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CROP SCIENCE

Response of Soybean Cultivars to Oxidative Stress caused by *Meloidogyne javanica*

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Abstract: The present study aimed to investigate the response of soybean cultivars with different susceptibility levels to the root-knot nematode *Meloidogyne javanica* at varied time intervals by analyzing the initial plant–nematode interaction using antioxidant enzymes as oxidative stress markers. A 4 × 4 × 2 factorial method with 5 repetitions was used to analyze 4 soybean cultivars at 4 different collection times—6, 12, 24, and 48 h—with and without *M. javanica* inoculation. The parameters evaluated were the activities of antioxidant enzymes phenol peroxidase (POX) and ascorbate peroxidase (APX); the concentrations of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA); and the number of *M. javanica* juveniles penetrated into each plant. H_2O_2 concentration varied among the cultivars with and without inoculation and at different collection times as indicated by MDA concentration and POX and APX activities, demonstrating a rapid response of the host to an infection by *M. javanica*. Oxidative stress caused by *M. javanica* did not vary among the soybean cultivars regardless of their susceptibility level; however, the antioxidant enzymes POX and APX responded according to the susceptibility level of the cultivars.

Key words: Glycine max, oxidative stress, phenol peroxidase, ascorbate peroxidase, hydrogen peroxide, malondialdehyde.

INTRODUCTION

Host–pathogen interactions involving biotrophic organisms are mediated by the specific interaction between pathogen avirulence genes and plant resistance genes. In this interaction, plants develop resistance as a rule and susceptibility as an exception. According to the gene-for-gene theory, recognition of the pathogenic avirulence allele by the host-specific allele, which is encoded by the R allele, results in an incompatible interaction—also known as disease resistance. On the other hand, a compatible interaction causes the disease (Flor 1971).

Phytonematodes are obligate parasites that exclusively feed on plant cell cytoplasm. Among these species, the root-knot nematodes (*Meloidogyne* spp.) have developed a complex relationship with the host, thereby creating a permanent feeding site in cells located in the central cylinder (Agrios 2005). Currently, several soybean cultivars are resistant or moderately resistant to *Meloidogyne javanica*; however, the resistance levels remain limited owing to a limited source of donors (Dias et al. 2010).

The phenotypic expression of plant resistance to nematodes is typically characterized by a hypersensitivity reaction that comprises programmed cell death at the site of

infection, thereby limiting their development and reproduction (Williamson 1999). With regard to physiological responses, the complex nematode–plant interaction involves a significant increase in the concentration of reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-); this is the first response of the plant to the attack (Melillo et al. 2006). This ROS accumulation in plant cells can be toxic to both the plant and the nematode. However, in plants, ROS can react as biological molecules, causing an irreversible damage to cell membranes, proteins, and nucleic acids (Apel & Hirt 2004). Although ROS accumulation may initially contribute toward nematode resistance (Kyndt et al. 2012), plants have developed non-enzyme and enzyme mechanisms (Apel & Hirt 2004) that can eliminate excess ROS generated during host–pathogen interaction, playing an important role in resistance to plant diseases. These enzymes include phenol peroxidase (POX) and ascorbate peroxidase (APX), which are commonly involved in host defense mechanisms against oxidative stress and are two of the most important enzymes involved in the H_2O_2 detoxification of cells, thereby preventing or minimizing oxidative damage (Mittler 2002, Sharma et al. 2012). POX is directly related to phenolic compound oxidation, polysaccharide binding, indole-3-acetic acid oxidation, monomer bonding, lignification, wound healing, and protection against pathogens (Resende et al. 2003). APX is a heme-containing protein that uses ascorbic acid as a reducing agent for H_2O_2 detoxification. It has a high affinity for H_2O_2 and eliminates it even at low concentrations (Sharma et al. 2012). These peroxidases together are important components of the oxidative protection system and their activity can be used as a biochemical marker of stress from biotic factors (Apel & Hirt 2004, Barbosa et al. 2014).

The present study aimed to investigate the response of soybean cultivars with different susceptibility levels to *M. javanica* at varied time intervals by analyzing the initial plant–nematode interaction using antioxidant enzymes as oxidative stress markers.

