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Expression of catalase, alcohol dehydrogenase, and malate dehydrogenase in rot grains upon fungicide use on maize hybrids grown at different spacings

E.R. Kluge[1], M.C. Mendes[1], M.V. Faria[1], H.O. Santos[2], L.A. Santos[1] and I.E. Sandini[1]

[1]Departamento de Agronomia, Setor de Ciências Agrárias e Ambientais, Universidade Estadual do Centro-Oeste, Guarapuava, PR, Brasil
[2]Departamento de Agricultura, Setor de Sementes, Universidade Federal de Lavras, Lavras, MG, Brasil

Corresponding author: E.R. Kluge
E-mail: elizandrokluge@gmail.com

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ABSTRACT. In this study, we evaluated the fungicide effect on the incidence of rot grains and expression of catalase (CAT), alcohol dehydrogenase (ADH), and malate dehydrogenase (MDH) enzymes in commercial maize hybrids grown with conventional and reduced spacing in Guarapuava, PR, Brazil. The experiment was designed in random blocks with a 3 × 8-factorial scheme, totaling 24 treatments. The first factor constituted three levels, the first with foliar fungicide application [150.0 g/L trifloxystrobin (15.0%, w/v) + 175.0 g/L prothioconazole (17.5%, w/v)] at a dose of 0.4 L/ha at V8-stage eight expanded leaves and the second with an application of 0.5 L/ha at VT-tasseling and check (no fungicide application) stage. The second factor comprised eight maize hybrids that were divided into two groups, complex (AG 9045PRO, AG 8041PRO, DKB245PRO2, and 2B707PW) and susceptible (P 32R48H, DKB390PRO, P 30F53H, and P 30R50H), according to their reaction to the causative fungus, totaling 72 plots at each site in the crop of 2013/2014. The percentage of rot grains and the expression of CAT, ADH, and MDH were evaluated for each hybrid. The percentage of rot grains was influenced by the hybrid and fungicide used. The (trifloxystrobin + prothioconazole) reduced the incidence of rot grains, with relatively higher reduction in the hybrids considered susceptible. The higher expression of CAT enzyme was related to the higher incidence of rot grains because of grain deterioration, depending on the hybrids evaluated. A higher expression of ADH and MDH enzymes was observed in the maize hybrids belonging to the group considered tolerant.

Key words: Chemical check; Catalase; Alcohol dehydrogenase; Malate dehydrogenase; Reduced spacing; *Zea mays*

INTRODUCTION

The Center-South region of Paraná is considered as the major producer of maize. Owing to its high climate suitability for maize production, this region has obtained high yields in maize production. The maize crop has a broad geographical scope, covering various soils and climatic conditions; therefore, the maize crop remains more exposed to the disease-causing pathogens, leading to a dynamic interaction between the pathogen, host, and cultivation environment. These pathogens cause a reduction in grain yield and quality. A fungal infection disrupts the normal process of grain filling, reducing the weight of maize cobs.

The stalk and cob rot are mainly caused by the fungal species *Stenocarpella macrospora*, *Fusarium vertillioides* and *F. graminearum*, which affect the crop yield and grain quality (Casa, 1997).

Thus, it becomes increasingly important to select the correct genetic material (Mendes et al., 2012). In recent years, several management strategies have been devised to reduce plant diseases in a sustainable manner, using crop rotation, disease-resistant genotype, and chemical control.

A sequential degradation of lipids, which are the primary products of the lipoxygenase reaction, occurs when plant tissues are damaged by pathogens or mechanically. After activation, these enzymes oxidize the fatty acids, producing a certain concentration of aldehydes and volatile compounds, which inhibit the formation and development of fungus in grains (Mendes et al., 2012). The deterioration is considered a degenerative change produced after the seed has reached its maximum quality level. Furthermore, respiratory enzymes, such as alcohol dehydrogenase (ADH) and malate dehydrogenase (MDH), are involved in the deterioration of seeds, besides those related to the removal of free radicals such as catalase (CAT), etc.

Currently, the adoption of reduced spacing associated with the use of modern and conventional maize hybrids has changed the spatial arrangement of maize plants in the field, promoting an alteration in the culture microclimate in a positive way. The reduced spacing increases the interception of photosynthetically active radiation by the canopy, leading to an increase in the absorption efficiency of nutrients and water, there by exerting influence on the maize grain yield. It might negatively influence the tolerance of hybrids to grain diseases and in the control efficiency of the fungicides active principles and applied preventively.

The efficiency of the chemical control to manage rot grains in maize, especially in relation to the efficiency of products and/or number of applications and susceptibility of the hybrid used, is not yet clear. Further research that can clarify the effects of fungicides in pathogens associated with cob diseases is required.

