ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 08, Issue, 01, pp.18468-18474, January, 2018

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

OXIDATIVE STRESS MEASUREMENTS CAN INDICATE THE BEST DOSE AND PERIOD OF NITROGEN FERTILIZER IN WHITE OAT CROP

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ARTICLE INFO

Article History:
Received 25th October, 2017
Received in revised form
06th November, 2017
Accepted 10th December, 2017
Published online 31st January, 2018

Key Words:

Oxidative stress,
Superoxide dismutase activity,
Malondialdehyde concentration,
Topdressing, Starter fertilizer,
White oat.

ABSTRACT

The white oat crop is highlightedas an important winter alternative for agriculture in the South of Brazil. Oxidative stress (OS) generated by increased reactive oxygen species under stress conditions may promote unavoidable outcomes for production. Thus, we aim to standardize a simple quantitative spectrophotometric protocol to verify OSparameters in white oat growing under different nitrogen fertilization conditions. We also intend to be able to predict the best time, amount and conditions for nitrogen fertilization, based on OS parameters. The white oat culture arrangementwas a 2x4 factorial scheme, in a randomized complete block design. The treatments consisted of starter fertilizer and different topdressing periods, thus, we had two experimental systems, soybean/oat and maize/oat. We estimated the level of grain yield, total protein concentration, superoxide dismutase activity and malondialdehydeconcentration produced by different doses of fertilizer. Databases were subjected to variance analysis, compared the average and regression equations one and two aiming right time adjustment of nitrogen application by the joint analysis of production traits and oxidative damage. Our data support the hypothesis that OS measurements can indicate the best dose of N fertilizer and best N fertilizer period in white oat culture.

Citation: Laura Mensch Pereira, Mara LisianeTissot-Squalli, Guilherme Wildner, Eldair Fabricio Dornelles, Thiago Gomes Heck and Jose Antonio Gonzalez da Silva, 2018. "Oxidative stress measurements can indicate the best dose and period of nitrogen fertilizer in white oat crop", *International Journal of Development Research*, 8, (01), 18468-18474

INTRODUCTION

The white oat (*Avena sativa* L.) crop is highlightedas an important winter alternative for agriculture in Southern Brazil. It is being used for pasture, silage, hay and as cover crop for soil protection and to improve soil´s chemical, physical and biological properties (Oliveira *et al*., 2011; Marolli *et al*., 2017). It is also an option as green cover and providing straw in no-tillage systems by blocking the cycle of many pathogens of standard crops (Floss *et al*., 2007; Spadotti *et al*., 2012). The oat grain is widely used in human nutrition because its high nutritional quality. Besides, oatmeal is considered a functional meal due tothe content and quality of 7.1% to 12.1%dietary fiber asβ-glucans, which contribute to the reduction of serum cholesterol, preventing heart diseases, and increasing the immune system(Gutkoski *et al*., 2007; Hawerroth *et al*., 2015). On the other hand, oat consumption is product based*in natura*, implying greater care in the production process, especially in the agrochemicals used (Silva *et al*., 2015)**.** The use of correct handling techniques and crop management are vital to the full development of the plant andto obtain high yields. Moreover, nitrogen is essential for the plant metabolism because it is part of important biomoleculessuch as ATP, NADH, NADPH, chlorophyll, proteins and several enzymes, influencing in the plants growth more than any other nutrient (Bredemeier and Mundstock, 2000; Arenhardt *et al*., 2017). The nitrogen (N) fertilizer is required due to insufficient amount of nitrogen provided by the soil. Among the nutrients that affect the plant growth, nitrogen is required in high quantity during the life

