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Article

Interference of Volunteer Corn on Stress Metabolism and Yield of Dry Beans

Interferência de Milho Voluntário no Metabolismo do Estresse e Produtividade do Feijão

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ABSTRACT - Dry bean cultivation after corn favors the occurrence of volunteer corn plants which interfere with the crop and cause yield losses of dry bean. Yield losses resulting from interferences caused by corn may be related to oxidative stress, which, in turn, is caused by the higher production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2). This work aimed to quantify H_2O_2 contents and the activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in dry beans under interference with densities of volunteer corn F_2 originated from individual plants and clumps (seven corn plants emerged at the same point). Two experiments were carried out in a randomized blocks design with three replicates in Passo Fundo – RS, Brazil. Enzyme analysis was performed in a laboratory at 21, 35 and 46 days after dry bean emergence (DAE). Corn densities were 0, 0.5, 1, 2, 4, 8 and 12 plants or clumps m^{-2}. The results show changes in H_2O_2 levels and in the activity of SOD, CAT, and APX enzymes with the increase of corn densities, in which the highest activity occurred for SOD. The interference of volunteer corn with dry beans alters the stress metabolism of dry bean but does not cause oxidative stress. The yield of dry beans reduced under interference with volunteer corn F_2, but it is higher when the corn was originated from clumps.

Keywords: *Phaseolus vulgaris*, *Zea mays*, clumps, oxidative stress.

RESUMO - O cultivo do feijão após milho favorece a ocorrência de plantas voluntárias de milho, que interferem e causam redução da produtividade da cultura. As perdas na produtividade em função das interferências causadas pelo milho podem estar relacionadas com o estresse oxidativo decorrente da maior produção de espécies reativas de oxigênio (EROs), como o peróxido de hidrogênio (H_2O_2). Os objetivos deste trabalho foram quantificar os teores de H_2O_2 e verificar a atividade das enzimas superóxido dismutase (SOD), catalase (CAT), ascorbato peroxidase (APX) e produtividade do feijão sob a interferência de densidades e origens de milho voluntário RR® F_2. Foram realizados dois experimentos em campo, no delineamento de blocos casualizados com três repetições. O milho voluntário foi originado de plantas individuais (experimento 1) e de touceiras (sete plantas de milho no mesmo ponto) (experimento 2). As densidades populacionais de milho interferindo com o feijão foram de 0, 0,5, 1, 2; 4, 8 e 12 plantas ou touceiras m^{-2}, respectivamente nos experimentos 1 e 2. As quantificações enzimáticas foram feitas em laboratório aos 21, 35 e 46 dias após a emergência (DAE) do feijão. Os resultados demonstram alterações nos teores de H_2O_2 e na atividade das enzimas SOD, CAT e APX em função das densidades do milho voluntário, sendo que a atividade da SOD foi mais alta. As interferências causadas por milho voluntário no feijão alteraram o metabolismo do estresse das plantas, porém, não resultaram em estresse oxidativo.

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A produtividade do feijão reduziu de forma significativa sob interferência com densidades e origens do milho voluntário RR® F_2. A análise conjunta dos experimentos demonstrou que touceiras causam maiores perdas na produtividade do feijão em relação a plantas individuais, entre as densidades estudadas.

Palavras-chave: *Phaseolus vulgaris*, *Zea mays*, touceiras, estresse oxidativo.

INTRODUCTION

Worldwide, bean plants (*Phaseolus vulgaris*) are grown in approximately 30 million hectares. India, Myanmar, and Brazil account for approximately 40% of the global production of 23.3 million tonnes (FAO, 2015). In Brazil, dry beans are cultivated in about 3 million hectares (first and second crop seasons) in virtually all regions of the country. The Middle-South region is particularly important as far as second crops are concerned: with approximately 46% of the area, it accounts for about 80% of all production (CONAB, 2017).