MATERIALS AND METHODS

Treatments and experimental conditions

An experiment was conducted in a greenhouse in Itaara, Rio Grande do Sul, located at 29°35'8"S latitude, 53°48'28"W longitude, and 444 m altitude in the harvest of 2018–2019. The maximum and minimum temperatures recorded inside the greenhouse were 28°C and 19°C, respectively.

This randomized study used a 4 × 4 × 2 factorial method with 5 repetitions to analyze 4 soybean cultivars—BMX-Ativa RR, NA-5909 RG, SYN-VTop RR, and BMX-Ícone IPRO—at 4 different collection times (6, 12, 24, and 48 h) with and without nematode inoculation. These cultivars were chosen because they presented different levels of reproduction factor (RF): two cultivars had a RF of >10 (BMX-Ativa RR: RF = 11.5; NA-5909 RG: RF = 15.3) and two cultivars had a RF of <7 (SYN-VTop RR: RF = 6.8 and BMX-Ícone IPRO: RF = 4.3).

Direct sowing was performed in 700-mL containers filled with a previously sterilized substrate of sand and soil in a ratio of 1:1. In each container, 3 soybean seeds were sown without chemical treatment, and after the emergence of seedlings, pruning was performed, leaving 1 seedling per experimental container.

Inoculation of *M. javanica*

The inoculum of *M. javanica* was obtained from the roots of infected soybean plants from the municipality of Júlio de Castilhos, Rio Grande do Sul, Brazil. After confirming the species via the

perineal pattern of 10 females and biochemical characterization via the esterase phenotype (Carneiro & Almeida 2001), egg masses were collected and inoculated into the Apollo F1 hybrid tomato plants for culturing the nematode. After 120 days, the tomato plants were collected and the inoculum was prepared according to the extraction techniques described by Hussey & Barker (1973) and modified by Boneti & Ferraz (1981).

To obtain second-stage juveniles (J_2), the funnel method described by Baermann (1917) was used to better standardize the inoculum, which was obtained from first-stage juveniles. This method adjustment was required owing to known variation in the hatching time of *M. javanica* eggs and the short study period. Thereafter, a population of 2,500 juveniles (J_2) per plant was inoculated. Inoculation was performed in the phenological stage V2 by making 3 holes in the soil that were 2 cm from the plant collar and at a depth of 2 cm.

Biochemical analysis

To determine the activity of the antioxidant complex, 5 plants were collected from each treatment, thoroughly washed, kept in liquid nitrogen during the sampling process, and subsequently stored in an ultrafreezer at −80°C until analysis.

Determination of POX activity, EC 1.11.1.7

POX activity was determined according to the method of pyrogallol oxidation described by Kar & Mishra (1976). For this analysis, 0.5-g root tissue was ground into fine powder with a mortar and pestle, and liquid nitrogen was added to this powder during this process. The fine powder was then homogenized into a 2000-µL solution containing 50 mM potassium phosphate buffer (pH 6.8). Thereafter, the solution was centrifuged at 12,000 ×*g* for 15 min at 4°C, and the supernatant was used as a crude enzyme extract. The reaction was initiated by addition of 50 µL of the crude enzyme extract to 950 µL of the reaction mixture along with 25 mM potassium phosphate buffer (pH 6.8), 20 mM pyrogallol, and 20 mM H_2O_2. POX activity was determined based on the absorbance of colored purpurogallin using a spectrophotometer at 420 nm for 1 min at 25°C. An extinction coefficient of 2.47 mM^{-1} cm^{-1} (Chance & Maehley 1955) was used to calculate POX activity, which was expressed as the micromole of purpurogallin produced per minute per milligram of protein.

Determination of APX activity, EC: 1.11.1.1

APX activity was determined by the oxidation rate of ascorbate, according to Nakano & Asada (1981), using the crude enzyme extract obtained for the previous POX analysis. The reaction was initiated by adding 50 µL crude enzyme extract to 950 µL of the reaction mixture along with 50 mM potassium phosphate buffer (pH 6.8), 0.8 mM ascorbate, and 1 mM H_2O_2. APX activity was measured based on the ascorbate oxidation rate using a spectrophotometer at 290 nm for 1 min at 25°C. An extinction coefficient of 2.8 mM^{-1} cm^{-1} was used to calculate APX activity (Fortunato et al. 2015), and the value was corrected according to the protein content (Bradford 1976).

