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Resistance induction anthracnose control in pepper plants using acibenzolar-S-methyl

Controle de antracnose por indução de resistência em plantas de pimentão usando acibenzolar-S-metil

Keila Aparecida Moreira[1]; João Tiago Correia Oliveira[2]; Euzanyr Gomes da Silva[3]; Alexandre Tavares da Rocha[4]; Erika Valente de Medeiros[5]; Josabete Salgueiro Bezerra de Carvalho[6]; José Romualdo de Sousa Lima[7]

[1]ORCID: https://orcid.org/0000-0002-7715-9285; Federal University of Agreste of Pernambuco, Associate Professor, Garanhuns, PE, BRAZIL, E-mail: keila.moreira@ufape.edu.br;
[2]ORCID: https://orcid.org/0000-0001-7469-5106; Institute of Studies of Humid Tropic, Federal University of the South and Southeast of Pará, Xinguara, PA, BRAZIL, E-mail: tiagocorreia@unifesspa.edu.br;
[3]ORCID: https://orcid.org/0000-0001-8284-4392; Federal University of Agreste of Pernambuco, Ms. Agricultural Production, BRAZIL, E-mail: euzanyrsilva@yahoo.com.br;
[4]ORCID: https://orcid.org/0000-0001-6070-1958; Federal University of Agreste of Pernambuco, Associate Professor, Garanhuns, PE, BRAZIL, E-mail: alexandre.rocha@ufape.edu.br;
[5]ORCID: https://orcid.org/0000-0001-5543-9414; Federal University of Agreste of Pernambuco, Associate Professor, Garanhuns, PE, BRAZIL, E-mail: erika.valente@ufape.edu.br;
[6]ORCID: https://orcid.org/0000-0002-8270-3548 Federal University of Agreste of Pernambuco, Associate Professor, Garanhuns, PE, BRAZIL,, E-mail: josabete.bezerra@ufape.edu.br;
[7]ORCID: https://orcid.org/0000-0003-2983-4650; Federal University of Agreste of Pernambuco, Associate Professor, Garanhuns, PE, BRAZIL, E-mail: romualdo.lima@ufape.edu.br.

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ABSTRACT: The resistance induction becomes an alternative to control microorganisms that attacks like plants. Little is known about the dose and its effect on the enzymatic activities associated with the induction of resistance of pepper plants under anthracnose attack. The objective of this study was to evaluate the severity and to estimate the enzymatic activity of Arcade F1 hybrid peppers infected with *Colletotrichum gloeosporioides* and submitted to different doses of the abiotic acibenzolar-S-methyl inducer. The experimental design was completely randomized blocks with five treatments, four doses of acibenzolar-S-methyl (0.15, 0.30, 0.45 and 0.60 g.L^{-1}), and the control with distilled water only. The evaluation of leaf severity and collection for enzymatic activity of β-1,3-glucanase, catalase, peroxidase, polyphenoloxidase and ascorbate peroxidase were performed on the 4th, 8th and 12th day after inoculation of the phytopathogen. The application of acibenzolar-S-methyl provided a reduction in anthracnose severity, with an increase in all the enzymatic activities evaluated, but there was no prevalence of a specific dose. However, all doses of the evaluated inducers were able to delay the development of the phytopathogen with elevated activity of one or more antioxidant enzyme.
KEYWORDS: Antioxidant enzymes, phytopathogenic fungus, *Colletotrichum gloeosporioides*.

RESUMO: A indução de resistência torna-se uma alternativa para controlar microrganismos que atacam as plantas. Pouco se sabe sobre a dose e seu efeito nas atividades enzimáticas associadas à indução de resistência de plantas de pimentão ao ataque da antracnose. O objetivo deste estudo foi avaliar a severidade e estimar a atividade enzimática de pimentões híbridos Arcade F1 infectados com *Colletotrichum gloeosporioides* e submetidas a diferentes doses do indutor abiótico acibenzolar-S-metil. O delineamento experimental foi em blocos inteiramente casualizados com cinco tratamentos, quatro doses de acibenzolar-S-metil (0,15; 0,30; 0,45 e 0,60 g.L^{-1}), sendo a testemunha apenas água destilada. A avaliação da severidade foliar e coleta quanto à atividade enzimática de β-1,3-glucanase, catalase, peroxidase, polifenoloxidase e ascorbato peroxidase foram realizadas no 4º, 8º e 12º dias após a inoculação do fitopatógeno. A aplicação de acibenzolar-S-metil proporcionou redução da severidade da antracnose, com aumento de todas as atividades enzimáticas avaliadas, mas não houve prevalência de dose específica. Porém, todas as doses dos indutores avaliados são capazes de retardar o desenvolvimento do fitopatógeno com elevada atividade de uma ou mais enzimas antioxidantes.
PALAVRAS-CHAVE: Enzimas antioxidantes, Fungos fitopatógenos, *Colletotrichum gloeosporioides*.