In most regions, second crops are planted in succession to corn, which favors the occurrence of volunteer corn plants that interfere in the growth and development of dry bean plants. Volunteer corn plants are originated from unharvested seeds (abandoned areas) or those lost during the poor harvest process from the field. They occur in the form of individual seeds and pieces or whole ears containing several seeds, which originate, respectively, individual plants and clumps of volunteer corn F_2 (Marquardt et al., 2013; Piasecki et al., 2018a). Volunteer corn is considered to be one of the most problematic weeds in beans (Sbatella et al., 2016) and soybean (Piasecki et al., 2018a). The competitive capacity of corn is high as compared to that of beans because yield is reduced in weed densities with less than one plant or clump m^{-2} (Sbatella et al., 2016).

Competition is a type o interference and is established when at least one environmental resource is limited for plant development (Radosevich et al., 1997). Competition between plants involves crop and weeds and is related to aspects such as species, distribution, density, a period of coexistence, edaphoclimatic conditions (Pitelli, 1985; Silva et al., 2009) and, in the case of volunteer corn, whether they are individual plants or clumps.

Plants in the field are subject to various biotic, abiotic or xenobiotic factors that lead to the production of reactive oxygen species (ROS) (Caverzan et al., 2016). When occurring an excessive accumulation of ROS molecules, they cause oxidative stress in plant tissues (Tripathy et al., 2007), which results in damage to cellular structures and loss of homeostasis, and even cell death may occur (Caverzan et al., 2016). Hydroxyl radical (HO^-), superoxide radical (O^{-2}), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) are the main ROS and can cause damage to DNA, protein oxidation, and lipid peroxidation (Gill and Tuteja, 2010).

Plants under oxidative stress are subject to membrane peroxidation, which affects their photosynthetic systems inactivating the reaction centers (Tripathy et al., 2007). The process of lipid peroxidation forms hydroperoxides that are then converted into secondary products, such as ROS, free radicals, aldehydes, alkanes, oxyacids, jasmonic acid and methyl jasmonate (Vick and Zimmerman, 1987).

In plants, the balance between production and scavenging of ROS is sustained by enzymatic and non-enzymatic antioxidant defense systems (Mittler, 2002). The enzymatic antioxidant system includes the enzymes glutathione-S-transferase (GST), ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX), peroxidase (POD) and peroxiredoxins (Prxs). Also, to assist in the detoxification of ROS, the enzymes of the glutathione-ascorbate cycle, the central pathway of ROS elimination in plants act in the chloroplast, cytosol, mitochondria, apoplast, and peroxisomes, work joined with the antioxidant enzymes to efficiently metabolize toxic molecules and reduces the stress (Cavalcanti et al., 2004). Non-enzymatic substances, such as anthocyanins, tocopherols, phenolic components, glutathione, and ascorbate, also play an essential role in the control of ROS levels (Gill and Tuteja, 2010; Sharma et al., 2012).

This study hypothesized that the interference caused by volunteer corn densities originated from individual plants and clumps can alter the stress metabolism in dry beans and cause

oxidative stress. Thus, the objective of this study was to quantify the levels of H_2O_2 and the activity of enzymes SOD, CAT, APX and yield of dry beans under the interference of densities of volunteer corn RR® F_2 originated from individual plants and clumps.

MATERIAL AND METHODS

The interferences of beans with the densities of volunteer corn RR® originated from individual plants or clumps was determined in two experiments conducted at the field, at the Agricultural Research and Extension Center (CEPAGRO), at University of Passo Fundo (UPF), Passo Fundo, RS, Brazil. Analyses were performed in the laboratory of plant virology, UPF. The two experiments used a randomized blocks design with three replications. In Experiment 1, individual plants of volunteer corn were originated from seeds while in Experiment 2 the volunteer corn was originated from clumps. Each clump consisted of a segment of ear containing, on average, seven corn plants. In both experiments, the studied corn densities were 0, 0.5, 1, 2, 4, 8 and 12 individual plants or clumps m^{-2}, respectively.