Concentration of H_2O_2

H_2O_2 concentration was determined using 0.1 g root tissue ground into a fine powder. The fine powder was homogenized at 1500 µL of 0.1% trichloroacetic acid (TCA). This solution was centrifuged at 12,000 ×*g* for 15 min at 4°C (Loreto & Velikova 2001), following which 500 µL of the supernatant was added to a reaction mixture containing 500 µL of 10 mM potassium phosphate buffer solution (pH 7.0) and 1000 µL potassium iodide (1M). Absorbance of the samples was determined at 390 nm. H_2O_2 concentration in the

samples was estimated according to a standard curve of H_2O_2 and expressed as millimole per gram of fresh weight.

Concentration of malondialdehyde

Oxidative damage in root cells was estimated as the concentration of total reactive substances to 2-thiobarbituric acid (TBA) and expressed as equivalent to malondialdehyde (MDA), according to Cakmak & Horst (1991). For this, 0.1 g of root tissue was ground with a mortar and pestle with liquid nitrogen until fine powder was obtained. The fine powder was homogenized in 2000 µL of 0.1% TCA (wt. vol^{-1}) in an ice bath. This solution was centrifuged at 12,000 ×*g* for 15 min at 4°C. Following centrifugation, 500 µL of the supernatant was added to 1500 µL of TBA/TCA solution (0.5% TBA in 20% TCA) and incubated in a water bath at 95°C for 30 min. Thereafter, the reaction was stopped using an ice bath. The samples were then centrifuged at 9000 ×*g* for 10 min, and specific absorbance was determined at 532 nm. Nonspecific absorbance was estimated at 600 nm and subtracted from the specific absorbance value. An extinction coefficient of 155 mM^{-1} cm^{-1} (Heath & Packer 1968) was used to calculate MDA concentration, which was expressed as micromole per kilogram of fresh weight.

Nematode analysis

Nematode analysis was performed at the plant roots; the collection times were the same as those of the enzyme analysis (6, 12, 24, and 48 h). In each collection time, 5 containers were sent to the laboratory, where the soybean plants were washed to remove soil adhered to the roots, following which the plants were sectioned at the collar area to separate the aerial part from the root system. Thereafter, the soybean plant roots of each repetition were submitted to the root staining technique described by Byrd Jr et al. (1983). After staining, the roots were arranged on glass slides on a microscope to count the number of *M. javanica* juveniles penetrated within the different time periods after inoculation in the different cultivars. The evaluation of the penetration index comprised a 4 × 4 two-factor method that analyzed 4 cultivars at 4 collection times with 5 repetitions.

Statistical analysis

Data were submitted to analysis of variance; the mean values were compared using the Scott–Knott test at 5% probability. Data analysis was conducted using the Sisvar software (Ferreira 2011).

RESULTS

On analyzing the study results, significant differences were observed in POX and APX activities, which varied for different soybean cultivars and collection times [6, 12, 24, and 48 h after inoculation (HAI)], with and without *M. javanica* inoculum (Table I). H_2O_2 and MDA concentrations presented similar variations, with a significant difference being noted for H_2O_2 at 12 and 48 HAI in terms of collection times versus cultivars and in terms of treatments without and with inoculum within each cultivar and collection time (Table I). Regarding MDA, significant differences were observed for different soybean cultivars at 6, 24, and 48 HAI in terms of treatments with and without inoculum for each cultivar and collection time (Table I). For POX activity and H_2O_2 and MDA concentrations, interaction was observed among cultivar, collection time, and the presence or absence of *M. javanica* inoculation.

For variable number of nematodes penetrated, a significant interaction was observed between cultivars and collection times, and a significant difference was observed

Table I. Activity of the enzymes peroxidase (POX) and ascorbate peroxidase (APX) and concentration of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in different soybean cultivars at different periods after *Meloidogyne javanica* inoculation.