Thus, due to the launch of new maize hybrids with high yield potential in the market every year, the importance of the research work to elucidate the effect of fungicide application in maize, especially its association with the activity of specific enzymes and control of the pathogens causing cob rot, is quite evident. However, the effect of chemical compounds on the activity of enzymes in maize with genotypes resistant to the pathogens causing rot grains should be better studied in Brazil. In this view, the aim of this work was to evaluate the effect of fungicide application in rot grains and the expression of catalase, alcohol dehydrogenase, and malate dehydrogenase enzymes in the grains of commercial maize hybrids grown with conventional and reduced spacing in Guarapuava, PR, Brazil.

MATERIAL AND METHODS

Two experiments were installed in the maize crop of 2013/14. The first experiment (environment 1) was installed in the experimental field (conventional row spacing of 0.70 m), Sector of Agricultural and Environmental Sciences, Department of Agronomy at the Universidade Estadual do Centro Oeste-UNICENTRO, Campus CEDETEG in Guarapuava county, PR, Brazil. It is located at an altitude of 1028 m, with 25°23'04.83"S latitude and 51°29'4432"W longitude. The installation of the experiment occurred in the no-tillage system, in an area with white oat (*Avena sativa*) as ground cover.

The second experiment (environment 2) was installed at Três Capões Farm (reduced row spacing of 0.45 m) in the county of Guarapuava, PR. This location has an average altitude of 948 m, 25°26'57.79"S latitude, and 51°38'29.18"W longitude. This experiment was conducted in the no-tillage system, in areas with white oat (*Avena sativa*) as ground cover. The topography was considered plane. This region has a humid mesothermal subtropical climate. According to Köppen classification, the climate was Cfb type, with an undefined dry season, cool summers, and winters with severe and frequent frosts. The annual average temperature was 16.8°C, ranging from 6.8°C (minimum average) to 36°C (maximum average), and the annual average total rainfall was 1500 mm with annual average relative humidity of 77.9%. The soil was classified as dystrophic haplohumoxoxisol with aclayey texture (Embrapa, 2006).

The experimental design was in random blocks, with three replications, in a 3 x 8-factorial scheme, totaling 24 treatments. The first factor was constituted by three levels, the first with foliar fungicide application [150.0 g/L trifloxystrobin (15.0%,w/v) + 175.0 g/L prothioconazole (17.5%,w/v)] at a dose of 0.4 L/ha atV8-stage with eight expanded leaves, and the second was done with the application of 0.5 L/ha at VT-stage with tasseling, considering the phenological scale of Ritchie et al. (1993), and check (no fungicide application). The second factor comprised eight maize hybrids that were divided into two groups, tolerant (AG 9045PRO, AG 8041PRO, DKB245PRO2, and 2B707PW) and susceptible (P 32R48H, DKB390PRO, P 30F53H, and P 30R50H), according to their reaction to the causative fungus of rot grains complex, totaling 72 plots in each site (environment) in the crop of 2013/2014.

The experiments were installed in the first fortnight of October 2013 and the harvest occurred after physiological maturity in the second fortnight of March 2014. The first experiment had four rows with a length of 5 m and a spacing of 0.70 m between rows, and a total area of 14 m^2 per plot. The second experiment had four rows with alength of 5 m and a spacing of 0.45 m between rows, and a total area of 9 m^2 per plot. However, in both experiments, only two central rows of each plot were used.

The fungicide applications were carried out with the aid of a CO_2-pressured costal sprayer, equipped with four nozzles, hollow cone spray nozzle (0.3) spaced 50 cm, with a consumption of 200 L/ha and a displacement speed of 3.6 km/h. The weather conditions at the time (start and end) of fungicide application were monitored by a digital anemometer, and the meteorological data was collected at the IAPAR/UNICENTRO/CEDETEG experimental station, located approximately 500 m from the area of the first experiment.

The agronomic characteristics, such as the incidence of rot grains and expression of CAT, ADH, and MDH enzymes, were evaluated in the laboratory. The incidence of rot grains was determined according to the procedure proposed in the ordinance No. 11, dated April 12, 1996 (Brasil, 1996).

This method involves the visual separation of the rot grains from the healthy grains followed by the determination of the percentage of rot grains showing discoloration in more than a quarter of the total area (Pinto, 2007). The grains were homogenized followed by the removal of a representative sample of 250 g from each harvested plot. The rot grains were weighed and the weight values were transformed into percentage values.

The enzymatic analyses were performed in the Central Laboratory of Seeds, belonging to the Department of Agriculture of the Universidade Federal de Lavras (UFLA). To perform the analysis, composite grain samples were used, derived from the three replicates of each treatment, and the gel analysis was performed according to the method described by Alfenas (2006).

