat subjected to the application of chlorotoluron (SONG *et al.*, 2007).

In general, major changes were caused by herbicides that inhibit PROTOX. These herbicides may have their action reduced by the increased activity of some antioxidant enzymes such as SOD, CAT and APX, which are able to mitigate oxidative stress (JUNG *et al.*, 2008). Rice plants treated with inhibitors of PROTOX from various chemical groups (acifluorfen, oxyfluorfen, carfentrazone-ethyl and oxadiazon) showed an increased activity of SOD, CAT and APX (60, 17 and 68%, respectively) if compared to non-treated plants (JUNG *et al.*, 2008).

It is noteworthy that over time, plants have evolved sophisticated strategies to withstand the adverse effects of herbicides and to reduce its phytotoxicity with a multiple detoxification system (KAWAHIGASHI, 2009). Numerous studies have shown that ROS (such as H_2O_2) may serve as signaling molecules involved in plant responses to biotic and abiotic stresses (GILL; TUTEJA, 2010). They may also activate enzymes of the antioxidant system (JIANG; YANG, 2009).

CONCLUSION

The herbicides pendimethalin and oxyfluorfen causes lower net photosynthesis, increase substomatic CO_2, a lower water use efficiency and carboxylation. Even selective herbicides registered for weed control in rice crops cause phytotoxicity, reduce height and alter the metabolism of plants, generating reactive oxygen species, which activate enzymatic and non-enzymatic defense systems and result in the degradation of photosynthetic pigments and in a reduced protein content.

REFERENCES

AHSAN, N. *et al.* Glyphosate-induced oxidative stress in rice leaves revealed by proteomic approach. **Plant Physiology and Biochemistry**, v. 46, n. 12, p. 1062-1070, 2008.

AMRI, E.; SHAHSAVAR, A. Response of lime seedlings (*Citrus aurantifolia* L.) to exogenous spermidine treatments under drought stress. **Australian Journal of Basic and Applied Sciences**, v. 4, n. 9, p. 4483-4489, 2010.

ARNON, D. I. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. **Plant physiology**, v. 24, n. 1, p. 1-15, 1949.

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. **Physiologia Plantarum**, v. 104, n. 2, p. 280-292, 1998.

BATES, L. S.; WALDREN, R. P.; TEARE, I. D. Rapid determination of free proline for water-stress studies. **Plant and Soil**, v. 39, n. 1, p. 205-207, 1973.

BOWLER, C.; MONTAGU, M. V.; INZE, D. Superoxide dismutase and Stress tolerance. **Annual Review of Plant Physiology and Plant Molecular Biology**, v. 43, p. 83-11, 1992.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 7, n. 1, p. 248-254, 1976.

CATANEO, A. C. *et al*. Atividade de superóxido dismutase em plantas de soja (*Glycine max* L.) cultivadas sob estresse oxidativo causado por herbicida. **Revista Brasileira de Herbicidas**, v. 4, n. 2, p. 23-31, 2010.

DAMANIK, R. I. *et al*. Response of antioxidant systems in oxygen deprived suspension cultures of rice (*Oryza sativa* L.). **Plant Growth Regulation**, v. 67, n. 1, p. 83-92, 2012.

ELLA, E. S.; KAWANO, N.; ITO, O. Importance of active oxygen-scavenging system in the recovery of rice seedlings after submergence. **Plant Science**, v. 165, n. 1, p. 85-93, 2003.

FLECK, N. G. et al. Competitividade relativa entre cultivares de arroz irrigado e biótipo de arroz-vermelho. **Planta Daninha**, v. 26, n. 1, p. 101-111, 2008.

GIBERTI, S.; FUNCK, D.; FORLANI, G. D1-pyrroline-5-carboxylate reductase from *Arabidopsis thaliana*: stimulation or inhibition by chloride ions and feedback regulation by proline depend on whether NADPH or NADH acts as co-substrate. **New Phytologist**, v. 202, n. 3, p. 911-919, 2014.