The experiments were carried in the field, in a no-tillage system, in an area with residues of black oat (*Avena strigosa*) and ryegrass (*Lolium multiflorum*) previously controlled with herbicides clethodim (76.2 g i.a. ha^{-1}) and glyphosate 720 g a.e. ha^{-1}. In both experiments, corn and beans were sown on the same day, and corn emergence occurred one day before bean. Fertilization used for planting the beans consisted of 5.6 kg ha^{-1} of N, 78.4 kg ha^{-1} of P_2O_5 and 50.4 kg ha^{-1} of K_2O. Aditional nitrogen fertilization was performed at the stage V_3-V_4 (three to four trifoliolate leaves), with a broadcast application of 250 kg ha^{-1} of urea (112.5 kg ha^{-1} of N). The treatment of seeds and the management of insects – pest and pathogens – were performed according to the recommendations for the cultivation of beans in the South region of Brazil (EPAGRI, 2012). Manual weeding controlled other weeds.

The corn hybrid AG 8088 PRO_2® was harvested in the previous season. After storage in the form of ears, it originated the F_2 densities used in the experiments. The densities of volunteer corn were randomly distributed and, after that, they were manually buried to approximately 3.5 cm of depth, in 17.5 m^2 plots (3.5 x 5 m). Immediately after sowing of corn, the bean cultivar BRS Pérola (mid-cycle, relative maturity of 8.8, semi-erect stem) was sowed mechanically in a density value which resulted in the establishment of 24 bean plants m^{-2}, distributed in the inter-row spacing of 0.50 m (EPAGRI, 2012). After corn emergence, the densities were manually adjusted, according to each treatment.

In the two experiments, the leaves of bean plants were collected for quantification of H_2O_2 levels and enzyme activity for SOD, CAT, and APX at 21, 35, and 46 days after emergence (DAE) of the dry bean, which corresponded to the stages V_6-V_7, R_1, and R_3, respectively. In each experimental unit, five fully expanded trifoliolate leaves of the bean plants were randomly collected from the apex, forming the composite sample. Immediately after collection, the leaves were packed in plastic bags and frozen at -18 °C.

H_2O_2 concentration was determined by following the methodology of Sergier et al. (1997), with adaptations, and the samples were centrifuged at 5,600 rpm for 25 minutes. H_2O_2 concentration was determined by using the standard curve with known concentrations of H_2O_2 and expressed in mMol per gram of fresh weight (mM g^{-1} of FW).

The activity of antioxidant enzyme SOD, CAT, and APX was determined through the extraction in 0.2 g of sample macerated in a porcelain mortar, in the presence of liquid nitrogen and 0.02 g of polyvinylpyrrolidone (PVPP). Then, 900 µL of 200 mM phosphate buffer (pH 7.8), 18 µL of 10 mM EDTA, 180 µL of 200 mM ascorbic acid and 702 µL of ultrapure water were added and centrifuged at 5,600 rpm, at 4 °C for 25 minutes. The results were expressed in active units (AU) per milligram of fresh weight (mg^{-1} FW) per minute (min^{-1}).

SOD activity was determined according to the methodology adapted from Peixoto (1999), in which such activity was determined by calculating the amount of extract that inhibited 50% of the NBT reaction and then expressed in AU mg^{-1} fresh weight $minute^{-1}$. By this method, the inhibition of NBT reduction (ρ-Nitro Blue Tetrazolium) was determined by using the enzymatic extract, thus preventing the formation of the chromophore. In this experiment, one unit of enzyme

activity (AU) of SOD was defined as the amount of enzyme required to inhibit 50% of the NBT reduction by SOD contained in the enzyme extract. For calculation purposes, the reagent blank was considered to be in the tube that contained no extract, exposed and not exposed to light.

APX and CAT activities were determined according to the methodology described by Azevedo et al. (1998), by consumption of H_2O_2 (extinction coefficient of APX of 2.9 mM cm^{-1} and extinction coefficient of CAT of 39.4 mM cm^{-1}). For calculation of the activities of CAT and APX, it was considered that the decline of an absorbance unit was equivalent to an active unit (AU). The activity of the total extract was determined from the quantity of extract which reduced the reading of absorbance in an AU, expressed in AU mg^{-1} fresh weight per minute.