The dry seeds were manually macerated in the presence of the antioxidant polyvinylpyrrolidone (PVP) and liquid nitrogen in porcelain crucibles until a thin powder is obtained and stored in a deep freezer at -86°C. From this material, 100 -mg samples were weighed and used for analysis. The samples were placed in a microcentrifuge tube (Eppendorf, USA) to which 250 µL extraction buffer (0.2 M Tris-HCl, pH 8.0) and 0.1% β-mercap to ethanol were added for the extraction of the isoenzymes ADH, MDH, and CAT. These were kept in the refrigerator for 12 h. Subsequently, the tubes were centrifuged at 14,000 g for 30 min at 4°C. The polyacrylamide gel electrophoresis was carried out in a discontinued system (7.5% separation gel and 4.5% concentration gel). The Tris-glycine buffer system, pH 8.9, was used for gel electrophoresis. To carry out the electrophoretic run, 50 µL of each supernatant was applied on the gel groove and the running was made at 4°C at 150 V, for approximately 4 h. Subsequently, the gels revealed the expression of particular enzymes in the presence of specific substrates (Alfenas, 1998).

All data obtained from the evaluated characteristics were submitted to the homogeneity of variances by Harley test (Ramalho et al., 2000). Thereafter, the individual and joint variance analyses were performed on the cultivation environments, and the averages were grouped by the Scott-Knott test at 5% probability level by using the statistical software SISVAR® (Ferreira, 2011).

The contrasts among the averages were carried out, and eight hybrids were separated into two groups, one considered tolerant (T) and the other considered susceptible (S) to the rot grain complex. Five orthogonal contrasts (T *vs* V8, T *vs* V8+VT, V8 *vs* V8+VT, G1 *vs* G2, and AMB1 *vs* AMB2) were performed to compare the hybrids, groups of hybrids, treatments, and environments, in relation to the incidence of rot grains, obtained for the different hybrids and fungicide treatments (T-check, V8-eight expanded leaves and VT-tasseling) in the two environments studied.

RESULTS AND DISCUSSION

It is important to emphasize that in the crop of 2013/14, in environment 1 - CEDETEG (conventional spacing), an accumulated rainfall level of 1008 mm was observed throughout the culture cycle. During the initial phase of the experiment, shortly after the sowing in October and November, an accumulation of 230 mm of rainfall was verified, ensuring a good initial development of the culture. A cumulative of 778 mm of rainfall was observed during the fungicides application and in the posterior months until the harvest. The rainfalls during the experiment implementation in environment 1 were obtained from the meteorological station of the Campus CEDETEG.

In environment 2 - Três Capões Farm (reduced spacing), also located in Guarapuava-PR, an accumulated rainfall of 896 mm was observed during the culture cycle. During the initial phase of experiment, shortly after the sowingin October and November, a good rainfall volume of 215 mm was observed, ensuring good initial development for the culture. During fungicide application and in the posterior months until harvest, was observed rainfall for the study area, with a cumulative of 681 mm. The rainfall data during experiment implementation in environment 2 were obtained from the meteorological station of Três Capões Farm.

The different results obtained for foliar diseases can be explained by the rainfall accumulation values, and for the appearance of most of these diseases, the weather and environment play an important role in the plant infection. In this study, we have pointed out the importance of the relative humidity, temperature, precipitation and different spacings as important factors, which might influence the appearance of foliar diseases and rot grains, especially those caused by fungus, in the culture.

In this regard, it is noteworthy that these characteristics can be used to evaluate the efficacy of the active principle as a method of foliar disease management and the pathogens that cause rot grains in maize.

The use of contrasts is a simple way to analyze the experimental data related to the main effects and the effects of comparison among groups of evaluated treatments (Nogueira, 2004).

For the contrasts involving the agronomic characteristic of the incidence of rot grains, the following contrasts were significant with over 95% of probability, T *vs* V8, T *vs* V8 + VT, V8 *vs* V8+VT, G1 *vs* G2, and AMB1 *vs* AMB2 (Table 1). The estimation of the contrast to this evaluated characteristic, involving the treatment T, V8 and AMB1 indicate their numerical superiority. For the contrast G1 *vs* G2, it was negative for G2, implying numerical superiority, and therefore, higher incidence of rot grains.

Table 1. Probability significance of the contrast for the incidence of rot grains, obtained for the different hybrids and treatments with fungicide (T - check, V8-eight expanded leaves and VT- tasseling), in two environments, Guarapuava-PR, in the crop of 2013/14. UNICENTRO. 2016.