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cycle of the plants (Scalco*et al*., 2002; Krysczun *et al*., 2017). TheN-fertilizer application amount and timing are fundamental for increasing grain yield and biomass production. However, the nitrogen supply to the plants depends, among other factors, such as, on the amount of organic matterin the soil, the plant residues composition, the yield and moisture expectation, aeration, and temperature interaction in cropping systems (Mantai *et al*., 2015). Because of its high mobility in soil and importance of N for the development of the white oat plant and, it is fundamental touse it efficiently to reduce the losses in the soil and improving the absorption and metabolizing of N. The dose of N-fertilizer that the plant can use is based on theability of the genotype to absorb the nutrient from the soil, and its biological efficiency (Caixeta *et al*.,2015; Silva *et al*., 2016).Because of this, it is necessary to pay attention to the nutrient handling, thus transport of nitrogen derived from fertilizers and decaying matter used in agriculture is one of the factors that mostly affects water quality (Guedes *et al*., 2015)**.** Environmental challenges such as temperature, humidity, water availability, salt stress or light intensity can lead to increased reactive oxygen species that promotes oxidative stress (OS) in plant cells(Alscher *et al*., 1997; Jajic *et al*., 2015). These modifications on plantmetabolism are related to tolerance against environmental challenges, development of physiological responses in cell metabolism,as wellas the hormonesand enzymes production in order to minimize cell damage.Long term or high intensity challenges can promotea negative influence on the plant, mediated by OS condition (You and Chan, 2015). Oxygen is continuously produced during light-driven photosynthetic electron transport and simultaneously removed from chloroplasts throughreduction and assimilation (Apel and Hirt, 2004; Sewelam *et al*., 2016).The reactive oxygen species (ROS) production, as superoxide (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hydroxyl radical (·OH),is an inevitable consequence of aerobic metabolism.

In plants, ROS are generated in the mitochondria, chloroplasts, and nitrogen-fixing nodules. These species work as a defense against pathogens but can lead to the damage in proteins, lipids and DNA, so the production and removal of ROS have to be strictly controlled and balanced in thecell (Møller, 2001; Castilhos, 2010). The cells have mechanisms to prevent and repair the damage caused by the ROS, developing defense strategies, which consist in three defense lines. The firstdefense line is the ROS annulment, keeping the balance between the availability of substrate and the ATP requirement. The second line is the process named detoxification, with the acting of the superoxide dismutase (SOD), catalase (CAT) enzymes, ascorbate peroxidase (APX), and glutathione peroxidase (GPX). Finally, the third line is damage repairing caused by the ROS (Møller, 2001; Hasanuzzaman *et al*., 2013).SODs act as the first line of defense against ROS, by dismutating superoxide to H_2O_2. APX, GPX, and CAT, subsequently detoxifying H_2O_2 (Apel and Hirt, 2004; Sewelam *et al*., 2016).Increased levels of oxidative metabolism can be identified by the quantification of O_2^-, H_2O_2 and radical ·OH in cells, or by enzymes activities measurements directly related to antioxidant defense. Depending on the crop plant genotype, the dose of N-fertilizer used can cause oxidative stress. Since OS parameters can be measured and are related to plant health, culture management can be accompanied by biochemical analyses and answer some practical and theoretical questions: Can the N-fertilizerbean OS inducer during the oat crop? In addition, if that happens, will it influence on yield? What is the best crop condition with the least cellular damage? To answer these questions we aimed to discuss theOS parameters in white oat plants, highlighting their importance as injuries indicative and relating it to the nitrogen fertilization. Thus, we aim to standardize a simple quantitative spectrophotometric protocol to verify OSparameters in white oat growing under different conditions of nitrogen fertilization. Also, we intend to be able to predict the best time, amount and condition for nitrogen fertilization, based on OS parameters.

MATERIALS AND METHODS

The white oatcrop was growing in the experimental area of theRegional Northwest University of Rio Grande do Sul (UNIJUÍ), located in Augusto Pestana, RS, Brazil. The growing soil is an OxisolDistroferric Typical. According to the climatic classification underKöppen´s system(Alvares *et al*., 2013) the climate of the experimentalarea matches to CFA (humid subtropical), with the occurrence of hot summers, no occurrence of prolonged droughts, and cold wet winters.The experimental area iscategorized by a direct seeding system with fifteen years of implementation, featured as a consolidated no-tillage system. In the summer period, the area is occupied with soybeans and maize, the cultural precedents used to compound the experiment.In this research the following steps were performed:

- Plot design:the white oatculture arrangementwas a 2x4 factorial scheme, in a randomized complete block design. The treatments consisted ofstarter fertilizer25kg ha^{-1} and 5kg ha^{-1} and different topdressing periods (0, 10, 30, 60 days after the emergence), in an area previouslycropped with soybean and maize.This way, we had two experimental systems, soybean/oat and maize/oat.We used the Brisasul cultivar that is characterized by high grain yield, reduced size and high resistance to lodging(Oliveira *et al*., 2011). The fertilizer methodology followed the fertilizer and liming manualfor oat crop to the states Rio Grande do Sul and Santa Catarina (SociedadeBrasileira de Ciência do Solo, 2004). The total dosesof nitrogen used in all the application periods were60 kg ha^{-1} in the soybean/oat system and 90 Kg ha^{-1}in the maize/oat system to the 3 t ha^{-1} yield. The seeds were sown in 2013/May with starter fertilizerin 5 lines of 5 m length and line spacing of 0.20 m. The population density was 300 viable seeds per square meterin accordance with the technical indications of the culture. Thegrain yield [GY (kg ha^{-1})]had been estimatedfrom the grain mass of the harvest of the three central rows of each plot. The leaves were collected before the inflorescence developmentand submitted to oxidative stress analysis.
- Oxidative stress analysis:wastested some protocolswhich had been used in the laboratory to animal tissue analysis, based on it, we adjusted them to use on vegetal tissue.
- Tissue preparation: was used 0.100g of fresh vegetal tissue homogenized (1:7 w/v) in sodium phosphate buffer (pH = 7.4), containing protease inhibitor PMSF (Phenyl-Methyl-Sulfonyl Fluoride, 100 µM, final concentration). Afterwards, the homogenates were centrifuged at 600 x g for 10 minutes at room temperature and the supernatant fractions were saved (-20°C) for further analyses by the spectrophotometric methods by following tests:

- Lipid peroxidation level: thelipid peroxidation levels measurement followed theThiobarbituric Acid Reactive Substances Method (TBARS)(Buege and Aust, 1974). First, in 180µL of homogenized samples540µL TCAwas added,kept on ice for 30 minutes. After centrifugation(3,000rpm for 10 min)the supernatant was collected (300µL) and equal volume of TBA was added. The tubes were covered and boiledin a water bath for 15 minutes, cooled and centrifuged at 2,000rpm for 10 minutes. The absorbance was measured in the supernatant at 535 nm. The amount of lipidperoxidationformed wasexpressed in nanomoles of malondialdehyde per milliliters of solution (nmol MDA/ml).
- Protein concentration: the protein concentrations were measured by the Bradford method(Bradford, 1976)using bovine serum albumin as standard curve (1mg/ml). Homogenized samples werediluted in distilled water(1:3 w/v) and wereadded Bradford assay buffer(1:1000). The absorbance was measured at 595nm and the results are expressed in milligrams of protein per milliliter (mg/ml).

RESULTS

The variablesgrain yield (GY), protein (PRT), MDA and SOD, were analyzed regarding the interaction with the fertilizer time (0, 10, 30, 60 days after the emergence treatment). The results of soybean/oat system presented interaction by GY, PRT, and MDA. On the other hand, the results of maize/oat system exhibited interaction in all parameters (tab.1). By the parameter results related to starter fertilizer N, the soybean/oat system had showed PRT, MDA and SOD with high significance, however, only PRT showed interaction in the maize/oat system (tab.1). It is highlighted that all the variables tested had significance between the topdressing time and starter dressing in both systems (Tab. 1). The Table 2 exhibits the results of average comparison obtained in the time of N application and N dose, with the decomposition of this interactions by analyzing the simple effects of starter fertilizer and topdressing. In the soybean/oat system, the lowest dose of starter fertilizer (5 kg ha^{-1}) showed the highest grain yield in 30 days after emergence (DAE) (2638 Kg ha^{-1}). However, in the highest dose of starter fertilizer (25 kg ha^{-1}) the highest grain yield was obtained in 10 DAE (2,687 Kg ha^{-1}) (tab.2).