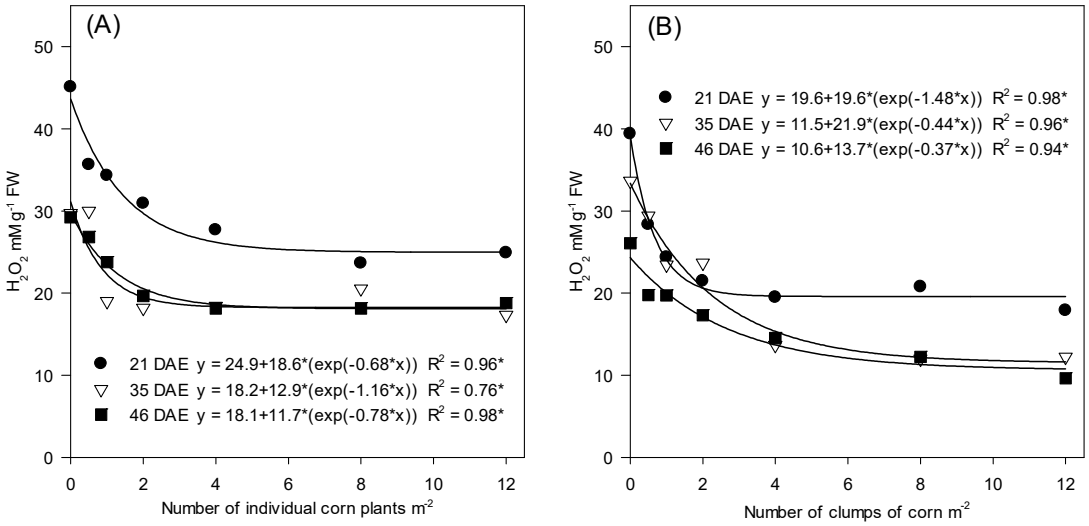
A sample area of 7.5 m^2 in each experimental unit was harvested and used to calculate the dry bean yield. Following the harvest, the grain moisture content was measured and corrected to 13%, and the grain yield per hectare (kg ha^{-1}) was estimated.

All collected data were analyzed for normality with the Shapiro-Wilk's test and homoscedasticity with Hartley's test and, subsequently, submitted to analysis of variance (ANOVA, $p<0.05$). For analyses of enzyme activity and bean yield, the effect of the densities was calculated using regression analysis at $p<0.05$. The qualitative results of the origins of volunteer corn on dry beans yield were assessed with a joint analysis performed of the experiments, according to Banzatto and Kronka (2006). The analyses were performed in routines generated in the software program SAS® version 9.00 (SAS, 2002).

RESULTS AND DISCUSSION

H_2O_2 levels in bean plants analyzed at 21, 35, and 46 DAE (days after emergence) decreased with the increase of the density of individual plants (Exp. 1) and corn clumps (Exp. 2) (Figure 1). In both experiments, the effects tended to stabilize from the density of four individual plants and two clumps m^{-2} (Figure 1).

According to these results, the factors that could be responsible for the highest levels of H_2O_2 in beans under low interference against individual plants or clumps of corn would be higher incident radiation and temperature intercepted by the crop, because of under the highest corn density more shading was given to the bean plants. In this way, the lower incident radiation on the beans in interference with the largest densities of corn probably formed a microclimate with mild environmental conditions in the region of plant canopies. The fact that H_2O_2 levels were



* Significant at $p<0.05$. Passo Fundo, RS.

Figure 1 - Levels of hydrogen peroxide (H_2O_2 mM g^{-1} FW) in dry bean leaves, cultivar BRS Pérola, resulting from the interference of densities of individual plants (A) and clumps (B) of volunteer corn RR® F_2, analyzed at 21, 35 and 46 days after emergence (DAE).