	CONTRASTS[1]				
	T *vs* V8	T *vs* V8 + VT	V8 *vs* V8 + VT	G1 *vs* G2	AMB1 *vs* AMB2
Rot Grains	0.03	0.01	0.01	0.01	0.01

[1]T (check); V8 (Trifloxystrobin + Prothioconazole at 0.4 L/ha by foliar application at V8-stage eight expanded leaves); VT (Trifloxystrobin + Prothioconazole at 0.5 L/ha at VT stage - tasseling); G1 (Group 1 - tolerant); G2 (Group 2-susceptible); AMB1 (Environment 1 - conventional spacing); AMB2 (Environment 2 - reduced spacing).

The significance of the contrast between the treatments (T *vs* V8) for the percentage of rot grains, shows the efficiency of the fungicide trifloxystrobin (strobilurin) + prothioconazole (triazolinthiones) in an application at V8 stage, to the control of the fungal species, *Puccinia sorghi* and *S. maydis*, the causative agents of foliar diseases and rot grains.

The contrasts among the treatments (T *vs* V8 + VT) and (V8 *vs* V8 + VT) were significant for the percentage of rot grains, showing the efficiency of the fungicide trifloxystrobin+ prothioconazole in the two applications for controlling *Puccinia sorghi* and *S. maydis*, which might have affected the grain filling.

In the contrast between the hybrids considered tolerant *vs* those considered susceptible (G1 *vs* G2), the percentage of rot grains was significant, indicating the existence of genotypes with higher resistance to *S. maydis* that harms the quality of grains. According to Mendes et al. (2012), comparing the hybrids considered resistant with those considered susceptible to the rot grains complex and evaluating their grain yield and percentage of rot grains, showed the existence of genotypes with higher resistance to the fungal species *F. verticillioides*, *S. maydis*, and *S. macrospora*. The same author found no association between the grain yield and percentage of rot grains, showing that the losses caused by the incidence of rot grains in maize were not quantitative. The development of tolerant cultivars is the most effective way to control foliar diseases in maize, and the discrimination of resistance is more reliable when the genotypes are evaluated based on the area under the disease progress curve, since it includes many measurements of the disease severity throughout the production cycle (Vieira et al., 2009).

The significance of the contrast between AMB1 *vs* AMB2 for the percentage of rot grains showed the existence of climatic factors in different cultivation environments and interference of the sowing spacingin the occurrence of the fungus *S. maydis*. These data corroborate the study of Zanatta (2013) in which the same fungicide (trifloxystrobin+ prothioconazole) was used in reduced spacing and the two crops were compared at V8 stage reported no reduction in the incidence of rot grains in the evaluated hybrids.

It is important to emphasize that this high significance, more than 95% of probability, for the contrasts involving one and two fungicide applications and the use of hybrids considered tolerant to the rot grain complex (*S. macrospora*) justifies the use of fungicides in maize, as shown by the evaluation of foliar diseases and grain quality. Taking into consideration the active ingredients of the new fungicide, these results were already expected, especially the response of this new fungicide as well as the responses of the selected hybrids and chosen environments.

The gel showing the expression of CAT, front to the treatments evaluated with fungicides obtained in environment 1, in the crop of 2013/14, is shown in Figure 1.

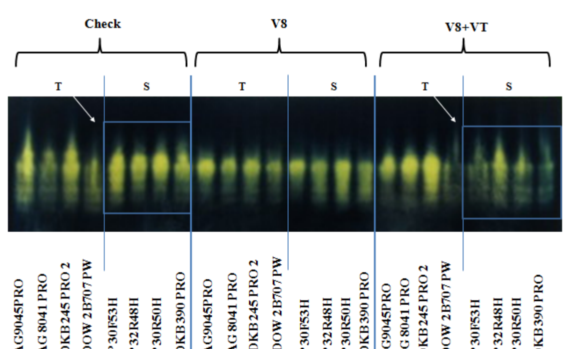

Figure 1. Catalase (CAT) electrophoretic pattern in maize hybrids, considered tolerant and susceptible to the causative agent of rot grains, produced in environment 1, in Guarapuava-PR, crop 2013/14. UNICENTRO. 2016.

