METABOLIC ACTIVITY OF WHEAT AND RYEGRASS PLANTS IN COMPETITION

ABSTRACT - Ryegrass is an annual cycle Poacea, often considered as a weed in wheat crops. The goal of this study was to analyze oxidative stress and enzyme activity of wheat and ryegrass cultures in response to stress caused by the competition between crop and weed, in two development stages. The experiment was conducted in completely randomized design, with eight replications, in replacement series, with a population of 64 plants per pot. Treatments consisted of proportions of ryegrass and wheat plants arranged in replacement series. The tested proportions were: 100/0 (pure wheat stand), 75/25, 50/50, 25/75 and 0/100% (pure ryegrass stand). The evaluated variables were: hydrogen peroxide (H$_2$O$_2$) content, thiobarbituric acid-reactive species (TBARS), electrolyte leakage and activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) antioxidant enzymes. Culture and the damages caused by ryegrass competition varied according to the plant developmental stage. Both for wheat and ryegrass culture, intraspecies competition caused higher oxidative stress, with increasing H$_2$O$_2$ and TBARS contents and electrolyte leakage. SOD, CAT and APX activity increased in the culture during interspecies competition. In ryegrass, interspecies competition also increased SOD and CAT activity, whereas weed monoculture caused an increase in the APX enzyme. Generally speaking, variable behaviors were similar between the tillering and stem elongation stages.

Keywords: Triticum aestivum, Lolium multiflorum, stress, competitiveness.

Atividade Metabólica das Plantas de Trigo e Azevém sob Competição

RESUMO - O azevém é uma Poacea de ciclo anual, constituindo-se, com frequência, em planta daninha na cultura do trigo. O objetivo deste estudo foi analisar o estresse oxidativo e a atividade enzimática das culturas do trigo e do azevém, em resposta ao estresse causado pela competição entre cultura e planta daninha, em dois estádios de desenvolvimento. O experimento foi conduzido em delineamento completamente casualizado, com oito repetições, em série de substituição com população de 64 plantas por vaso. Os tratamentos constaram de proporções de plantas de azevém e trigo. As proporções testadas foram: 100/0 (estande puro de trigo), 75/25, 50/50, 25/75 e 0/100% (estande puro de azevém). As variáveis avaliadas foram teor de peróxido de hidrogênio (H$_2$O$_2$), espécies reativas ao ácido tiobarbitúrico (TBARS), extravasamento de eletrólitos e atividade das enzimas antioxidantes superóxido dismutase (SOD), ascorbato peroxidase (APX) e catalase (CAT). Tanto para a cultura do trigo quanto para o azevém, a competição intraspecífica ocasionou maior estresse oxidativo, com o aumento do teor de H$_2$O$_2$, TBARS e extravasamento de eletrólitos. A atividade das enzimas SOD, CAT e APX aumentou na cultura quando em competição interspecífica. No azevém, a competição interspecífica aumentou a atividade da SOD e da CAT, enquanto para APX o monocultivo de daninha ocasionou aumento da enzima. Em geral, o comportamento das variáveis foi similar entre o estádio de afilhamento e o de alongamento do colmo.

Palavras chave: Triticum aestivum, Lolium multiflorum, estresse, competitividade.

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INTRODUCTION

During the 2015/16 harvest, the wheat area in Brazil was approximately 2.5 million hectares; Rio Grande do Sul (RS) state was responsible for 50% of it, with average yield of 2,260 kg ha⁻¹ (Conab, 2016). However, yield has fallen short of culture productive potential; partially, this is the result of unsatisfactory weed control, which causes quantitative and qualitative damages to wheat production (Paula et al., 2011).

Competition among plants is a fundamental part in plant ecology, and it occurs when two or more plants use or withdraw resources that are limited within the niche for their growth and development. Moreover, the more morphological similarities the competitors will have, the more intense the competition will be (Radosevich et al., 2007).