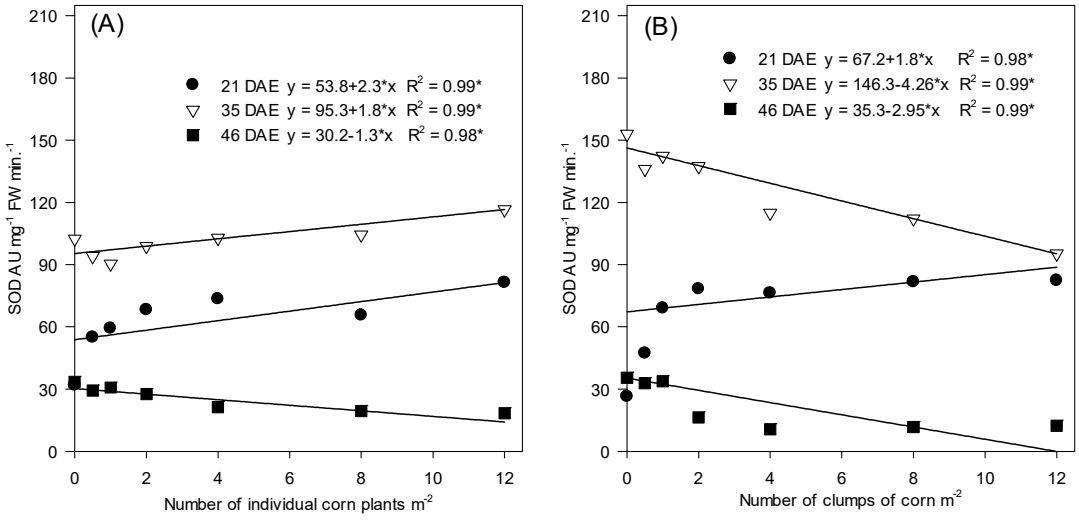
higher at 21 DAE than at 35 and 46 DAE corroborates this assertion because, in the initial phase of development, there was less shading because the corn plants were shorter (Figure 1).

Environmental stresses cause disorders in the metabolism of plants and oxidative injury as a result of increased production of ROS (Mittler, 2002; Caverzan et al., 2016). H_2O_2 diffuses into cells and is regarded as a stress messenger, spreading the oxidative damage to plant cells (Barbosa et al., 2014). Redox homeostasis is the balance between production and elimination of ROS (Sharma et al., 2012). On the other hand, an imbalance in redox homeostasis results in fast and transient excess of ROS, known as oxidative stress (Mullineaux and Baker, 2010). In this way, defense mechanisms are activated against oxidative damage to regulate the toxic levels of ROS (Caverzan et al., 2016). To prevent oxidative damage to tissues, even at low levels of H_2O_2, there is an increase in the activity of antioxidant enzymes in plant cells (Agostinetto et al., 2016).

The activity of enzyme SOD in leaf tissues of beans increased linearly at 21 and 35 DAE when in under interference with individual plants (Figure 2A) and at 21 DAE with clumps (Figure 2B). On the other hand, SOD activity decreased linearly at 46 DAE under interference with individual plants (Figure 2A) at 35 and 46 DAE with clumps (Figure 2B).

SOD participates in the modulation of the level of H_2O_2 in chloroplasts, mitochondria, cytosol and peroxisomes (Mittler, 2002), and it is the first enzyme which acts in the process of detoxification by catalyzing the dismutation of O^{2-} into H_2O_2 and O_2 (Gill and Tuteja, 2010). The produced H_2O_2 is converted into H_2O by enzymes APX and CAT (Wang et al., 2004). Thus, it is expected that, when SOD activity increase, there may also occur an increase in the activity of enzymes CAT and APX, which metabolize H_2O_2 (Agostinetto et al., 2016), but this increase did not happen in this study. In the present study, there was the higher activity of the enzyme SOD in the detoxification of ROS, which shows that it has a role in oxidative protection in bean plants.