The expression patterns of CAT in the grains produced in environment 1 are shown in Figure 1. A higher expression of the CAT enzyme was observed in the chemical check for the hybrids belonging to the group considered susceptible to rot grains (group 2). In group 1, a lower expression of the enzyme was observed, highlighting the hybrids AG8041PRO and 2B707PW. Based on the data presented in Table 1, the percentage of rot grains was significant, showing the existence of genotypes with higher resistance to *S. maydis*. The hybrids belonging to group 1 showed a lower incidence of rot grains, compared to those belonging to group 2. Thus, it can be stated that the higher expression of CAT enzyme is related to the higher incidence of rot grains because of the deterioration of grains. According to Timoteo and Marcos - Filho (2013), while evaluating the seed storage in different environments, a higher CAT activity was observed in the controlled environment when compared to the laboratory and cold chamber. This increase might have occurred due to the environmental conditions, 20°C and 70% of relative humidity, which contributed to the acceleration of seed deterioration. The deteriorated seeds either showed CAT activity or lacked it; it was also observed in the seeds of the hybrids stored for 15 months in a controlled environment. The CAT removes free radicals, and the loss of its activity might partially explain the fact that these aged seeds accumulate more hydrogen peroxide. The results of this study reinforced the data analyzed by Corte et al. (2010) and Nakada et al. (2010), who observed an increase in the lipid peroxidation with an increase in the seed deterioration. Thus, the lower expression of CAT can make the seeds more sensitive to the effects of free radicals and enhance the formation of cellular peroxide, and with that become more sensitive to the loss of viability. This can be confirmed by the reduction in the final germination periods of storage.

In the V8 treatment, the same expression of the CAT was observed for the two groups of hybrids. However, in the treatments V8 + VT, the same expression was not observed; however, a higher expression of CAT enzyme was observed for the hybrids belonging to the group considered tolerant to rot grains, AG9045PRO, AG8041PRO, DKB245PRO2, with the exception of 2B707PW hybrid, from the same group (Figure 1).

This increase in the enzyme expression might be related to the application of trifloxystrobin+ prothioconazole. It can be inferred that one of the possible contribution of the fungicide used, when applied at V8 + VT, is on the route of the expression of the enzyme. The reduction in the CAT activity might make the seed more sensitive to the effects of O_2 and free radicals on membrane unsaturated fatty acids and the products of the peroxidation of secondary lipids (Gomes et al., 2000). The catalases are oxidoreductase enzymes, which are present in all plant cells, animals, and aerobic microorganisms (Scandalios, 1990). They can be found in the cytoplasm, mitochondria, leaf peroxisomes, and tissue glyoxysomes (Frugoli et al., 1996).

They are the important catalysts for converting of hydrogen peroxide (H_2O_2) into H_2O and O_2 in cells.

The gel revealing the expression of CAT, front to the treatments evaluated with fungicides obtained in environment 2, in the crop of 2013/14, is shown in Figure 2.

The expression of catalase enzyme (CAT) was determined for all hybrids studied and all treatments evaluated in environment 2 in the crop of 2013/14 (Figure 2). The check treatment in environment 1 led to the observation of a higher expression of CAT enzyme in the hybrids belonging to group 2 and considered susceptible to rot grains. In group 1, a lower expression of the enzyme was observed, highlighting the hybrids AG9045PRO, AG8041PRO, and 2B707PW. Based on the data presented in Table 1, the percentage of rot grains was significant, which showed the existence of genotypes with higher resistance to *S. maydis* in the hybrids belonging to group 1 and have a lower incidence of rot grains as compared to group 2. Thus, it can be stated that a relationship exists between the higher expression of CAT enzyme and the higher incidence of rot grains because of the deterioration of grains. According to Aebi (1984), the catalase enzyme has two functions: the catalytic activity for decomposing hydrogen peroxide into water and oxygen and the oxidation of hydrogen donor compounds, such as methanol, ethanol, formic acid, phenols, with the consumption of one-mole peroxide.

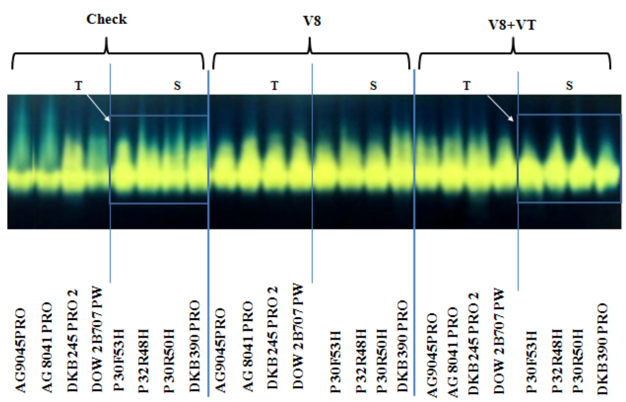

Figure 2. Catalase electrophoretic pattern in maize hybrids, tolerant and susceptible to the causative agent of rot grains, produced in environment 2, Guarapuava-PR, in the crop of 2013/14. UNICENTRO. 2016.

In treatment V8, the same expression of CAT was observed for the two groups of hybrids. However, for treatment V8 + VT, the same fact did not occur; a higher expression of CAT enzyme was observed for the hybrids belonging to the group considered tolerant to rot grains, AG9045PRO, AG8041PRO, and DKB245PRO2, with the exception of hybrid 2B707PW from the same group (Figure 2). This increase in the enzyme expression might be related to the application of the fungicide trifloxystrobin+ prothioconazole. It can be inferred that one of the possible contributions of the fungicide used, when applied in treatment V8 + VT, is on the route of the expression of the enzyme. The activity of this enzyme is related to the decomposition of hydrogen peroxide formed by the superoxide dismutase (SOD) in the cells, functioning as a second line of cellular defense (Mallick and Mohn, 2000).