One of the main weed in wheat culture is ryegrass (*Lolium multiflorum*), which, similarly to wheat, belongs to the Poaceae family. The occurrence of similar morphological characteristics hampers the adoption of chemical control, on account of the selectivity for the culture and the effectiveness of herbicides to control ryegrass.

Normally, studies about plant competitive abilities evaluate characteristics about growth, like height, leaf area, tillering and dry matter mass production of the aerial part. In addition to morphological responses, plants can also respond to stress conditions caused by changes in their secondary metabolism (Wojtaszek, 1997).

Under normal conditions, plants have relative species of oxygen (EROs), such as singlet oxygen (\(^{1}\text{O}_2\)), hydrogen peroxide (\(\text{H}_2\text{O}_2\)), superoxide radicals (\(\text{O}_2^{-}\)) and hydroxyl radicals (\(\text{OH}^{-}\)), which are detoxified by various antioxidant defense mechanisms (Foyer and Noctor, 2005). However, in stressful situations caused by biotic, abiotic and xenobiotic factors, such as salinity, radiation, water deficiency, extreme temperatures, herbicides, pathogen attacks and competition for resources, EROs increase occurs, generating oxidative stress, which forces plants to action defense responses as a way of overcoming these stresses and going back to normal metabolism conditions.

The negative effect of stress mediated by oxidative damages started by EROs results into oxidative damages to proteins, DNA, lipids and membrane structure and its organization, changing the function of enzymes and receptors connected to them (Gill and Tuteja, 2010).

Plants present defense systems against oxidative stress, constituted by antioxidant enzymes, like superoxide dismutase, ascorbate peroxidase and catalase, and/or through compounds with antioxidant activity, such as carotenoids, anthocyanins and phenolic compounds (Noctor and Foyer, 1998; Selote et al., 2004). Despite the fact that secondary metabolism is not always necessary for plants to complete their life cycle, it plays an important role in the interaction between plants and environment, since it acts as an interface for signals coming from primary metabolism and environment, in response to different stresses.

Knowing plant biochemical and metabolic changes, according to competitive interactions, is important to create strategies in order to obtain plants that are more tolerant to stress conditions. Thus, the goal of this study was to analyze oxidative stress and enzymatic activity in wheat and ryegrass cultures, in response to stress caused by the competition between culture and weed, in two development stages.

MATERIAL AND METHODS

Two experiments were conducted in a greenhouse, in completely randomized design, with four and eight replications for the first and second experiment, respectively. The first had the goal to determine plant population m⁻² from which dry matter mass from the aerial part (MMSPA) per area unit (g m⁻²) becomes independent from the population (Radosevich et al., 2007). The tested populations, in wheat and ryegrass monocultures, had from 4 to 128 plants per pot⁻¹. Competitor species were Horizonte wheat (precocious cycle) and ryegrass biotype (*Lolium multiflorum*) crops, planted in vases with eight liters volumetric capacity and 23 cm diameter. MMSPA variable was quantified on day 30 and 60 after emergence (DAE), by weighing the aerial part of plants after being dried in a greenhouse with forced air circulation at 60 °C for 72 hours.
Constant MMSPA was observed in the averages of the two evaluation periods, with population of 64 plants per vase, that is, 1,542 plants m⁻² for culture and weed (data not presented).

The second experiment was conducted in replacement series with population of 64 plants per vase, as determined in the first experiment. Treatments consisted of proportions of wheat and ryegrass plants. The tested proportions were: 100/0 (pure wheat stand), 75/25, 50/50, 25/75 and 0/100% (pure ryegrass stand). Collections were performed on day 30 (tillering stage) and 60 (stem elongation stage) after emergence (DAE), using four replications for each period of the collection.