INTRODUCTION

The pepper (*Capsicum annuum* L.) comes from South and Central America, now is considered the second most cultivated vegetable in the world, behind the tomato (CORTÉS-ESTRADA et al., 2020). Despite the care and inclusion of new technologies in the production system, several pests and diseases limit pepper production (ZAMLJEN et al., 2020). Among the biotic stresses, the anthracnose caused by *Colletotrichum* spp. presented prominence, compromising the pre- and post-harvest (WANG et al., 2017).

In the search for forms of control of the phytopathogen, concomitantly with reduced use of agrochemicals and environmental problems, in addition to increasing food safety, the use of chemical products that demonstrate the capacity to induce the expression of systemic resistance acquired in the vegetable has gained evidence (AKKÖPRÜ, 2020). Among these products, acibenzolar-S-methyl is outstanding, because it does not have direct antimicrobial action, it interferes in the physiological and/or biochemical processes of the plants, activating the systemic resistance without any alteration in the genome of the plant (GE et al., 2019).

Plants, when infected by pathogens, tend in response to altering their biochemistry, increasing the production of several enzymes in the plant, mainly antioxidants (SILVA et al., 2017), this fact is elevated after the application of resistance inducers (AKKÖPRÜ, 2020). Although the effects of acibenzolar-S-methyl in the scientific environment are known, little is cognized about the dose and its effect on the enzymatic activity associated with resistance induction of pepper plants under the anthracnose attack caused by *Colletotrichum* spp. In this sense, the objective was to evaluate the severity and to estimate the enzymatic activity of pepper plants, Arcade F1 hybrid, infected with *C. gloeosporioides* CMM 0811 and submitted to different doses of the abiotic acibenzolar-S-methyl inducer.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Federal University of Agreste of Pernambuco, Brazil. The climate of the municipality is of type Cs'a,

mesothermic tropical of altitude according to the classification of Köppen-Geiger (ALVARES et al., 2013), with an average annual temperature of 20 °C and the average annual precipitation of 1,300 mm (ANDRADE et al., 2008).

The experimental design was completely randomized blocks, with five treatments, four doses of acibenzolar-S-methyl, 0.15; 0.30; 0.45 and 0.60 $g.L^{-1}$, and the control with only distilled water. Each treatment per block contained three 4 L pots, each containing one plant. The pathogen *C. gloeosporioides* CMM 0811 used in the infection of the pepper plants to cause anthracnose was obtained from the Collection of Cultures of Phytopathogenic Fungi of the Federal Rural University of Pernambuco.

The pepper commercial seeds were grown on a sowing tray containing soil, sand and organic matter mixture (2:1:2) and kept in a greenhouse under a relative humidity of approximately 70%, a value which was maintained until the end of the experimental period. The pepper hybrid Arcade F1 was used due to its local commercialization, fairs and outlets throughout the municipality of Garanhuns-PE, besides the high productivity, excellent fruit uniformity and high resistance of and tolerance to pests and diseases according to the references of the producer TopSeed Premium®. In order to control the moisture of the vessels, its capacity of field of the vessel was determined, is the replenishment of the water realized daily.

The seedlings were transplanted at 35 days after germination and 60 days after germination were sprayed with different doses of acibenzolar-S-methyl (Bion®, Syngenta) and distilled water, all in a volume of 10 mL in the first leaves of the plant. Two days after spraying, the conidia of *C. gloeosporioides* CMM 0811, at the concentration of 1.6 x 10^6 mL^{-1} conidia in the first leaves of the plants, were inoculated and kept in plastic bags for four days to favor fungus development. The severity evaluation and leaf collection for enzymatic activity were performed on the 4th, 8th and 12th day after the inoculation of *C. gloeosporioides* CMM 0811.

