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RESPONSE OF MELON CULTIVARS TO INFECTION BY *Macrophomina pseudophaseolina* ISOLATES AND ITS EFFECT ON PROTEIN EXPRESSION

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ABSTRACT

It was studied the pathogenicity of *Macrophomina pseudophaseolina* isolates in melon seedlings and their influence on protein expression of the crop. For this purpose, two isolates of the fungus were inoculated in Golden and Cantaloupe melon seedlings using the toothpick method. Thirty days after inoculation, disease incidence and severity were assessed using pre-established scales; additionally, stem and root length and fresh weight was evaluated. Protein extraction from plant tissues was performed using the phenol/SDS precipitation method and quantification by the Bradford method. The protein samples were subjected to denaturing polyacrylamide gel electrophoresis (SDS-PAGE). Then, the existence of polymorphism and molecular weight of bands detected in the samples were evaluated. *M. pseudophaseolina* isolates caused root rot in all inoculated melon seedlings. However, the two isolates exhibited different degrees of aggressiveness to the seedlings. The isolates CMM-4801 and CMM-4771 caused an average symptom severity of 3.40 and 2.60, respectively, in both cultivars. The disease negatively affected seedling development by reducing root length, and stem and root fresh weight. Different polypeptide band patterns were verified between inoculated and control seedlings, indicating different protein expression due to biotic stress caused by the fungus. Moreover, different protein expression patterns were found between the inoculated seedlings, indicating a correlation between pathogen aggressiveness and host response.

Additional Keywords: *Cucumis melo*, root rot, SDS-PAGE, vine decline

RESUMEN

Respuesta de cultivares de melón a infección por *Macrophomina pseudophaseolina* y su efecto en la expresión proteica

En este estudio se evaluó la patogenicidad de aislados de *Macrophomina pseudophaseolina* en plántulas de melón y su influencia en la expresión proteica. Con este fin, se inocularon dos aislados del hongo en plántulas de melón Golden y Cantaloupe mediante el método del palillo. Treinta días después de la inoculación se evaluó la incidencia y severidad de la enfermedad con la ayuda de una escala pre-establecida; adicionalmente, se evaluó la longitud del tallo, raíz y su peso fresco. La extracción de proteínas de los tejidos vegetales se realizó mediante el método de precipitación con fenol/SDS y la cuantificación mediante el método de Bradford. Las muestras de proteínas se sometieron a electroforesis en gel de poliacrilamida desnaturalizante (SDS-PAGE). Posteriomente, se evaluó la existencia de polimorfismo y peso molecular de las bandas detectadas en las muestras. Los aislamientos de *M. pseudophaseolina* causaron pudrición de la raíz en todas las plántulas de melón inoculadas. Sin embargo, los dos aislamientos exhibieron diferentes grados de agresividad en las plántulas. Los aislamientos CMM-4801 y CMM-4771 provocaron una severidad de síntomas promedio de 3.40 y 2.60, respectivamente, en ambos cultivares. La enfermedad afectó negativamente el desarrollo de las plántulas al reducir la longitud de la raíz y el peso fresco del tallo y raíz. Se verificaron diferentes patrones de bandas polipeptídicas entre las plántulas inoculadas y el control, lo que indica una expresión proteica diferente debida al estrés biótico causado por el hongo. Además, se encontraron diferentes patrones de expresión de proteínas entre las plántulas inoculadas, lo que indica una correlación entre la agresividad del patógeno y la respuesta del huésped.

Palabras clave: *Cucumis melo*, decadencia de las ramas, pudrición de la raíz, SDS-PAGE

INTRODUCTION

Melon (*Cucumis melo* L.), belonging to the Cucurbitaceae family, is a crop of great economic importance. According to FAO (2023), 27.5 million Mg of melons were produced in 2021. The crop is adapted to regions of high light intensity, low rainfall, and dry climate, which has intensified its cultivation (Costa et al., 2020).

Continuous cropping without preventive and cultural control of weeds and diseases can decrease melon productivity and fruit quality. Studying prevailing weeds in cucurbit fields, Sales Júnior et al. (2012; 2019) found the fungus *Macrophomina phaseolina* present in 13 weed

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species infesting melon cultivation areas in Northeastern Brazil. Later, through phylogeny studies, Negreiros et al. (2019) identified a new species, *Macrophomina pseudophaseolina*, in *Trianthema portulacastrum* L. and *Boerhavia diffusa* L, common weed species in cucurbit cultivation areas in the states of Rio Grande do Norte and Ceará.