The aerial parts of wheat and ryegrass plants were collected separately, picking the youngest leaves. Composite samples of each replication were made with all plants from the experimental unit, which were stored at -80 °C until variable quantification. The analyzed variables were cell damages in tissues, determined by hydrogen peroxide (H₂O₂) content, as described by Sergier et al. (1997); the ones from thiobarbituric acid-reactive species (TBARS), were determined by malondialdehyde (MDA) accumulation, as described by Health and Packer (1968); and electrolyte leakage, as described by Tarhanen et al. (1999). In order to evaluate antioxidant enzyme activity, superoxide dismutase (SOD) was determined according to Peixoto’s adapted methodology (1999), starting from Del Longo et al. (1993); ascorbate peroxidase (APX) was determined by Nakano and Asada’s method (1981); and catalase (CAT) was determined according to the methodology described by Azevedo et al. (1998).

Results obtained for the metabolic variables of plants were analyzed in terms of normality by Shapiro-Wilk’s test and, subsequently, submitted to analysis of variance (p ≤ 0.05). After that, the effects of proportions, in relation to monoculture (control sample), were evaluated by Dunnett’s test (p ≤ 0.05) and, among mix proportions, by Tukey’s test (p ≤ 0.05), separately for each competitor.

**RESULTS AND DISCUSSION**

H₂O₂ content in wheat competing with ryegrass presented higher content of this reactive species in monoculture, both in tillering (30 DAE) and elongation (60 DAE) (Table 1). In general, during both evaluation periods, the 75/25 proportion presented increased contents of this variable, compared to the others, when analyzing the effect of interspecies competition. This increase is justified by the fact that H₂O₂ plays a double role in plants: in low concentrations, it acts as a signaling molecule, involved in tolerance and signaling, causing adaptation to various biotic and abiotic stresses; and, in high concentrations, it can lead to plant death (Halliwell, 2006).

As for ryegrass competing with wheat, H₂O₂ content presented higher value for the monoculture, in both development stages (Table 1). Among plant proportions, the 25/75 one, on

<table>
<thead>
<tr>
<th>Proportion (wheat/ryegrass)</th>
<th>H₂O₂ (mM g⁻¹) 30 DAE</th>
<th>H₂O₂ (mM g⁻¹) 60 DAE</th>
<th>TBARS (nM MDA g⁻¹ MF) 30 DAE</th>
<th>TBARS (nM MDA g⁻¹ MF) 60 DAE</th>
<th>Leakage (%) 30 DAE</th>
<th>Leakage (%) 60 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>100/0 (T)</td>
<td>0.92</td>
<td>1.44</td>
<td>12.27</td>
<td>17.25</td>
<td>45.99</td>
<td>47.07</td>
</tr>
<tr>
<td>75/25</td>
<td>a1.09*</td>
<td>a1.14*</td>
<td>a11.26*</td>
<td>a14.48*</td>
<td>a46.29*</td>
<td>a46.60*</td>
</tr>
<tr>
<td>50/50</td>
<td>ab0.52*</td>
<td>b0.66*</td>
<td>a11.28*</td>
<td>b12.93*</td>
<td>b41.28*</td>
<td>b43.56*</td>
</tr>
<tr>
<td>25/75</td>
<td>b0.47*</td>
<td>a0.95*</td>
<td>a12.24*</td>
<td>a15.58*</td>
<td>b41.73*</td>
<td>b44.00*</td>
</tr>
<tr>
<td>Ryegrass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/100 (A)</td>
<td>0.08</td>
<td>0.15</td>
<td>11.24</td>
<td>8.76</td>
<td>44.0</td>
<td>45.00</td>
</tr>
<tr>
<td>25/75</td>
<td>a0.07*</td>
<td>a0.44*</td>
<td>a7.66*</td>
<td>a8.84*</td>
<td>a43.33*</td>
<td>a47.61*</td>
</tr>
<tr>
<td>50/50</td>
<td>b0.05*</td>
<td>a0.03*</td>
<td>a8.10*</td>
<td>a8.32*</td>
<td>a42.32*</td>
<td>a47.36*</td>
</tr>
<tr>
<td>75/25</td>
<td>c0.03*</td>
<td>a0.04*</td>
<td>a7.43*</td>
<td>a9.10*</td>
<td>a42.01*</td>
<td>a43.52*</td>
</tr>
</tbody>
</table>