The soil used to fill the pots was collected in the 0.0-0.2 m layer of native forest area (Caatinga) of the Municipality of São João, Pernambuco. The soil is classified as a typical eutrophic Regolithic (Santos et al., 2012). Chemical analysis indicating, pH 4.5; 16.6 mg Kg^{-1} of P; 0.8 cmolc dm^{-3} of Ca^{2+}; 0.8 cmolc dm^{-3} of Mg^{2+}; 0.15 cmolc Kg^{-1} of Al^{3+}; 1.8 cmolc dm^{-3} of H+Al, the physical analysis characterized the soil as sandy, with 880 g Kg^{-1} of sand, 40 g Kg^{-1} of clay and 80 g Kg^{-1} of silt according to the evaluation methodology of Donagema et al. (2011).

To perform the experiment, the soil was air-dried, sieved at 2 mm, homogenized and autoclaved at 121 °C for two hours, and then dried at rest for two weeks, aiming at the elimination of fungal propagules and the stabilization of the contents of heavy metals, adapted methodology of Silva et al. (2016).

The evaluation of the severity of the anthracnose in the pepper plants was performed according to a scale of 1 to 5, according to Pereira et al. (2011), in which: 1 = no symptoms; 2 = traits at 10% severity; 3 = 11 to 25%; 4 = 26 to 50% and 5 = greater than 50% plant severity or death. For extraction and estimation of the enzymatic activity two leaves of each plant per pot were collected, and later homogenized by block and maintained at 4 °C. The vegetable sample was macerated in liquid N_2 and 4 mL of 50 mM potassium phosphate buffer (pH 7.0) to avoid oxidative effects were added 0.05 g of polyvinylpyrrolidone. The concentrates were centrifuged at 10,000 x g for 10 min at 4 °C and the supernatants stored at -20 °C (ANDRADE et al., 2013).

Estimates of the enzymatic activities of β-1,3-glucanase (GLU) and catalase (CAT) were determined according to Lever (1972) and, Havir and Mchale (1987), respectively. For the peroxidase activity (POX) the method described by Urbanek et al. (1991), using guaiacol and H_2O_2 as substrates. The activity of polyphenoloxidase (PPO) was verified by the oxidation of pyrogallol according to Kar and Mishra (1976) and the activity of ascorbate peroxidase (APX) measured according to Nakano and Asada (1981). The enzymatic activities were expressed in units of μmol $min^{-1}.g^{-1}$ of foliar mass.

The data of severity and enzymatic activity were analyzed by orthogonal contrast using the t test ($p \leq 0.05$), later analyzed in time-subdivided plot form, when significant differences in the analysis of variance were detected by Fisher-Snedecor's F statistic the Tukey test ($p \leq 0.05$) using the statistical software SISVAR 5.6.

The data of the enzymatic activities after transformation, square root, were used to construct the dispersion graph in the main component using the statistical software PAST 1.9. Regression analyzes were performed in order to verify if there was a relationship between disease severity and enzymatic activity by treatment.

RESULTS AND DISCUSSION

The application of acibenzolar-S-methyl provided a reduction in the severity of the anthracnose, with an elevation of all the enzymatic activities evaluated (Table 1). Elevation of the inducer doses reduced the severity of the disease in the plant and the progression of the infection. It should be noted that none of the doses of the inductors evaluated allowed the severe attack of the phytopathogen (Table 2).

Table 1. Comparison between groups of averages by orthogonal contrast for the severity of the anthracnose and enzymatic activities in pepper leaves (*Capsicum annuum* L.) hybrid Arcade F1 submitted to different doses of the abiotic acibenzolar-S-methyl inducer and later infected with *Colletotrichum gloeosporioides* CMM 0811, causing anthracnose.