Table 1. Analysis of variance of grain yield (GY), protein (PRT); malondialdehyde (MDA); superoxide dismutase (SOD) in starter fertilizer dose and topdressing period

		Mean Square			
VariationSource	DF	GY (Kg ha^{-1})	PRT (mg/ml)	MDA (nmol/ml)	SOD (U/ml)
		Soybean/oat system			
Block	3	180993	0.00022	0.00005	0.01038
NPT	3	1347826*	0.0156*	0.02129*	0.01745^{ns}
NSF	1	21218^{ns}	0.0482*	0.11858*	1.10707*
NSF x NPT	3	194498*	0.0040*	0.01687*	1.97897*
Error	21	18138	0.0002	0.00009	0.010901
Total	31				
General average		2146	0.27	0.10	3.63
CV (%)		12.27	5.73	8.99	3.78
		Maize/oat system			
Block	3	10133	0.00005	0.00004	2.45792
NPT	3	4010401*	0.0064*	0.00364*	4.59421*
NSF	1	3549^{ns}	0.00874*	0.00008^{ns}	0.13364^{ns}
NSF x NPT	3	80955*	0.00524*	0.00081*	16.77399*
Error	21	19143	0.00014	0.00017	0.54719
Total	31				
General average		1802	0.19	0.22	12.54
CV (%)		14.67	6.09	5.76	5.89

*Significant at 0.05 probability level; DF (degrees of freedom);CV (coefficient of variation); NPT (N-period-topdressing); NSF (N-dose-starter fertilizer).

- Superoxide dismutase (SOD) activity:SOD activity was performed by auto-oxidation inhibition of pyrogallol(Marklund and Marklund, 1974). Briefly, in a cuvette, 930 µL of 50 mM Tris/1mM EDTA Buffer (pH 8.2), 4 µL of catalase (CAT 30 µM) and 50 µL of homogenate were added and mixed. After, pyrogallol (24 mM in HCl 10 mM) was added and SOD activity determined at 25ºC in spectrophotometer (420 nm) for 120 seconds. Results were expressed in units of SOD/ml.
- Statistical Analysis:databaseswere subjected to variance analysis (ANOVA) for the presence or absence of interaction between the factors. Based on this, wecompared the average test by Scott and Knott (1974) at the 5% level of probability usingthe GENES software. It was adjusted for degree of regression equations one and two aiming right time adjustment of application of nitrogen by the joint analysis of production traits and oxidative damage.

The analysis of the leaf demonstrated the highest expression of protein when N was applied at 60 DAE (0.371 mg/ml), butin the highest dose of starter fertilizer, the highest protein content was obtained in the absence of topdressing (0.289 mg/ml). The MDA analysis in the leaf showed the highest content in 30 DAE (0.055 mg/ml) in the lowest dose of starter fertilizer and in 10 DAE (0.263 mg/ml) for the highest dose of starter fertilizer. SOD analysis showed similar results than MDA in the lowest dose of starter fertilizer (3.515U/ml). Finally, the highest SOD results were in the highest dose of starter fertilizer on the absence of topdressing (4.336 U/ml) and in 10 DAE (4.146 U/ml) (tab.2). The maize/oat system showed the highest grain yield in 30 DAE in both condition of higher or lower dose of starter fertilizer (2,523 Kg ha^{-1}, 2,253 Kg ha^{-1}) (tab. 2). The highest levels of protein in the leaf were found in 60 DAE in both conditions of high or low dose of starter fertilizer (0.251 mg/ml, 0.202 mg/ml), and the highest levels of MDA were found in 30 DAE in both conditions (0.267 U/ml, 0.246 U/ml). SOD analysis showed the highest level for both

Table 2. Average comparison test of grain yield (GY), protein (PRT); malondialdehyde (MDA); superoxide dismutase (SOD) in starter fertilizer dose and topdressing period