(1) Days after emergence; (2) Averages preceded by the same lowcase letter in the column, in proportions with plant mixes, do not differ among themselves by Tukey’s test (p ≤ 0.05); (3) Averages followed in the column by * and * indicate non-significant and significant difference, respectively, by Dunnet’s test (p ≤ 0.05) in relation to wheat (T) and ryegrass (A) monoculture.
Results demonstrated that both for wheat and ryegrass cultures, intraspecies competition has greater abilities of causing cell damage than interspecies competition. Studies demonstrated that even low concentrations of hydrogen peroxide can induce changes in the phosphorylation of specific regulating proteins (Apel and Hirt, 2004). On the other hand, the excess of $H_2O_2$ in plant cells leads to oxidative stress, being able to inactivate enzymes such as SOD, CAT, APX, guaiacol peroxidase and glutathione $s$-transferase (Gill and Tuteja, 2010). Thus, even if reactive, many of the cell damages caused by hydrogen peroxide and/or radical peroxide result from the conversion of these molecules for even more reactive species (Moller, 2001).

As for TBARS variables, in general, wheat presented higher value in monoculture, compared to proportions with ryegrass in both evaluated stages (Table 1); this supports the results that were observed for $H_2O_2$ and highlights that intraspecies competition caused higher oxidative stress in wheat. As for interspecies competition analysis, there was no difference among proportions on day 30 DAE, whereas on day 60 DAE there was higher production for the 25/75 proportion of wheat/ryegrass, not different from the 75/25 proportion.

On day 30 DAE, ryegrass competing with wheat presented higher TBARS concentration in monoculture, and on day 60 DAE in the 75/25 proportion, compared with the weed monoculture. As for the analysis of competition among proportions, no differences were observed among plants with the competitor presence in both evaluation periods (Table 1). TBARS increase may derive from ryegrass intraspecies competition on day 30 DAE, and on day 60 DAE, it may be due to the higher number of wheat plants in the 75/25 proportion, enhancing wheat greater ability in causing oxidative stress on ryegrass.

The increase in contents of lipid peroxidation may be due to the fact that plant intraspecies competition cause changes such as the increase in membrane permeability, modifying the flow of ions and other substances and resulting in the loss of selectivity for the inlet and/or outlet of nutrients and substances that are toxic for the cell. Therefore, lipid peroxidation causes the rupture of lipid bilayers, making cell leakage happen.

Cell leakage in the culture was higher for the monoculture in both stages, with the exception of the 75/25 proportion (Table 1). In the analysis of interspecies competition, there was a difference for the 75/25 proportion in both evaluation periods, highlighting that the culture presents greater cell damages when competing within itself and, in lower population, in relation to ryegrass. Results indicate that, under this condition, intraspecies competition generates higher stress for the culture, causing an increase in production of species that are reactive to oxygen and, consequently, the rupture of membranes, originating cell leakage. The direct consequence of damages to cell membranes by lipid peroxidation is the leakage of cell content to the medium involving damaged tissues (Kruse et al., 2006). Higher indices of electrolyte leakage reflect higher permeability - which is also connected to oxidative stress (Li et al., 2000).

As for ryegrass, in the comparison between monoculture and proportions there was no difference in leakage on day 30 DAE, whereas on day 60 DAE, the highest values were in the 25/75 and 50/50 proportions (Table 1). Results indicate that, when weeds compete with proportions that are higher than or equal to the culture, both situations may cause higher cell leakage. During the occurrence of stress factors, both of abiotic and biotic origin, there are changes in membrane permeability and a reduction in solute retention abilities (Zhou et al., 2007).