Averages	Severity	Enzymatic activities				
		β-1,3-glucanase	Catalase	Peroxidase	Poly-phenoloxidase	Ascorbate peroxidase
		μmol min^{-1} g^{-1} of leaf mass				
Resistance Inducers	1.666	15.313	8.374	122.492	1.996	58.892
Control	2.833	2.274	3.834	105.092	1.086	27.460
General	1.900	12.705	7.466	119.012	1.814	52.606
Resistance Inducers vs. Control						
t test	-4.08*	10.384*	5.742*	2.026*	5.106*	7.480*
CV (%)	11.25	6.52	8.42	9.37	6.34	11.43

CV - Coefficient of variation; ns - Not significant and * - Significant at 5% probability by the t test.

Table 2. Evaluation of severity in pepper leaves, *Capsicum annuum* L. hybrid F1 Arcade submitted to different doses of abiotic inducer acibenzolar-S-methyl and subsequently infected with *Colletotrichum gloeosporioides* CMM 0811, causing anthracnose.

Doses of inductors	Days after infection with *C. gloeosporioides* CMM 0811			CV (%)
	4th	8th	12th	
0.15 $g.L^{-1}$	1.500 Ba	2.000 Ba	2.500 Ba	
0.30 $g.L^{-1}$	1.250 Ba	2.000 Ba	2.500 Ba	
0.45 $g.L^{-1}$	1.000 Ba	1.250 Ba	2.000 Ba	7.00
0.60 $g.L^{-1}$	1.000 Ba	1.000 Ba	2.000 Ba	
Control	2.000 Ab	2.750 Ab	3.750 Aa	
CV (%)	4.50			

CV: Coefficient of variation; Means followed by the same uppercase letter in the column and lowercase in the row do not differ from each other by the Tukey test at 5% probability.

The use of products that induce mechanisms of resistance in plants is an alternative for the integrated management of phytopathogens and has been gaining prominence (AKKÖPRÜ, 2020). Among these products, acibenzolar-S-methyl is used in several plant species, in a wide range of pathogens, including fungi, viruses and bacteria (LI et al., 2020), has no direct antimicrobial action, interferes with the physiological and/or biochemical processes of plants, such as the production of phenols, activating systemic resistance (GE et al., 2019).

After stress, be it of a biotic or abiotic nature, plants produce several reactive oxygen species quickly. Among these species are singlet oxygen, superoxide anion, hydrogen peroxide and the hydroxyl radical, with the accumulation of these substances in the cells causing effects toxic to the plant (FRANZENER et al., 2018). Aiming at plant protection against oxygen intermediates, there is enzymatic production of antioxidative and other non-enzymatic molecules, among them, carotenoids, flavonoids, vitamin E, ascorbic acid, besides the induction of defense genes, the polymerization of proteins that make up the cell wall (WANG et al., 2017).

The different doses of the inducer provided an increase in the activity of all enzymes evaluated, with no specific dose being emphasized (Table 3). However, with the increase of the time of infection of the phytopathogen there was an increase in the enzymatic activity. Different from the control plants, these with similar enzymatic activities between the times and the enzymes evaluated. The distancing of the control treatment becomes clear when evaluating the dispersion of the main components (Figure 1), occurring mainly on the 8th and 12th days after infection of the phytopathogen.

Table 3. The enzyme activity (μmol $min^{-1}.g^{-1}$ of leaf mass) of pepper leaves (*Capsicum annuum* L.) hybrid Arcade F1 submitted to different doses of abiotic inducer acibenzolar-S-methyl and subsequently infected with *Colletotrichum gloeosporioides* CMM 0811, causing anthracnose.