Variable	Starter Fertilizer (Kg-ha^{-1})	Topdressing (Days after emergence/DAE)			
		0	10	30	60
		Soybean/oat system			
GY	5	1777Ca	2432Bb	2638Aa	1840Ca
(Kg ha^{-1})	25	1935Ca	2687Aa	2227Bb	1631Db
PRT	5	0.337Ba	0.295Ca	0.238Da	0.371Aa
(mg/ml)	25	0.289Ab	0.214Bb	0.196Cb	0.231Bb
MDA	5	0.031Da	0.040Cb	0.055Ab	0.041Bb
(nmol/ml)	25	0.032Da	0.263Aa	0.197Ba	0.161Ca
SOD	5	2.990Db	3.213Cb	3.515Ba	4.096Aa
(U/ml)	25	4.336Aa	4.146Aa	3.632Ba	3.187Cb
		Maize/oat system			
GY	5	865Ca	2369Aa	2523Aa	1496Ba
(Kg ha^{-1})	25	910Ca	2301Aa	2253Aa	1705Ba
PRT	5	0.216Ba	0.192Ca	0.177Ca	0.251Aa
(mg/ml)	25	0.191Ab	0.160Bb	0.150Ba	0.202Ab
MDA	5	0.199Ba	0.223Ba	0.267Aa	0.212Ba
(nmol/ml)	25	0.209Ba	0.222Ba	0.246Aa	0.210Ba
SOD	5	10.131Cb	12.368Ba	13.420Aa	13.982Aa
(U/ml)	25	14.584Aa	10.754Ca	12.483Ba	12.597Ba

Means followed by the same lowercase letters in the column and capital in line are not statistically different from each other at 5% error probability level by Scott & Knott test.

Table 3. Regression equation of grain yield (GY), protein (PRT); malondialdehyde (MDA); superoxide dismutase (SOD) in starter fertilizer, dose and topdressing period

Y	Starter Fertilizer (Kg-ha^{-1})	VS^{y}	y = a±bx±cx^2	[bi$_x^{(n)}$]	R^2	Ideal Period	YE
			Soybean/oat system				
GY (Kg ha^{-1})	5	L^{ns}	-	-	-	-	-
		Q*	1839+57.6x-0.96x^2	*	96	30	2703
	25	L*	2364-9.73x	*	32	-	-
		Q*	2125+28.3x-0.61x^2	*	68	23	3098
PRT (mg/ml)	5	L*	0.2947+0.00062x	*	8	-	-
		Q*	0.3430-0.0070x+0.00012x^2	*	98	29	0.2409
	25	L*	0.2492-0.00065x	*	18	-	-
		Q*	0.2794-0.0054x+0.00007x^2	*	88	38	0.1752
MDA (nmol/ml)	5	L*	0.0382+0.00015x	*	16	-	-
		Q*	0.0302+0.0014x-0.00002x^2	*	97	35	0.0547
	25	L*	0.1413+0.0008x	*	5	-	-
		Q*	0.0848+0.0098x-0.00014x^2	*	48	35	0.2563
SOD (U/ml)	5	L*	3.0017+0.0180x	*	99	-	-
		Q^{ns}	-	^{ns}	-	-	-
	25	L*	4.3125-0.0194x	*	98	-	-
		Q^{ns}	-	^{ns}	-	-	-
			Maize/oat system				
GY (Kg ha^{-1})	5	L*	1720+3.72x	*	1	-	-
		Q*	1087+104.45x-1.63x^2	*	85	32	2760
	25	L*	1629+6.51x	*	7	-	-
		Q*	1160+81.29x-1.21x^2	*	73	33	2524
PRT (mg/ml)	5	L*	0.1927+0.00066x	*	29	-	-
		Q*	0.2169-0.0031x+0.00006x^2	*	99	25	0.1769
	25	L	0.1684+0.00031x	*	11	-	-
		Q	0.1888-0.0029x+0.00005x^2	*	98	29	0.1467
MDA (nmol/ml)	5	L^{ns}	-	^{ns}	-	-	-
		Q*	0.1952+0.00042x-0.00006x^2	*	96	35	0.1364
	25	L^{ns}	-	^{ns}	-	-	-
		Q*	0.2063+0.0024x-0.00003x^2	*	95	40	0.2543
SOD (U/ml)	5	L*	11.0751+0.0560x	*	76	-	-
		Q*	10.4173+0.1607x-0.0017x^2	*	95	47	14.214
	25	L^{ns}	-	^{ns}	-	-	-
		Q*	13.6014-0.1265x+0.0018x^2	*	30	35	15.363