GILL, S. S.; TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiology and Biochemistry**, v. 48, n. 12, p. 909-930, 2010.

HEATH, R. L.; PACKER, L. Photoperoxidation in isolated chloroplasts. I. kinetics and stoichiometry of fatty acid peroxidation. **Archives of Biochemistry and Biophysics**, v. 125, n. 1, p. 189-198, 1968.

HEMAPRABHA, G. *et al*. Evaluation of drought tolerance potential of elite genotypes and progenies of sugarcane (*Saccharum* sp. hybrids). **Sugar Technology**, v. 15, n. 1, p. 9-16, 2013.

JIANG, L.; YANG, H. Prometryne-induced oxidative stress and impact on antioxidant enzymes in wheat. **Ecotoxicology and Environmental Safety**, v. 72, n. 6, p. 1687-1693, 2009.

JUNG, H. I. *et al*. Resistance pattern and antioxidant enzyme profiles of protoporphyrinogen oxidase (PROTOX) inhibitor-resistant transgenic rice. **Pesticide Biochemistry and Physiology**, v. 91, n. 1, p. 53-65, 2008.

KAWAHIGASHI, H. Transgenic plants for phytoremediation of herbicides. **Current Opinion in Biotechnology**, v. 20, n. 2, p. 225-230, 2009.

MOLINARI, H. B. C. *et al*. Evaluation of the stress-induce production of proline in transgenic sugarcane (*Saccharum* spp.): osmotic adjustment, chlorophyll fluorescence and oxidative stress. **Physiologia Plantarum**, v. 130, n. 2, p. 218-229, 2007.

PEIXOTO, P. H. P. Aluminum effects on lipid peroxidation and on the activities of enzymes of oxidative metabolism in sorghum. **Revista Brasileira de Fisiologia Vegetal**, v. 11, n. 3, p. 137-143, 1999.

RODZIEWICZ, P. *et al*. Influence of abiotic stresses on plant proteome and metabolome changes. **Acta Physiologiae Plantarum**, v. 36, n. 1, p. 1-19, 2014.

SERGIEV, I.; ALEXIEVA, V.; KARANOV, E. Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plant. **Comptes rendus de l'Académie bulgare des Sciences**, v. 51, p. 121-124, 1997.

SHARMA, P. *et al*. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. **Journal of Botany**, v. 2012, p. 1-26, 2012.

SHARMA, S. S.; DIETZ, K. J. The significance of amino acids and amino acid-derivated molecules in plant responses and adaptations to heavy metal stress. **Journal of Experimental Botany**, v. 57, n. 4, p. 711-726, 2006.

SIGNORELLI, S. *et al*. Molecular mechanisms for the reaction between OH radicals and proline: insights on the role as reactive oxygen species scavenger in plant stress. **The Journal of Physical Chemistry B**, v. 118, n. 1, p. 37-47, 2014.

SONG, N. H. *et al*. Biological responses of wheat (*Triticum aestivum*) plants to the herbicide chlorotoluron in soils. **Chemosphere**, v. 68, n. 9, p. 1779-1787, 2007.

SOOD, A. *et al*. Differential responses of hydrogen peroxide, lipid peroxidation and antioxidant enzymes in *Azolla microphylla* exposed to paraquat and nitric oxide. **Biologia**, v. 67, n. 6, p. 1119-1128, 2012.

TRIPATHY, B. C.; MOHAPATRA, A.; GUPTA. I. Impairment of the photosynthetic apparatus by oxidase stress induced by photosensitization reaction of protoporphyrin IX. **Biochimica et Biophysica Acta**, v. 1767, n. 6, p. 860-868, 2007.

WANG, W. N. *et al*. Evaluating regional mean optimal nitrogen rates in combination with indigenous nitrogen supply for rice production. **Field Crops Research**, v. 137, p. 37-48, 2012.

WU, G. L. *et al*. Fluroxypyr triggers oxidative damage by producing superoxide and hydrogen peroxide in rice (*Oryza sativa*). **Ecotoxicology**, v. 19, n. 1, p. 124-132, 2010.