Cultivars	POX (phenol peroxidase) µM min^{-1} mg Ptn^{-1}															
	Time 1: 6 h				Time 2: 12 h				Time 3: 24 h				Time 4: 48 h			
	N/ I		W/ I		N/ I		W/ I		N/ I		W/ I		N/ I		W/ I	
BMX-Ativa	12.76	Aaα	15.59	Aaα	15.33	Baα	17.66	Aaα	14.84	Aaα	16.99	Aaα	16.68	Aaα	18.38	Aaα
NA-5909	14.77	Aaα	15.02	Aaα	10.20	Aaα	18.39	Aaβ	13.04	Aaα	17.53	Aaβ	13.44	Aaα	20.28	Aaβ
SYN-VTop	11.83	Abα	25.99	Baβ	19.55	Bbα	23.61	Baβ	15.01	Abα	26.28	Baβ	18.00	Abα	25.90	Baβ
BMX-Ícone	17.89	Baα	23.51	Baβ	16.51	Baα	23.02	Baβ	19.82	Baα	20.99	Aaα	17.38	Aaα	23.46	Baβ
Cultivars	APX (ascorbate peroxidase) µM min^{-1} mg Ptn^{-1}															
	Time 1: 6 h				Time 2: 12 h				Time 3: 24 h				Time 4: 48 h			
	N/ I		W/ I		N/ I		W/ I		N/ I		W/ I		N/ I		W/ I	
BMX-Ativa	34.16	Aaα	48.05	Aaα	48.05	Abα	57.22	Aaα	48.88	Abα	50.97	Aaα	47.93	Abα	53.03	Aaα
NA-5909	51.57	Baα	62.38	Baα	45.73	Aaα	51.20	Aaα	50.37	Aaα	50.86	Aaα	41.30	Aaα	52.59	Aaα
SYN-VTop	50.48	Baα	62.53	Baβ	57.61	Aaα	60.21	Aaα	55.68	Aaα	65.43	Baα	48.29	Abα	61.25	Aaβ
BMX-Ícone	45.75	Baα	47.26	Aaα	50.74	Aaα	53.46	Aaα	52.81	Aaα	60.64	Bbα	39.87	Aaα	61.43	Abβ
	H_2O_2 (hydrogen peroxide) mM H_2O_2g mF^{-1}															
Cultivars	Time 1: 6 h				Time 2: 12 h				Time 3: 24 h				Time 4: 48 h			
	N/ I		W/ I		N/ I		W/ I		N/ I		W/ I		N/ I		W/ I	
BMX-Ativa	27.34	Aaα	31.16	Aaβ	25.74	Aaα	40.14	Bcβ	26.98	Aaα	35.65	Abβ	28.00	Aaα	34.46	Abβ
NA-5909	26.03	Aaα	30.59	Aaβ	26.60	Aaα	30.57	Aaβ	26.94	Aaα	34.04	Abβ	27.04	Aaα	40.68	Bcβ
SYN-VTop	24.04	Aaα	33.38	Aaβ	27.84	Abα	31.83	Aaβ	26.14	Aaα	32.91	Aaβ	29.49	Abα	37.09	Abβ
BMX-Ícone	26.81	Aaα	29.72	Aaα	26.66	Aaα	33.09	Abβ	27.81	Aaα	33.19	Abβ	25.54	Aaα	33.78	Abβ
	MDA (malondialdehyde) µm MDA kg MF^{-1}															
Cultivars	Time 1: 6 h				Time 2: 12 h				Time 3: 24 h				Time 4: 48 h			
	N/ I		W/ I		N/ I		W/ I		N/ I		W/ I		N/ I		W/ I	
BMX-Ativa	13.36	Aaα	16.75	Bbβ	12.40	Aaα	12.17	Aaα	11.04	Aaα	10.06	Aaα	11.58	Aaα	11.53	Aaα
NA-5909	12.70	Aaα	23.01	Ccβ	10.52	Aaα	12.06	Aaα	14.45	Baα	17.36	Bbβ	12.42	Aaα	17.44	Bbβ
SYN-VTop	14.29	Aaα	16.89	Bbα	13.14	Aaα	12.89	Aaα	12.48	Aaα	11.90	Aaα	11.80	Aaα	12.60	Aaα
BMX-Ícone	15.70	Abβ	11.74	Aaα	19.38	Bcβ	12.28	Aaα	16.38	Bbα	25.08	Ccβ	12.74	Aaα	21.81	Cbβ

Means followed by the same capital letter compare the cultivars within each time and inoculum, lowercase letters compare the times within each cultivar and each inoculum, Greek alphabet letters compare inoculum within each cultivar and each time and do not differ from each other according to Scott–Knott test at 5% probability. N/I: no inoculation; W/I: with inoculation.

in terms of the penetration of *M. javanica* juveniles into the roots of soybean cultivars at the respective collection times (Figure 1).