VS (variation source); R2 (coefficient of determination); bix (parameter that measures the statistical significance of linear coefficient of the equation at a 5% probability level); L(linear); Q (quadratic); ns (not statistically significant at 0.05 level of error probability); * (statistically significant at 0.05 level of error probability).

without topdressing in high dose of starter fertilizer (14.5845U/ml) and with the lowest dose of starter fertilizer in 60 DAE (13.982U/ml) (tab.2). In table 2, we can observe in both systems that the highest levels of grain yield match with the highest levels of MDA, evidencing that the highest grain yield also generates a higher cellular damage, and for that, it is necessary a cellular damage protector. In the same table, wenoticed in the highest dose of starter fertilizer had the highest SOD levels in the first periods of topdressing, with this SOD is acting as a cellular damage protector, decreasing the ROS effect. The quadratic equation is the most effective in explaining the grain yield behavior demonstratedin the soybean/oat system, in lower dose of starter fertilizer, with 30 DAE as the ideal time to supply N, and 23 DAE for the highest dose of starter fertilizer (tab.3). Quadratic equation was observed also in protein when N applied in 38 DAE in the highest dose of starter fertilizer. By the analysis of biochemical variables in relation to oxidative damage, quadratic equation

explains the parameter MDA in the two starter fertilizer conditions, with 35 DAE as the ideal time to supply N. The SOD analysis presented unexpected result to evidence a uniquely linear equation, reporting that every day of delay in topdressing there was an increase in low doses of starter fertilizer and a reduction in high doses of starter fertilizer front of the expression of this variable (Tab.3). Through themaize/oat system analysis, quadratic equation explains thegrain yield behavior in the two fertilizer conditions, when the ideal time to supply N was 32 DAE in low dose of starter fertilizer and 33 DAE to high dose of starter fertilizer. Quadratic equation was observed also by the protein analysis, with the highest results when N applied at 29 DAE in the highest dose of starter fertilizer. All the biochemical variables presented quadratic equations. The best MDA condition was observed when N applied at 40 DAE in the highest dose of starter fertilizer. The SOD analysis is better explained by a quadratic equation, differently than observed in soybean/oat system, in both fertilizer conditions, with ideal N supply at 47 DAE in low dose of starter fertilizer (Tab.3).

DISCUSSION

We observed differences in GY when using starter fertilizer, with increased results of N supply in different top dressing periods. GY variation for soybean was independent of period of N supply, since that with the dose of 60 kg ha^{-1}, this variation is observed without nitrogen and with application of 30 kg ha^{-1} in the previous survey (Bahry *et al*., 2014). In the white oat crop, starter starting fertilizer with 30kg ha^{-1} of N may be appropriate to prevent nutrientloss (Ceccon *et al*., 2004), results confirmed by our work, wherein database show effectiveness for oats GY with basic fertilization 25 kg ha^{-1}, indicating a great nutrient consumption. Our analysis indicted quadratic equation as the most effective to explain protein levels in leaves, evidencing the ideal period to supply N to achieve the best expression and GY. On the other hand, it was proposed that the grass protein content increases with the amount of N applied, indicating that the protein levels increased linearly as a function of nitrogen levels in napier grass, with the lowest doses providing smaller proteins contents (Andrade *et al*., 2003). It was estimated that 1% of the oxygen consumed in the plant tissue participated on the production of ROS, which are often produced as a respiration and photosynthesis sub product (Møller, 2001). The ROS reacted with lipid membrane, caused lipid peroxidation and formed new lipid radicals, with irreversibly damage to membranes barrier, modifying the permeability to other cell damaging toxic products (Chagas, 2007). The conversion of oxidant $H_2O_2$to H_2O by APX occurs by oxidation of ascorbate to MDA, which can be regenerated by MDA reductase (MDAR) and MDA can spontaneously dismutate into dehydroascorbate. Upon abiotic stresses, ROS scavenging enzymes are induced to decrease the concentration of toxic intracellular ROS levels (Apel andHirt, 2004), explainingthe MDA cellular behavior.