Doses of inductors	Days after infection with *C. gloeosporioides* CMM 0811			CV (%)
	4th	8th	12th	
β-1,3-glucanase				
0.15 $g.L^{-1}$	8.196 Cb	10.405 Db	17.639 Ba	
0.30 $g.L^{-1}$	10.920 Bc	13.792 Cb	18.307 Aba	
0.45 $g.L^{-1}$	11.779 Bc	17.424 Bb	20.418 Aa	8.83
0.60 $g.L^{-1}$	16.000 Ab	22.160 Aa	16.663 Bb	
Control	1.717 Da	3.141 Ea	1.963 Ca	
CV (%)		7.63		
Catalase				
0.15 $g.L^{-1}$	4.549 Bb	5.846 Bb	10.075 Aba	
0.30 $g.L^{-1}$	5.639 Bb	10.676 Aa	10.075 Aba	
0.45 $g.L^{-1}$	8.934 Ab	9.887 Ab	11.842 Aa	10.82
0.60 $g.L^{-1}$	4.342 Bb	8.722 Aa	9.568 Ba	
Control	4.417 Ba	4.022 Ba	3.064 Ca	
CV (%)		10.52		
Peroxidase				
0.15 $g.L^{-1}$	119.444 Aa	137.731 Aa	136.111 Aba	
0.30 g,L^{-1}	87.731 BCc	114.120 Ab	153.703 Aa	
0.45 $g.L^{-1}$	109.259 ABb	118.287 Aab	138.426 Aba	9.65
0.60 $g.L^{-1}$	109.259 ABb	141.435 Aa	111.574 BCb	
Control	80.731 Cb	140.046 Aa	87.500 Cb	
CV (%)		9.02		
Polyphenoloxidase				
0.15 $g.L^{-1}$	1.777 Ab	2.412 Aba	1.608 Bb	
0.30 $g.L^{-1}$	1.322 ABb	2.644 Aa	2.269 Aa	
0.45 $g.L^{-1}$	1.715 Aab	2.018 Ba	1.411 BCb	14.00
0.60 $g.L^{-1}$	1.492 ABb	2.805 Aa	2.484 Aa	
Control	1.045 Ba	1.322 Ca	0.893 Ca	
CV (%)		8.90		
Ascorbate peroxidase				
0.15 $g.L^{-1}$	49.305 Ab	68.750 Aa	67.129 Aba	
0.30 $g.L^{-1}$	41.203 Ab	54.629 Ab	75.000 Aa	
0.45 $g.L^{-1}$	50.694 Ab	56.944 Aab	68.287 Aba	13.88
0.60 $g.L^{-1}$	50.694 Ab	69.213 Aa	54.861 Bab	
Control	34.712 Ba	26.751 Ba	20.918 Ca	
CV (%)		8.13		

CV: Coefficient of variation; Means followed by the same uppercase letter in the column and lowercase in the row do not differ from each other by the Tukey test at 5% probability.