The possible action of non-enzymatic factors or non-evaluated enzymes which can eliminate H_2O_2 produced by SOD activity is likely to have controlled the levels of H_2O_2, particularly under the conditions studied in beans. In addition to CAT and APX, H_2O_2 formed by the action of SOD can be converted into H_2O_2 by the action of the enzymes POD (Locato et al., 2010), PRX and GPX (Barbosa et al., 2014). Another possibility is that the action of SOD on O^{2-} may have produced higher levels of $^1O_2$ and, for this reason, the concentrations of H_2O_2 did not increase despite the increased interference of volunteer corn densities with dry beans. Although $^1O_2$ is less reactive than $OH^{\bullet}$, it is more reactive than H_2O_2 and O_2, hence it is considered to be a highly toxic molecule (Barbosa et al., 2014).

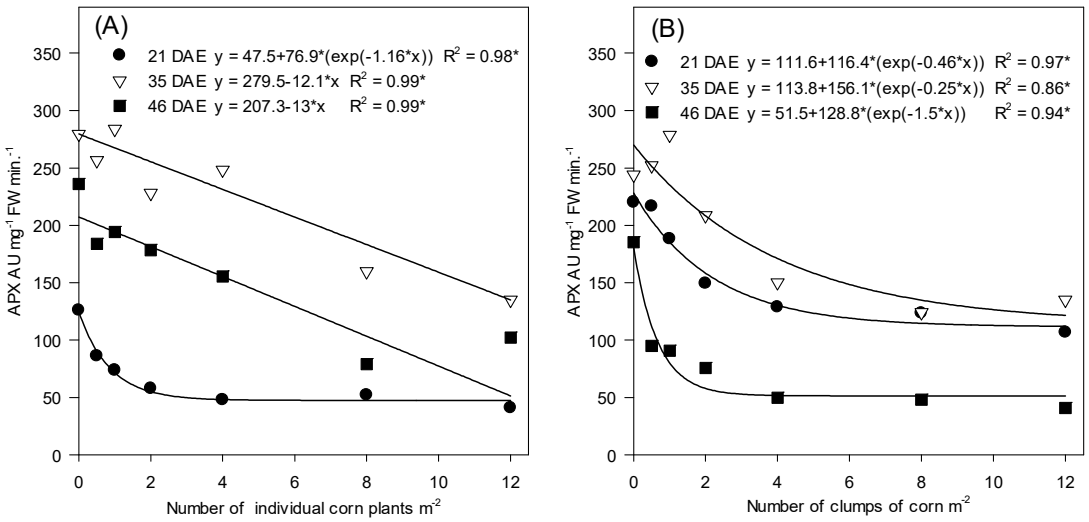


* Significant at $p<0.05$. Passo Fundo, RS.

Figure 2 - Activity of the enzyme superoxide dismutase (SOD) (AU mg^{-1} FW $min.^{-1}$) in dry bean leaves, cultivar BRS Pérola, resulting from the interference of densities of individual plants (A) and clumps (B) of volunteer corn RR® F_2, analyzed at 21, 35 and 46 days after emergence (DAE).