The antioxidant defense system of plants includes different enzymes located in the different cellular compartments. Among the main enzymes, it is possible to highlight superoxide dismutase, ascorbate peroxidase and catalase, which promote EROs elimination (Apel and Hirt, 2004; Cavalcanti et al., 2004). The level of oxidative stress in the cell is determined by the quantity of hydrogen peroxide and superoxide and hydroxyle radicals. Therefore, the balance of superoxide dismutase and peroxidase activities is important in the suppression of EROs toxic levels in cells (Apel and Hirt, 2004). The regulation of SOD activity seems to be very sensitive to environmental changes, presumably as a consequence of the increase in the formation of oxygen radicals, since SOD is the first enzyme that acts in the detoxification process of superoxide radical ($O_2^-$) in $H_2O_2$(Gupta et al., 1991).
The activity of SOD enzyme for the culture was higher when this was associated to ryegrass, compared to monoculture, in the two evaluated stages (Table 2). Comparing proportions on day 30 and 60 DAE, the 25/75 proportion presented higher SOD activity, that is, the activity of this enzyme increased when interspecies competition occurred.

As for ryegrass, comparing monoculture and proportions, higher enzyme activity was verified for the weed monoculture on day 30 DAE, in relation to the 75/25 proportion, whereas on day 60 DAE, lower activity was observed in monoculture in relation to proportions. In the comparison between proportions, the highest activity was observed in the 25/75 and 50/50 associations on day 30 DAE, and in the 75/25 association on day 60 DAE.

Environment adversity often leads to an increase in the generation of EROs and, consequently, of SOD, which is an important enzyme in plant stress tolerance. The reduction or increase in enzyme activity may occur according to the function of other detoxifying enzymes. To minimize the effects of oxidative stress, plants have an antioxidant system and can activate other enzymes, such as guaiacol peroxidase (GPX) and ascorbate peroxidase (APX), which actively participate in the elimination and rupture of H$_2$O$_2$ molecules (Foyer and Noctor, 2003). Being the first line of defense against EROs, because of superoxide decomposition into H$_2$O$_2$, it is assumed that, when SOD activity increases, there is also an increase in the activity of enzymes that metabolize H$_2$O$_2$.

On day 30 DAE, higher CAT activity was observed in wheat for monoculture and 75/25 of the culture in relation to higher weed proportions; on the other hand, on day 60 DAE, when the culture was in interspecies competition, higher enzyme activity occurred (Table 2). A similar result was found for soybean competing with ryegrass (Oliveira et al., 2014).

In the comparison among associations, the highest activities were observed when wheat was in higher proportion than ryegrass (75/25, on day 30 DAE, whereas on day 60 DAE the highest activity was observed in equal proportion (50/50) between plants and in the higher proportion of weed competing with wheat (25/75) (Table 2). As for ryegrass on day 30 and 60 DAE, CAT activity was higher for interspecies competition in comparison with monoculture. Among proportions, both on day 30 and 60 DAE, the 75/25 proportion presented higher enzyme activity, that is, ryegrass presented higher enzyme activity when the culture had more individuals, causing culture competition over weed to intensify CAT production in ryegrass plants.

CAT is an enzyme that turns H$_2$O$_2$ into H$_2$O and O$_2$, working as a cleaning channel of cellular H$_2$O$_2$ (Moller, 2001). Wheat plants under cadmium stress presented increased CAT activity (Khan et al., 2007). The enzyme increase in this study possibly occurred due to H$_2$O$_2$ increase, making signaling occur and increasing CAT production to detoxify hydrogen peroxide in cellular compartments.

### Table 2 - Activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) enzymes, extracted from wheat and ryegrass leaves, in response to the competition in experiments in replacement series, on day 30 and 60 DAE