When analyzing POX activity at 6 h, significant values were observed in SYN-VTop RR (119.7%) and BMX-Ícone IPRO (31.4%) cultivars in relation to non-inoculated cultivars (Table I). This may be related to the susceptibility of these soybean cultivars, because at this collection time, the mean number of *M. javanica* juveniles penetrated into the roots of these cultivars ranged from 3.75 to 4.0 (Figure 1). APX activity showed greater variation among the cultivars with and without inoculum; however, a similar

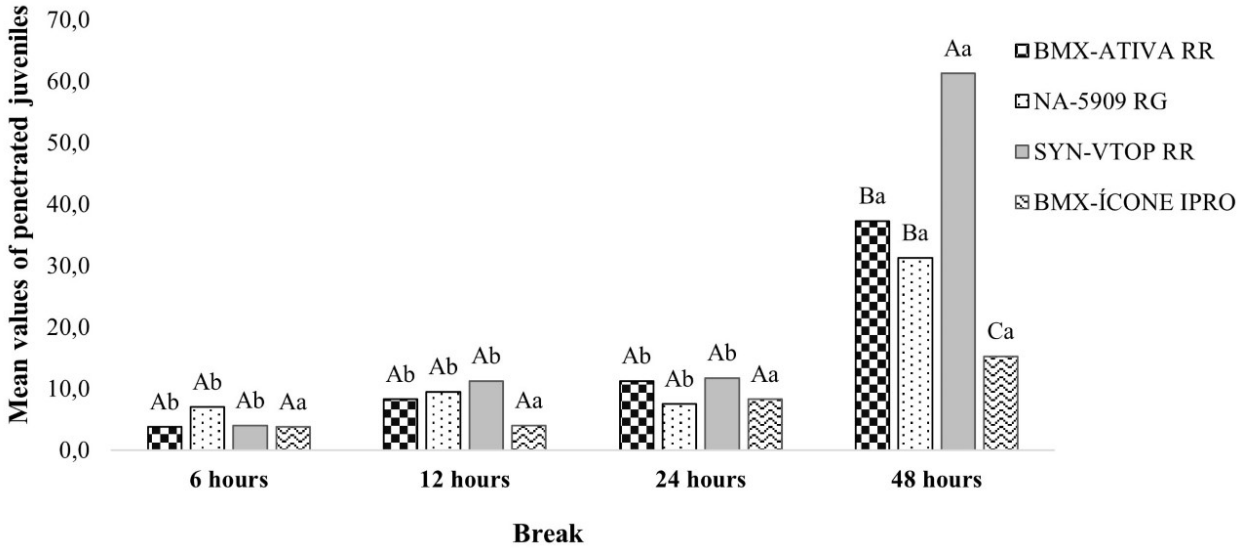

Figure 1. Mean values of the number of *Meloidogyne javanica* juveniles that penetrated into soybean cultivars at different collection times after inoculation.
Means followed by the same capital letter compare cultivars within each time, and lowercase letters compare the times within each cultivar and do not differ from each other, as per the Scott–Knott test at 5% probability.

trend was observed in terms of the increased activity of this enzyme, with values ranging from 3.3% to 40.7% (Table I).

These results observed for POX and APX exhibited a direct relationship with H_2O_2 concentration, which presented an increase between 10.9% and 38.9% in relation to non-inoculated cultivars, with significant increase being observed in the cultivars BMX-Ativa RR, NA-5909 RG, and SYN-VTop RR (Table I). MDA concentration followed the same increasing trend as that of H_2O_2, with the respective values of 25.4%, 81.2%, 18.2% for the abovementioned cultivars, indicating a possible stress at this time (Table I).

For the 12-h evaluation, POX activity in all cultivars presented higher percentage values compared with non-inoculated cultivars, with values ranging from 20.8% to 80.3%, which were significant for the cultivars NA-5909 RG, SYN-VTop RR, and BMX-Ícone IPRO (Table I). This POX response is directly linked to the high H_2O_2 concentration in soybean cultivars, with values between 14.3% and 55.9% (Table I). On analyzing the mean number of *M. javanica* juveniles that penetrated into the roots of the abovementioned cultivars, the respective infection rates were observed: 8.25, 9.50, 11.25 individuals; however, no significant difference was noted (Figure 1).