A higher expression of the CAT enzyme was observed that in environment 2. In the second experiment, group 1 showed lower expression of the enzyme, highlighting the hybrids AG9045PRO, AG8041PRO, and 2B707PW, and in environment 1, a relatively lower expression of the enzyme was observed in group 1, highlighting only the hybrids AG8041PRO and 2B707PW. These findings showed that an interference of the environment in reduced spacing possibly created a microclimate, and alower expression of CAT was observed in a greater number of evaluated hybrids.

It can be inferred from the evaluation of environments 1 and 2 and the contrast for the agronomic characteristic of rot grains presented in Table 1 that in the check treatments, V8 and V8 + VT, the contrasts were significant with more than 95% probability, and in the contrast G1 *vs* G2, the value of G2 was negative, indicating numerical superiority and a higher incidence of rot grains. In other words, the hybrids belonging to group 1 (considered tolerant to rot grains) showed a relatively lower incidence of rot grains. However, we can infer that a relationship exists between the higher expression of CAT enzyme and a higher incidence of rot grains because of grain deterioration, especially in the check treatment. In Figures 1 and 2, evaluating the application of fungicide (trifloxystrobin+ prothioconazole) in treatments V8 and V8 + VT, a variation was observed in the expression of CAT enzyme, mainly in treatment V8 + VT, in which a higher CAT expression was observed in the hybrids belonging to the group 1in both environments 1 and 2, with the exception of the hybrid 2B707PW, in which no higher incidence of rot grains was observed. Thus, this increase in the expression of the CAT enzyme might be related to the application of the fungicide trifloxystrobin+ prothioconazole during V8 + VT treatment, indicating that one of the possible forms of fungicide contribution is the expression of CAT enzyme.

The gel revealing the expression of alcohol dehydrogenase (ADH) enzyme, front to the evaluated treatments with fungicides obtained in environment 1 in the crop of 2013/14 is shown in Figure 3.

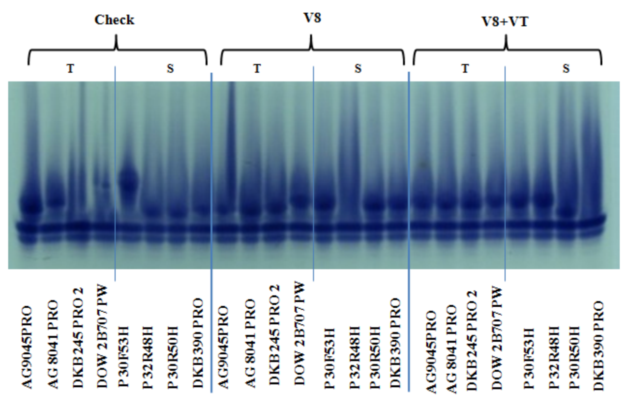

Figure 3. Alcohol dehydrogenase (ADH) electrophoretic pattern in maize hybrids, tolerant and susceptible to the causative agent of rot grains, produced in environment 1, Guarapuava-PR, in the crop of 2013/14. UNICENTRO. 2016.

As shown in Figure 3, the expression of ADH enzyme was determined for all hybrids and treatments that were evaluated in environment 2 in the crop of 2013/14. In the check treatment, a higher expression of the (ADH) enzyme was observed for the hybrids belonging to the group considered tolerant to rot grains. Based on the data presented in Table 1, the percentage of rot grains was significant, showing the existence of genotypes with higher resistance to *S. maydis*. The hybrids belonging to the group 1 showed a lower incidence of rot grains compared to group 2; therefore, it can be stated that a relationship exists between the lower incidence of rot grains and a higher enzyme (ADH) expression. When the activity of ADH decreases, the seed becomes more susceptible to the deleterious effects of acetaldehyde (Zhang et al., 1994); therefore, the ADH enzyme acts in the anaerobic metabolism of plants reducing acetaldehyde to ethanol (Vantoai et al., 1987; Pertel, 2001).