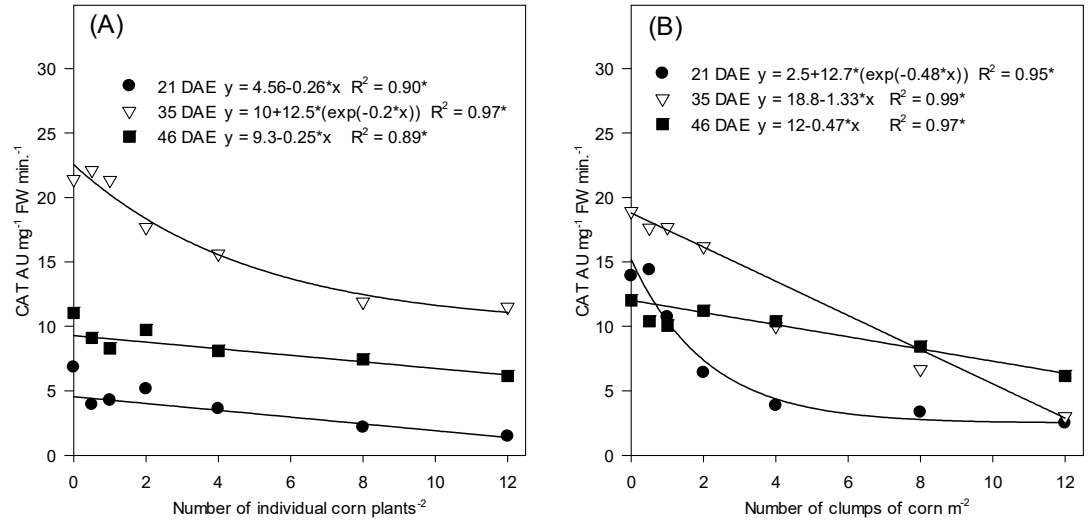
The activities of APX and CAT reduced in dry beans as a result of the increase in corn density at the evaluation times (Figures 3 and 4). As the levels of H_2O_2 in beans also decreased with the interference of corn increase, this may be a possible explanation for the reduction in the activity of APX and CAT (Figures 3 and 4). Also, APX and CAT are not enzymes that act on the front line in the process of extinction of $^1O_2$, which was probably generated in a higher amount through the action of SOD over O^-_2 (Triantaphylides and Havaux, 2009). For this reason, there was probably the no greater production of enzymes APX and CAT (Figures 3 and 4).

APXs are present in the cytosol, mitochondria, peroxisomes, and chloroplasts (Dabrowska et al., 2007). In chloroplasts and mitochondria, APX reduces H_2O_2 formed by the action of SOD and water through the use of ascorbate as an electron donor (Locato et al., 2010). CAT acts on glyoxysomes and peroxisomes, and it can be found in mitochondria (Barbosa et al., 2014). It converts two molecules of H_2O_2 into the water and molecular oxygen (Dubey, 2011).



* Significant at p<0.05. Passo Fundo, RS.

Figure 3 - Activity of the enzyme ascorbate peroxidase (APX) (AU mg^{-1} FW min.$^{-1}$) in dry bean leaves, cultivar BRS Pérola, resulting from the interference of densities of individual plants (A) and clumps (B) of volunteer corn RR® F_2, analyzed at 21, 35 and 46 days after emergence (DAE).



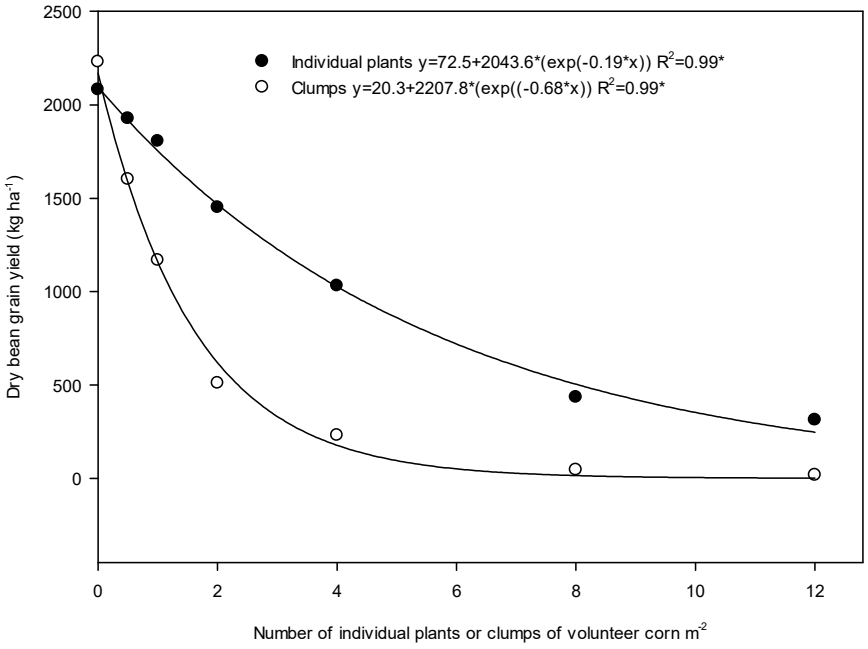
* Significant at p<0.05. Passo Fundo, RS.