At the collection time of 24 h, on comparing cultivars with and without *M. javanica* inoculation, an increase in POX activity was observed in all inoculated cultivars; however, it was significant only in the cultivars NA-5909 RG (34.4%) and SYN-VTop RR (75.1%) (Table I). On comparing all cultivars, APX activity showed a significant difference, with the cultivars SYN-VTop RR (17.5%) and BMX-Ícone IPRO (14.8%) presenting a higher percentage of activity compared with those not inoculated (Table I). These results obtained for POX and APX activities were directly linked to H_2O_2 concentration, which presented a significant increase (mean value, 25.9%) in all inoculated versus non-inoculated cultivars (Table I). MDA concentration significantly varied in the cultivars NA-5909 RG (20.1%) and BMX-Ícone IPRO (53.1%). On comparing these results of POX and APX activities and H_2O_2 and MDA

concentrations to the mean number of juveniles of *M. javanica* that penetrated into the roots of the cultivars, the number of individuals was found to be between 7.50 and 11.75; however, no significant difference was found in relation to cultivars (Figure 1).

For the 48-h evaluation, the highest values of POX activity were observed in the cultivars SYN-VTop RR and BMX-Ícone IPRO, with significant differences from the remaining cultivars. However, compared with cultivars with and without *M. javanica* inoculation, an increase in POX activity was observed in all inoculated cultivars versus non-inoculated cultivars, and this significant increase was reported in the cultivars NA-5909 RG (50.9%), SYN-VTop RR (43.9%), and BMX-Ícone IPRO (35.0%) (Table I). For the enzyme APX, significant difference was observed in the cultivars SYN-VTop RR (26.8%) and BMX-Ícone IPRO (54.1%) compared with non-inoculated cultivars (Table I). POX and APX activities can be explained based on H_2O_2 concentration, which showed significant increases in all inoculated cultivars as follows: BMX-Ativa RR (23.1%), NA-5909 RG (50.4%), SYN-VTop RR (25.8%), and BMX-Ícone IPRO (32.3%) (Table I). Moreover, H_2O_2 concentration was directly related to MDA accumulation, as observed in the cultivars NA-5909 RG (40.4%) and BMX-Ícone IPRO (71.2%), which presented the highest H_2O_2 values and highest MDA accumulation, indicating possible stress. When analyzing these results with the number of juveniles of *M. javanica* that penetrated into the roots, a significant difference was observed in the cultivars: 37.25 (BMX-Ativa RR), 31.25 (NA-5909 RG), 61.25 (SYN-VTop RR), and 15.25 (BMX-Ícone IPRO) (Figure 1).

A correlation test was applied to the following variables: RF, POX, APX, H_2O_2, MDA, number of juveniles penetrated at 6 h (NP 6 h), number of juveniles penetrated at 12 h (NP 12 h), number of juveniles penetrated at 24 h (NP 12 h), and number of juveniles penetrated at 48 h (NP 48 h). Table II shows the correlation coefficients. On analyzing these results, a negative correlation was observed between RF of soybean cultivars in relation to the antioxidant enzymes POX ($r = -0.96^{*}$) and APX ($r = -0.73^{ns}$), showing that in a soybean cultivar with higher susceptibility, the activation of these enzymes was lesser (Table II).

H_2O_2 concentration showed a similar correlation with the enzymes POX ($r = -0.61^{ns}$) and APX ($r = -0.36^{ns}$). However, MDA was not significantly correlated with both enzymes (POX and APX) but was significantly correlated with H_2O_2 ($r = -0.90^{ns}$) (Table II). At the collection time of 6 h, a high correlation with POX ($r = -0.62^{ns}$) and H_2O_2 ($r = -0.71^{ns}$) was observed. This pattern remained consistent for POX in subsequent collection times; however, for H_2O_2, a mild reduction was observed in the subsequent collection times ($r = -0.65^{ns}$, -0.66^{ns}, and -0.54^{ns} for 12, 24, and 48 h, respectively; Table II).