<table>
<thead>
<tr>
<th>Proportion</th>
<th>SOD (UA mg$^{-1}$ prot.min$^{-1}$)</th>
<th>CAT (UA mg$^{-1}$ prot. min$^{-1}$)</th>
<th>APX (UA mg$^{-1}$ prot. min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100/0 (T)</td>
<td>30 DAE</td>
<td>60 DAE</td>
<td></td>
</tr>
<tr>
<td>1.54</td>
<td>3.07</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>75/25</td>
<td>b5.76*</td>
<td>b6.21*</td>
<td>a0.61*</td>
</tr>
<tr>
<td>50/50</td>
<td>a7.39*</td>
<td>b6.52*</td>
<td>b0.49*</td>
</tr>
<tr>
<td>25/75</td>
<td>a7.24*</td>
<td>a9.11*</td>
<td>b0.53*</td>
</tr>
<tr>
<td>Ryegrass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/100 (A)</td>
<td>30 DAE</td>
<td>60 DAE</td>
<td></td>
</tr>
<tr>
<td>7.68</td>
<td>9.53</td>
<td>0.43</td>
<td>0.20</td>
</tr>
<tr>
<td>25/75</td>
<td>a7.61</td>
<td>b18.78*</td>
<td>b0.43*</td>
</tr>
<tr>
<td>50/50</td>
<td>a6.69</td>
<td>b20.34*</td>
<td>a0.48*</td>
</tr>
<tr>
<td>75/25</td>
<td>b3.34*</td>
<td>a31.40*</td>
<td>a0.65*</td>
</tr>
</tbody>
</table>

(1) Days after emergence; (2) Averages preceded by the same lowercase letter in the column, in proportions with plant mixes, do not differ among themselves by Tukey’s test (p≤0.05); (3) Averages followed in the column by * and + indicate non-significant and significant difference, respectively, by Dunnet’s test (p≤0.05) in relation to wheat (T) and ryegrass (A) monoculture.
The different affinities of APX and CAT for $\text{H}_2\text{O}_2$ suggest that APX is responsible for the fine signal modulation of reactive species, whereas CAT removes the excess during stress (Mittler, 2002). In wheat, APX did not show any difference between monoculture and proportions with ryegrass on day 30 DAE, whereas on day 60 DAE the interspecies competition caused higher enzyme activity than the monoculture (Table 2). As for proportion comparison, the increase in the competitor increased activity during tillering, whereas in the elongation stage there was no difference among associations.

With ryegrass, on day 30 and 60 DAE there was higher APX activity in monoculture, compared to proportions with wheat (Table 2). In the comparisons among associations, no difference was noticed on day 30 DAE, whereas on day 60 DAE the 25/75 proportion was the one presenting higher APX activity.

APXs are the main peroxidases in $\text{H}_2\text{O}_2$ removal from inside the cell, and they work synchronically with other enzymes that participate in the ascorbate-glutathione cycle (Foyer and Noctor, 2005). APX may have its activity increased in response to environmental stresses, such as salinity, low temperatures, metal poisoning, water deficit, high temperatures, ozone, high light intensity, pathogen attack, among others, as described for different plant species (Yoshimura et al., 2000; Sharma and Dubey, 2004).

Plants have different antioxidant system behaviors in specific stress situations, due to their adaptation ability when exposed to some stress factor (Carvalho et al., 2011). The observed results show that secondary metabolites, under stress conditions, are connected and present relation in the reduction and/or increase in contents of the studied compounds, as a response to oxidative stress.

The way in which plants recognize and respond to the environment will influence their competitive ability in a determined ecosystem and, thus, it has important consequences for the species’ success or failure. Competition among plants from the same species can be notably different from the one among plants from different species; this factor can change growth habits, metabolism and the expression of genes from the involved plants. Based on the above, competition may stimulate the secondary metabolism of wheat and ryegrass plants, through the antioxidant system, comparing with stresses induced by temperature, pathogens, insects, UV radiation, water deficit and nutrient deficiency.

Results demonstrated that both for wheat and ryegrass cultures, intraspecies competition has higher abilities of causing cell damages than interspecies competition. The activity of SOD, CAT and APX enzymes in the culture increased during interspecies competition. In ryegrass, interspecies competition also increased SOD and CAT activity, whereas for APX, weed monoculture created enzyme increase. Generally speaking, variable behaviors were similar between the tillering and stem elongation stages.

REFERENCES


AGOSTINETTO, D. et al. Metabolic activity of wheat and ryegrass plants in competition