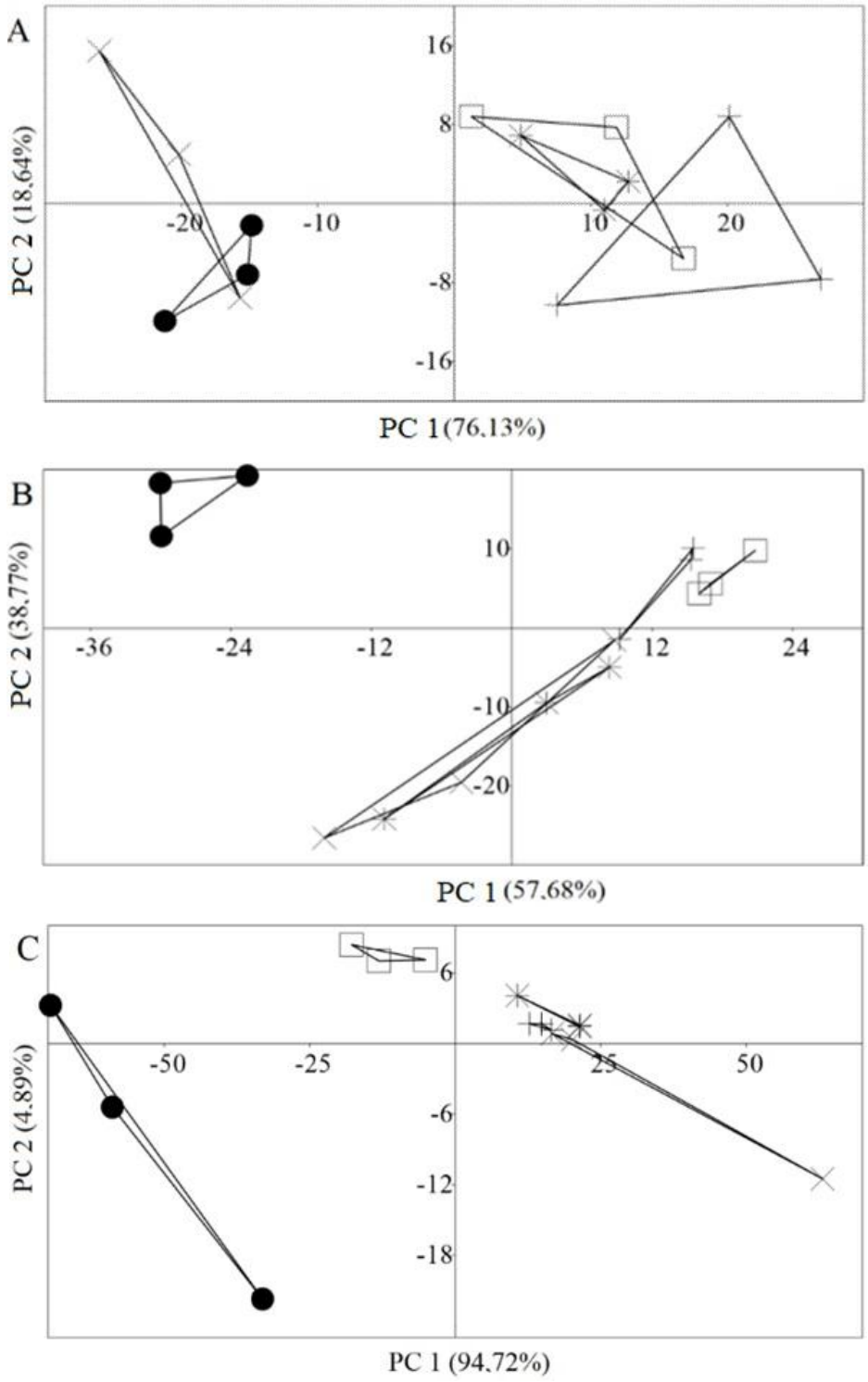


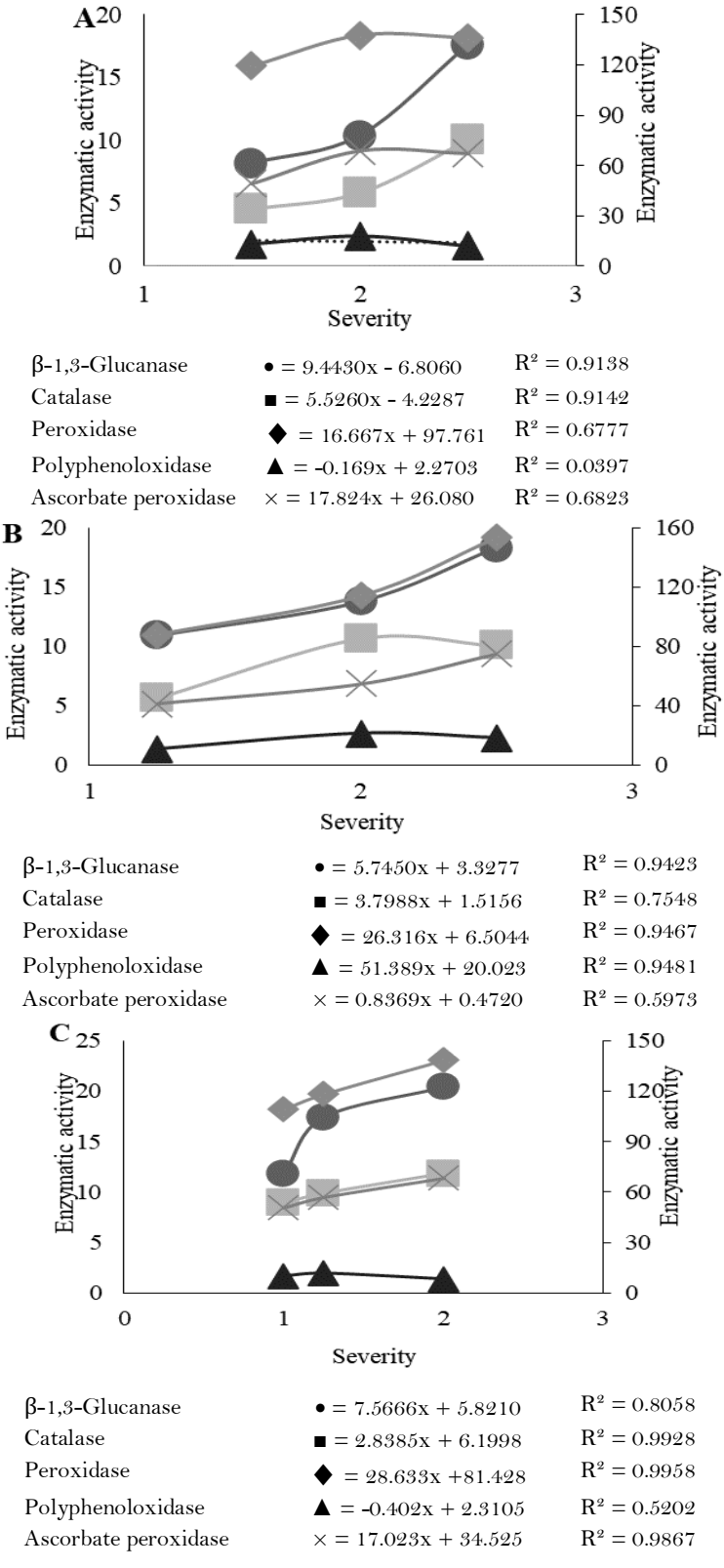
Figure 1. Principal component analysis (PC) of the enzymatic activities β-1,3-glucanase, catalase, peroxidase, polyphenoloxidase and ascorbate peroxidase (μmol.min^{-1}.g^{-1} foliar mass) in pepper leaves, *Capsicum a*nnuum L. F1 Arcade hybrid subjected to different doses of the abiotic inducer, acibenzolar-S-methyl and subsequently infected with *Colletotrichum gloeosporioides* CMM 0811, days after infection.
A: 4th day; B: 8th day and C: 12th day. Treatments (+) 0.15 g.L^{-1} of acibenzolar-S-methyl; (×) 0.30 g.L^{-1} of acibenzolar-S-methyl; (○) 0.45 g.L^{-1} of acibenzolar-S-methyl; (□) 0.60 g.L^{-1} of acibenzolar-S-methyl, and (●) Control with distilled water