DISCUSSION

Infection caused by *M. javanica* increased H_2O_2 production in all soybean cultivars and at all collection times compared with non-inoculated plants. Although different cultivars with different RFs were used, H_2O_2 concentration remained high at all collection times, regardless of the cultivar. A response associated with the RF of cultivars was anticipated—the lower the RF, the faster the antioxidant enzyme activity (POX and APX); this plays an important role in the host defense mechanism by promoting H_2O_2 detoxification, which was observed in the results above.

When infecting the roots of plants, the damage caused by *M. javanica* juveniles as they migrate between cells is lesser than that caused by other species; despite this, ROS production (such as H_2O_2 production) could have already

Table II. Pearson correlation coefficient among variables—reproduction factor (RF), phenol peroxidase (POX), ascorbate peroxidase (APX), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), number of juveniles penetrated at 6 h (NP 6 h), number of juveniles penetrated at 12 h (NP 12 h), number of juveniles penetrated at 24 h (NP 24 h), and number of juveniles penetrated at 48 h (NP 48 h) in different soybean cultivars at different periods after *Meloidogyne javanica* inoculation.

	RF	POX	APX	H_2O_2	MDA	NP 6 h	NP 12 h	NP 24 h	NP 48 h
RF	1								
POX	−0.96*	1							
APX	-0.731^{ns}	0.74^{ns}	1						
H_2O_2	0.38^{ns}	-0.61^{ns}	-0.36^{ns}	1					
MDA	-0.053^{ns}	0.29^{ns}	-0.05^{ns}	-0.901^{ns}	1				
NP 6 h	0.75^{ns}	-0.62^{ns}	-0.15^{ns}	-0.71^{ns}	0.17^{ns}	1			
NP 12 h	0.068^{ns}	-0.17^{ns}	0.48^{ns}	-0.65^{ns}	-0.71^{ns}	0.64^{ns}	1		
NP 24 h	-0.52^{ns}	0.28^{ns}	0.38^{ns}	-0.66^{ns}	-0.81^{ns}	0.13^{ns}	0.71^{ns}	1	
NP 48 h	-0.35^{ns}	0.20^{ns}	0.69^{ns}	-0.54^{ns}	-0.74^{ns}	0.32^{ns}	0,92*	0.84^{ns}	1

Significance of F values: * = 5%; and NS = not significant.

occurred in the initial phase of penetration into the root (Gheysen & Mitchum 2011). This accumulation in plant cells can be toxic to both the plant and the nematode (Gillet et al. 2017, Sato et al. 2019). However, according to Apel & Hirt (2004), this is one of the fastest defense mechanisms to the pathogenic infection—known as oxidative burst—and is mediated mainly by H_2O_2 accumulation at the site of penetration.

In this context, the initial reaction of a susceptible cultivar is similar to that of a resistant cultivar and may be the result of nematode secretions in plant tissues during their migration (Davis et al. 2000, Silva 2001). Melillo et al. (2006), when studying ROS production and H_2O_2 concentration during the early stages of plant–nematode interaction and comparing the responses of susceptible and resistant tomato plants to avirulent and virulent populations of *M. incognita*, found a more significant increase in ROS levels in the roots of resistant cultivars.

Different responses were observed among cultivars with regard to biochemical parameters, particularly when compared with non-inoculated cultivars. In inoculated plants, H_2O_2 concentration in all soybean cultivars increased in response to the *M. javanica* infection, regardless of RF. This H_2O_2 concentration is generated by a reaction catalyzed by superoxide dismutase or as a normal product of plant cellular metabolism (Sharma et al. 2012). According to Melillo et al. (2011), the oxidative burst in plants occurs in the early stages of infection by the parasitic nematode of plants, which is consistent with our results. Despite a low interaction at 6 HAI of *M. javanica* juveniles and the roots, even with inoculation of only infective juveniles (J_2), this increase in penetrated juveniles occurred in subsequent times (12, 24 and 48 HAI) (Figure 1).

The enzyme POX is directly involved in the removal of H_2O_2 and plays an important role in the defense of plants against pathogens, because it participates in lignin biosynthesis and wound healing (Resende et al. 2003). Increased activity of this enzyme is necessary to reduce H_2O_2 concentrations (Apel & Hirt 2004).