Previously, *M. pseudophaseolina* was described by Sarr et al. (2014) in fungal isolates from okra (*Abelmoschus esculentus* L.), peanut (*Arachis hypogaea* L.), sorrel (*Hibiscus sabdarifa* L.), and cowpea (*Vigna unguiculata* L.) roots in cultivation fields of Senegal. The species has shown to have a greater geographic distribution and it has been reported in India acting as plant probiotic fungus (Mastan et al., 2019), and also in Brazil, infecting cassava (Brito et al., 2019), and oilseed crops like physic nut (*Jatropha curcas* L.), castor bean (*Ricinus communis* L.), Mexican cotton (*Gossypium hirsutum* L.) and peanut (Machado et al., 2019).

Species from the genus *Macrophomina* are among the causal agents of root rot and vine decline (RRVD) in melon, causing serious economic problems in the cultivation of cucurbits worldwide (Porto et al., 2019). Control of *Macrophomina* is problematic for producers due to the high genetic diversity of the species, which allows its adaptation to different agroecological conditions and a variety of hosts (Iqbal and Mukhtar, 2014). Such genetic variations are expressed at different disease severity levels in plants (Purkayastha et al., 2006).

M. pseudophaseolina can negatively influence plant growth and development. By infecting roots, the pathogen hinders plants from expressing their defense mechanisms, thereby increasing their susceptibility. Thus, this work aimed to evaluate the severity of *M. pseudophaseolina* isolates (collected from weeds) infesting melon cultivars and verify the influence of this interaction on protein expression.

MATERIALS AND METHODS

Plant material and experiment location. Seeds from two commercial melon cultivars, Golden (Golden, Feltrin Seeds) and Cantaloupe (Hale's Best Jumbo, Top Seed Garden), were used in this study. The seeds were disinfested with 2% sodium hypochlorite solution for one minute, then washed twice with sterile distilled water for one minute. After washing, the seeds were placed in Petri dishes with filter paper and cotton soaked with distilled water, then kept in a bacteriological oven for ± 48 hours at 37 °C in darkness.

After germination, the seeds were transferred to 1.0 L pots filled with soil and organic substrate at 2:1 ratio. Soil and substrate were previously autoclaved before use. The experiments were carried out at the Biotechnology Laboratory and Phytopathology Laboratory II, Department of Agronomic and Forest Sciences, Federal Rural University of Semiarid, located in Mossoró, RN, Brazil.

Pathogenicity and virulence in melon. The *M. pseudophaseolina* isolates CMM-4771, collected from *T. portulacastrum*, and CMM-4801, from *B. diffusa*, deposited in the Culture Collection of Phytopathogenic Fungi Prof. Maria Menezes (CMM) at Federal Rural University of Pernambuco, Brazil, were used in this study. These specimens were proved as genetically distant in previous studies on genetic characterization (Costa et al., 2020).

The inoculation technique used was the toothpick method, as described by Ambrósio et al. (2015). Pieces of 12 mm long toothpicks, with the sharpened end up, were placed in holes made in a 90 mm diameter filter paper. The toothpicks were then placed in Petri dishes with the same diameter as the filter paper and autoclaved twice for 30 min at 121 °C, with an interval of 24 h between the autoclaving. Afterward, 20 mL of PDA (potato-dextrose-agar) medium supplemented with streptomycin sulfate were added to each toothpick-containing Petri dish. Once solidified, the PDA plates were inoculated with five mycelial plugs (6 mm in diameter) from pure cultures of each *M. pseudophaseolina* isolate and were incubated for eight days at 28±2 ºC in darkness in a bio-oxygen demand (BOD) incubator.

Melon seedlings were inoculated ten days after planting by inserting the toothpicks infested with mycelia and microsclerotia in the hypocotyl of each seedling, 1 cm above the soil. Non infested and autoclaved toothpicks were used as control. The seedlings were kept in the greenhouse for 30 days under natural light conditions and at 33 °C average temperature. The experimental design was completely randomized in a 2 × 2 + 2 factorial

arrangement, with two melon cultivars, two *M. pseudophaseolina* isolates, and two controls, totaling six treatments with five replicates each.