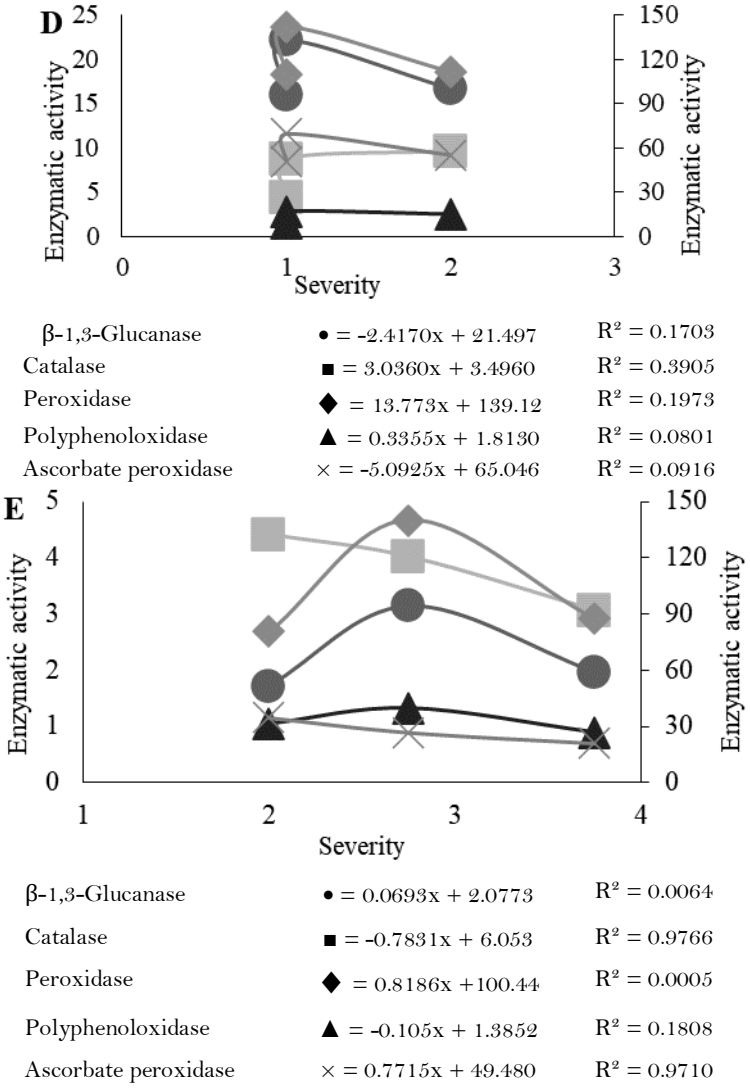
Among the enzymes capable of decomposing reactive oxygen species produced in plant cells, the most important are β-1,3-glucanase, catalase, peroxidase, polyphenoloxidase and ascorbate peroxidase (FRANZENER et al., 2018). Modifications in the enzymatic system of antioxidant defense in plants is the first response of the plant to the attack of the phytopathogen, as demonstrated by Silva et al. (2017) when evaluating an induction of biological force by *Trichoderma* in cassava plants was to the attack of *Scytalidium lignicola*, which causes black root rot.