Our results showed a significant increase in POX activity in cultivars with low RF (SYN-VTop RR and BMX-Ícone IPRO) in the early collection times, indicating a faster response of these cultivars to infection (Table II). Similarly, the enzyme APX showed significance for the same cultivars only at 48 HAI, although in all evaluations its activity was numerically higher than those observed in non-inoculated cultivars, with later response in this interaction, although APX had a higher affinity for the removal of H_2O_2 (Apel & Hirt 2004)—a situation that was inconsistent with the results presented above (Table II).

MDA concentration has a direct relationship with increased H_2O_2 concentration (Kruse et al. 2006, Sharma et al. 2012). Our results were partially consistent with this fact, considering that high MDA concentrations were observed at the first collection time in two cultivars: BMX-Ativa RR and NA-5909 RG, which presented higher RFs. Plant parasitic nematodes are obligate parasites that are completely dependent on their host to receive nutrients (Mitchum et al. 2007, Hofmann et al. 2010). Resistance to *M. javanica* is majorly based on the hypersensitivity reaction triggered by the programmed cell death at the site of infection (Williamson 1999). According to Ali et al. (2018), plants produce ROS to induce their defense responses against pathogens, stimulating programmed cell death or hypersensitive response to stop and kill the invading pathogen at the site of infection.

Das et al. (2010) conducted a transcriptional profiling study of feeding sites of root-knot nematodes in cowpea roots and reported that these microorganisms can reduce or neutralize the release of ROS in the host cell to prevent cell death via the use of antioxidant enzymes in the plant, because the ROS production exceeds their ability to eliminate it, leading to ROS accumulation and thus causing cell death. In this study, root-knot nematodes can modulate ROS production as signaling molecules to induce the antioxidant pathways rather than as toxic compounds (Gillet et al. 2017, Ali et al. 2018).

However, the ability of this group of pathogens to secrete effector molecules that can control cell signaling pathways and thus establish long-term relationships with the host (Kyndt et al. 2013, Mantelin et al. 2015) may explain the low correlation observed for the antioxidant enzymes POX and APX, which was inversely proportional to H_2O_2 accumulation, with POX presenting higher activity at collection times in cultivars of low RF, regardless of APX having a higher affinity with H_2O_2 than POX (Apel & Hirt 2004). Studies in this field using transcriptome analysis have reported that nematode parasites of plants influence ROS in apoplast, as it releases trapped ROS, such as glutathione peroxidase, peroxidase, peroxiredoxin, and catalases (Bellafiore & Briggs 2010).

As observed in the study results, H_2O_2 concentrations were more evident in all inoculated cultivars compared with non-inoculated cultivars; however, the behavior of MDA did not have a direct relation with this increase and may be insufficient to cause damage to cell membranes via lipid peroxidation. The products of lipid peroxidation of the membrane, such as MDA, can be an important indicator of the extent of cell damage caused by oxidative stress induced by pathogens (Silva et al. 2020)—a fact that was not observed in the present study; however, interestingly, this fact may be associated to the ability of this nematode to alter the initial signaling pathways of defense in plants to establish its feeding site (Goverse & Smant 2014).

Overall, these results indicated that the interaction between *M. javanica* and soybean cultivars resulted in ROS production, particularly H_2O_2, with no substantial differences observed in terms of H_2O_2 accumulation at the different

collection times (hours); further, the differences were relatively small and showed a similar response, regardless of the RF of the cultivar. However, the antioxidant enzymes POX and APX presented a particularly stronger correlation with the cultivars with lower RF (SYN-VTop RR and BMX-Ícone IPRO), indicating a possible faster response of the antioxidant complex, considering that both enzymes are a part of a network of host defense-related mechanisms against oxidative stress in the cell for preventing or minimizing damage.

Several studies have addressed the kinetics of oxidative stress associated with interaction with biotrophic pathogens; however, only a few studies have analyzed the pathosystem of *M. javanica* versus soybean. To the best of our knowledge, this is one of the first studies to provide information regarding the behavior of different soybean cultivars in response to *M. javanica* infection. Finally, these results showed that antioxidant enzymes can act as oxidative stress markers associated with the RF of cultivars; however, further studies with a higher number of soybean cultivars are warranted to confirm the results obtained in this study.

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