SOD is involved in several contexts in antioxidant regeneration throughout the plant cell. One of these is a metabolic cycle located both within the chloroplast stroma and in the cytosol that successively oxidizes and reduces again the antioxidant substrates. The enzyme activity preservation suggests that the SODs present in pea leaf cells are less susceptible to attack by ROS than other proteins and therefore less susceptible to proteolytic attack (Alscher *et al*., 1997). Thus, SOD can be considered an important cellular protector during the crop, evidenced by the increasing of its enzymatic activity inhigher starter fertilizer doses. Other researches also proved that SOD is an oxidative damage protectiveand demonstrated that the increase ofO^{-}_2 e H_2O_2 concentration in wheat infected with *Pyriculariaoryzae* corresponding to the highest concentration of MDA (Debona, 2012). The extent of cellular damage caused by oxidative stress related to the plant response to pathogen infection can be estimated from the membrane lipids peroxidation products. The results showed also higher SOD activity in inoculated plants, when compared with non-inoculated plants, which emphasize that high SOD activity corresponds to low oxidative damage. Higher lipid peroxidation levels in soybean treated with oxyfluorfen (herbicide) instead of control plants and correlated SOD activity increasesas herbicide dose is increased(Cataneo *et al*.,2006). These results agree with our research, on that the lipid peroxidation levels and SOD activity also increased with higher doses of N-fertilizer, in this case. High intensity of membrane lipid peroxidation was observed in sugarcane tissueexposed for 48 hours to Paraquat than for 24 hours (Chagas, 2007). The SOD results also indicated enzymatic activity increase for 48 hours, thus, SOD higher activity certainly could not be sufficient on plant detoxification. Biotic and abiotic stresses can both give rise to further increases in ROS levels. In the case of biotic stresses, the first attack site by most pathogens is outside the cell, whereas in the case of abiotic stresses like in herbicides, it changes the photodynamic inside chloroplast, being the first cell action spot. It has been shown that phospholipid membranes are impermeable to charged O^{-}_2 molecules (Palma *et al*., 1991).

Therefore, the presence of SOD activity is crucial for removing O^{-}_2 in the compartments where the radicals are formed (Alscher *et al*., 2002). As suggested by researches, MDA increase and accumulation may represent an oxidative stress biomarker of in plants (Montanari, 2006). Thesensitive maize shows less protection against oxidative damage under salt stress. However, the lipid peroxidation at low levels presented by the tolerant cultivar suggested a better efficiency of mechanisms of free radicals elimination. Thus, in our research it was observed stable MDA levels because higher SODactivity levelspromoted the cellular detoxification function. Our data also indicate that SOD may be useful asbiomarker of oxidative stress status in white oat, better than MDA. Thus, we demonstrated that SOD enzymatic activity, as the first line of defense against ROS, canbe a plant cell damage marker. This was evidenced in soybean/oat system in our study, on that SOD showed linear equation, being SOD aN supply function, which may indicate that the enzyme is acting against existing ROS to maintain plant ideal balance. The research could adapt protocols to quantify oxidative stress parameters in white oat. We demonstrated the relevance of N fertilizer on white-oat crop and demonstrated the performance of the antioxidant enzyme SOD in repairing the oxidative damage, stabilizing the lipidperoxidation levels, thus maintaining the balance of reactive oxygen species and ensuring proper cell function. Our work indicates the best N-fertilizer handling based on the enzyme SOD activity, suggesting this biochemical practice by agricultural management. Moreover, our results point to a future possibility to develop rapid diagnostic test for oxidative stress from the SOD activity measurements, to provide adequate and optimal interventions in the white oat crop.

Acknowledgment

Wethank the Programa de Educação Tutorial - PET (Ministério da Educação - MEC/Secretaria da Educação Superior – SESU, Brazil) for the partialsupport.

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