Figure 4 - Activity of the enzyme catalase (CAT) (AU mg^{-1} FW min.$^{-1}$) in dry bean leaves, cultivar BRS Pérola, resulting from the interference of densities of individual plants (A and clumps (B of volunteer corn RR® F_2, analyzed at 21, 35 and 46 days after emergence (DAE).

The enzyme APX and CAT work together against the excessive accumulation of H_2O_2: APX is responsible for the fine modulation of reactive oxygen species while CAT removes excess ROS (Mittler, 2002; Gill and Tuteja, 2010). APX has a higher affinity for H_2O_2 (Locato et al., 2010) as compared to CAT (Gill and Tuteja, 2010). Similar results were found by Piasecki et al. (2018b) when assessing these variables in the interference of volunteer corn with soybean.

The yield of dry beans under interference with densities and origins of volunteer corn decreased significantly in both experiments (Figure 5; Table 1). For beans under interference with 0.5, 1, and 2 individual plants m^{-2}, the reduction was 155, 276, and 631 kg ha^{-1}, respectively, compared to the weed-free control (Figure 5). When dry beans under the interference of 0.5, 1, and 2 clumps m^{-2}, yield loss was 480, 914, and 1,572 kg ha^{-1} in comparison to the control without interference, respectively (Figure 5).

From the eight individual plants or four clumps of volunteer corn m^{-2}, there was a tendency for stabilization of the effects of interference on bean yield losses, and virtually no grain from



* Significant at p<0.05. Passo Fundo, RS.

Figure 5 - Dry bean yield (kg ha^{-1}), cultivar BRS Pérola, resulting from the interference of densities of individual plants or clumps of corn RR® F_2.

Table 1 - Results of mean dry bean yield, cultivar Pérola (kg ha^{-1}) resulting from the interference of densities of individual plants or clumps of volunteer corn RR® F_2.

Volunteer corn population m^{-2}	Origin of volunteer corn*		p<0.05
	Individual plant	Clumps	
0	2081.3 B	2229.5 A	0.0494
0.5	1925.7 A	1601.4 B	0.0001
1	1805.1 A	1167.3 B	0.0001
2	1450.3 A	508.9 B	0.0001
4	1030.9 A	229.1 B	0.0001
8	433.6 A	44.2 B	0.0001
12	311.2 A	16.8 B	0.0004
VC (%)	8.29		
R^2	0.99*		

* Significant at p<0.05. Passo Fundo, RS. Means of separate analyses of simple effect, followed by the same letter on the row, differ statistically for the qualitative factor 'origins' at the probability of p<0.05.

this density was produced when under interference with clumps (Figure 5). A similar effect stabilization behavior was found in most of the evaluated metabolic variables.

The analysis of the qualitative variables (origins of corn) through the joint analysis of yield losses data of the two experiments showed that clumps are more competitive than individual plants (Table 1). In other words, in the same density with individual plants, clumps cause higher dry bean yield losses. These results are attributed to the higher number of corn plants that clumps have at one point. Thus, interference is more intense.

The results show the high competitive capacity of volunteer corn originated of individual plants and clumps against beans, even in densities with less than one plant or clump m^{-2} (Figure 5; Table 1). Also, the highest dry bean yield losses occurred because of the interference with corn and not by higher production of H_2O_2, which could indicate the occurrence of oxidative stress in bean plants. On the other hand, the times of quantification of enzymatic variables in beans may have influenced the results, since the last evaluation time (46 DAE) amounted to approximately one-third of the crop cycle. Thus, evaluations at later times in the crop cycle could show a differential behavior from those observed at that point.

Interference from volunteer corn in dry beans causes changes in their metabolism but does not oxidative stress. The interference of volunteer corn in dry bean reduces the yield significantly. Volunteer corn densities lesser than one plant or clump m^{-2} cause a significant reduction of yield; conversely, the dry bean yield losses is higher when corn is originated from clumps.

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