In treatment V8, an equal expression of the ADH enzyme was observed for the two groups of hybrids. The application of fungicide trifloxystrobin + prothioconazole did not change the expression of ADH, except for the hybrid AG9045PRO that showed the highest expression of ADH in group 1. For treatment V8 + VT, it was possible to notice a higher expression of the ADH enzyme for the hybrids belonging to the group considered susceptible to rot grains, that is, P30F53H, P32R48H, P30R50H, and DKB390PRO (Figure 3). These results do not corroborate with the ones found by Siqueira et al. (1985), who detected no activity in the garlic leaf tissue for the isoenzymes alcohol dehydrogenase, esterase, and peroxidase. When the aerobic pathway is compromised, the anaerobic pathway of respiration is activated, leading to the accumulation of toxic products, such as acetaldehyde and ethanol, in cells. In the anaerobic metabolism, the pyruvate first produced in the glycolysis is converted to acetaldehyde by the action of the enzyme pyruvate decarboxylase, and the acetaldehyde is then reduced to ethanol by ADH (Taiz and Zeiger, 2004).

The gel revealing the expression of the ADH enzyme, front to the treatments evaluated with fungicides obtained in environment 2, in the crop of 2013/14, is shown in Figure 4.

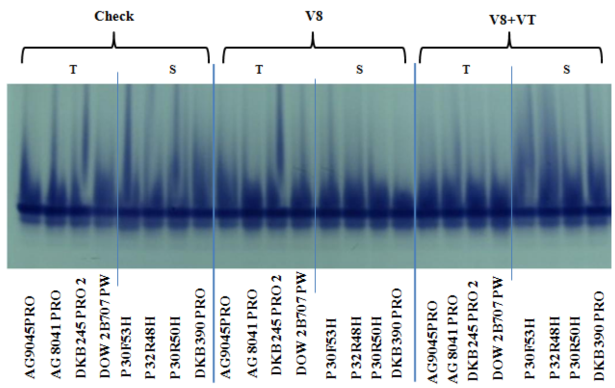

Figure 4. Alcohol dehydrogenase (ADH) electrophoretic pattern in maize hybrids, tolerant and susceptible to the causative agent of rot grains, produced in environment 2, Guarapuava-PR, in the crop of 2013/14. UNICENTRO. 2016.

As shown in Figure 4, the expression of the ADH enzyme was determined for all hybrids and all treatments evaluated in environment 2 in the crop of 2013/14. In the check treatment, a higher expression of the ADH enzyme was observed for the hybrids belonging to the group considered tolerant to rot grains. Based on the data presented in Table 1, the percentage of rot grains was significant, showing the existence of genotypes with higher resistance to *S. maydis*. The hybrids belonging to group 1 showed a lower incidence of rot grains as compared to group 2; therefore, it can be stated that a relationship exists between the lower incidence of rot grains and the higher expression of the ADH enzyme. The ADH enzyme acts in the respiratory process, removing toxic substances, such as acetaldehyde and ethanol, from the seeds that are produced when cells start anaerobic respiration (Faria et al., 2003).

In the treatment V8 was found an equal expression of ADH enzyme, for the two groups of hybrids, the fungicide application of trifloxystrobin + prothioconazole, did not change the ADH expression, as shown in Figure 4.

In treatment V8 + VT, a higher expression of the (ADH) enzyme was observed in the hybrids belonging to the group considered susceptible to rot grains. The application of fungicide trifloxystrobin + prothioconazole possibly affects the expression of ADH, as shown in Figure 4. The ADH enzyme is involved in the physiological quality and anaerobic respiration, promoting the reduction of acetaldehyde and ethanol (Buchanan et al., 2005). The acetaldehyde accelerates seeds deterioration (Zhang and Kirkham, 1994); therefore, with an ADH increase, the seeds are further protected against the harmful action of this compound. Similarly, Vidigal et al. (2009) observed a high ADH activity in pepper seeds with high physiological quality.

Baldoni (2013) observed a relatively higher expression of the respiratory enzymes, ADH and MDH, in the soybean seeds with high physiological quality.

The gel revealing the expression ofthe malate dehydrogenase (MDH) enzyme,front to the treatments evaluated with fungicides obtained in environment 1, in the crop of 2013/14, is shown in Figure 5.

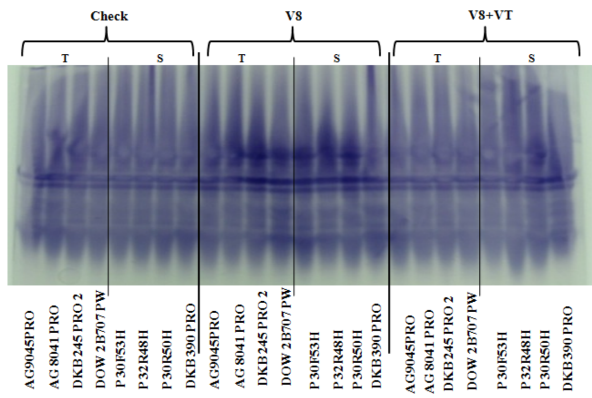

Figure 5. Malate dehydrogenase (MDH) electrophoretic pattern in maize hybrids, tolerant and susceptible to the causative agent of rot grains produced in environment 1, Guarapuava-PR, in the crop of 2013/14. UNICENTRO. 2016.