The β-1,3-glucanase degrades glucans, a major component of the cell wall of fungi, protecting the plant against infections (AKKÖPRÜ, 2020). Already the increase in the activity of antioxidant enzymes catalase, peroxidation and polyphenoloxidase contributes aided in the reduction of the toxic levels of hydrogen peroxide (WANG et al., 2017). The accumulation of these enzymes will occur depending on the physical conditions of the plant, the pathogen and induction (FRANZENER et al., 2018). Fact observed at all doses and times in this study.

High correlations were observed between the activities of the antioxidant enzymes and the severity of the disease at doses 0.15; 0.30 and 0.45 $g.L^{-1}$ of acibenzolar-S-methyl, making a possible inference of the increase of the enzymatic activity against the action of the phytopathogen. The 0.15 $g.L^{-1}$ dose of the inducer provided the highest values of determination coefficients (R^2) for the enzymes β-1,3-glucanase and catalase. Already the dose of 0.30 $g.L^{-1}$ of the inducer the largest R2 was in the enzymes β-1,3-glucanase, peroxidase and polyphenoloxidase, at the dose of 0.45 $g.L^{-1}$ inducer the largest R^2 was in the enzyme's catalase, peroxidase and ascorbate peroxidase. Some plants without the incorporation of external agents present high enzymatic activity to combat the phytopathogen in the enzyme catalase and ascorbate peroxidase (Figure 2).

Figure 2. Correlation between enzymatic activity (μmol.min^{-1}.g^{-1} leaf mass) and severity of anthracnose disease in the 4th, 8th and 12th day after infection by *Colletotrichum gloeosporioides* CMM 0811 in pepper leaves, *Capsicum annuum* L. F1 Arcade hybrid subjected to different doses of the abiotic inducer, acibenzolar-S-methyl.





(A) 0.15 g.L^{-1} of acibenzolar-S-methyl; (B) 0.30 g.L^{-1} of acibenzolar-S-methyl; (C) 0.45 g.L^{-1} of acibenzolar-S-methyl; (D) 0.60 g.L^{-1} of acibenzolar-S-methyl, and (E) Control with distilled water.

Understanding the defense mechanism of plants is a path that will enable the development of cultivars that are more resistant to the diversity of phytopathology and insects (WANG et al., 2017). The treatment of the plants with inducers allows increasing the resistance to the attack of phytopathogens, not only in the place of treatment but also in tissues distant from the initial infection sites, promoting physiological and biochemical changes in the vegetable, aiming to prevent and/or combat the attack of the pathogen (MISHRA et al., 2018).

The inducer doses of acibenzolar-S-methyl resistance were efficient in combating and retarding the development of anthracnose caused by *C. gloeosporioides* CMM 0811 in arid F1 hybrid pepper plants. All doses of acibenzolar-S-methyl increased the antioxidant enzymatic activity of β-1,3-glucanase, catalase, peroxidase, polyphenoloxidase and ascorbate peroxidase over time.

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