As shown in Figure 5, the malate dehydrogenase (MDH) expression was observed for all hybrids and evaluated treatments in environment 2 in the crop of 2013/14. No variation was observed in the MDH expression among the treatments and groups of hybrids. However, a variation existed between different environments. Abreu (2013) evaluated the drought tolerance of maize lines and observed no significant differences in the expression of the MDH enzyme in the seeds produced under different plant populations. This enzyme is related to aerobic respiration and is encoded by five loci. It is found in great abundance in different cell organelles, including mitochondria and cytoplasm (Goodman and Stuber, 1987). Because of this, a change in its expression is observed only in more advanced deterioration in seeds; therefore, it is considered an inefficient marker of physiological quality.

Brandão Júnior et al. (1999) observed that the activity of MDH was relatively less affected by the aging treatments on maize seeds. Higher enzyme stability has been observedfor MDH during the processing and drying of coffee beans, in which the expression of MDH enzyme was similar in all drying treatments, and thus for natural as well as pulped coffee (Ferreira et al., 2007).

In the treatments V8 and VT + V8, equal expression of the MDH enzyme was observed for the two groups of hybrids. The application of fungicide trifloxystrobin+ prothioconazole did not alter the expression of MDH (Figure 5). No significant differences were observed in the expression of this enzyme. Furthermore, Brandão Júnior et al. (1999) observed no correlation between the enzyme activity of MDH and the physiological quality of seeds.

This difference might be due to the expression of a gene or lack of expression in each organ or tissue of the plant. The MDH enzyme catalyzes the conversion of malate to oxaloacetate, playing an important role in the Krebs cycle, and participates in the malate movement across the mitochondrial membrane and CO_2 fixation in plants (Taiz and Zeiger, 2004). Thus, this enzyme is related to the aerobic respiration route.

The gel revealing the expression of MDH, front to the treatments evaluated with fungicide obtained in environment 2 in the crop of 2013/14 is shown in Figure 6.

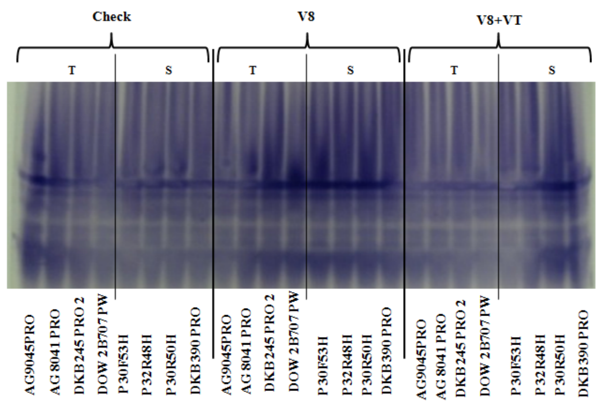

Figure 6. Enzymatic pattern of maize grains obtained from the different hybrids, considered tolerant and susceptible to the causative agent of rot grains and the treatments with fungicide (T-check, V8-eight expanded leaves and VT-tasseling), produced in environment 2, Guarapuava-PR, in the crop of 2013/14. UNICENTRO, 2016.

As shown in Figure 6, the expression of MDH was determined for all hybrids studied and all treatments evaluated in environment 2 in the crop of 2013/14. No variation in MDH expression was observed in any treatment and group of hybrids. Silva et al. (2009) noticed that MDH activity was very low as shown bythe presence of very clear bands.

In treatments V8 and V8 + VT, an equal expression of MDH was observed for the two groups of hybrids. The application of fungicide trifloxystrobin+ prothioconazole did not alter the expression of MDH (Figure 6). Ferreira et al. (2007) analyzed the enzymatic patterns of MDH in maize hybrids seeds. A relatively lower activity of the enzyme was observed in the treated seeds with the highest dose of Cellerate®, six months before sowing, compared to the check standards and the ones treated in pre-sowing, indicating lower respiratory activity in this condition.

CONCLUSIONS

The percentage of rot grains is influenced by the hybrid and the fungicide used.

The fungicide (trifloxystrobin+ prothioconazole) reduces the incidence of rot grains, with the higher reduction in the hybrids considered susceptible. A relationship exists between an increased expression of CAT enzyme and an increased incidence of rot grains because of the deterioration of grains, depending on the evaluated hybrids. A relatively higher expression of ADH and MDH enzymes was observed for maize hybrids belonging to the group considered tolerant to fungus that cause rot grains.

Conflicts of interest

The authors declare no conflict of interest.

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