Thirty days after inoculation of each isolate, disease severity was assessed using the scale described by Ambrósio et al. (2015), where 0 = symptomless, 1 = less than 3 % of shoot tissues infected, 2 = 3–10 % of shoot tissues infected, 3 = 11–25 % of shoot tissues infected, 4 = 26–50 % of shoot tissues infected and 5 = more than 50 % of shoot tissues infected. Disease incidence was determined by the percentage of infected plants from the number of plants in the experiment. Fresh weight was measured using an analytical scale and shoot height and root length were measured using a caliper. Moreover, tissue fragments from plants with symptoms were placed in PDA plates to isolate the inoculated fungi and complete Koch's postulate.

Disease severity data were analyzed by the Kruskal-Wallis test at 5% probability. In turn, shoot height, root length, and shoot and root fresh weight data were analyzed by Tukey's test at 5 % probability. All statistical analyzes were performed using the Assistat Software version 7.7 (Silva and Azevedo, 2016).

Protein extraction and analysis. After pathogenicity analysis, seedling roots and stems were collected and frozen in liquid nitrogen to avoid the action of proteases. Subsequently, the plant material was kept at -80 °C in an ultra-freezer. Protein extraction was performed using the method described by Wang et al. (2006). About 350 mg of each tissue sample was ground into a fine powder in a mortar and pestle under liquid nitrogen then transferred into 2 ml tubes and 1 ml of 10 % TCA/acetone solution was added to the tubes, mixed by vortexing, and centrifuged for 10 min at 6,000 g and 4 °C. Then the supernatant was discarded. This same procedure was repeated using 0.1 M methanolic ammonium acetate, 80% acetone, and 80% ethanol solutions. Afterward, the pellet was dried at room temperature to remove residual acetone, ethanol, and methanol.

Subsequently, 800 µL of dense SDS buffer (2 % SDS, 0.9 M sucrose, 0.1 M Tris-HCl pH 8.0, 5 % β-mercaptoethanol, 10 mM EDTA, 5% PVP, and ultrapure water were added to the tubes and mixed by vortexing. Then, 800 µL of phenol pH 8.0 were added and mixed again. The tubes were then centrifuged for 10 min at 6,000 g's and 4 °C. The upper (aqueous) phase was transferred into a new tube, 1 mL of 0.1 M ammonium acetate in methanol was added, and tubes were incubated at -20 °C for 12 h. The tubes were then centrifuged at 6.000 × g for 10 min at 4 ° C, and the supernatant was discarded. The pellet was washed with 0.1 M methanolic ammonium acetate and 80% acetone solutions, then dried at room temperature to remove residual acetone and methanol. The protein samples were solubilized in 50 µL of solubilization buffer (7 M Urea, 2 M Tiourea) and sonicated (10 % potency for 5 to 10 seconds) until complete homogenization, then stored at -20 °C in a freezer.

Protein was quantified using the Bradford method described by Kruger (2009). One µL sample was diluted in 99 µL of ultrapure water and 900 µL of Bradford reagent. Absorbance at 595 nm was measured in a visible light spectrophotometer (Biospectro SP-220). Then, absorbances were plotted on a standard curve previously constructed with known concentrations of BSA (Bovine Serum Albumin), thus determining the protein concentration in the samples.

Denaturing polyacrylamide gel electrophoresis (SDS-PAGE) The protein samples were submitted to denaturing polyacrylamide gel electrophoresis (SDS-PAGE) according to Brunelle and Green (2014). About 15 $\mu g \cdot ml^{-1}$ of each protein sample were added to the sample buffer (120 mM Tris-HCl pH 6.8, 4 % SDS, 20 % Glycerol, 750 mM β-mercaptoethanol, and 0.05 % bromophenol blue) in the proportion of 2:1 and were denatured by heating at 94 °C for 3 min. The samples were placed on 12 % acrylamide gel and submitted to electrophoresis in Tris-Glycine buffer at 80 V for 30 min and 150 V for 1 h 40 min. Subsequently, the gel was fixed in 40 % ethanol and 80% acetic acid solutions for ±12 h. The gel was then stained in Coomassie Brilliant Blue solution for ±48 h and then decolored in 10 % acetic acid and 30 % methanol for ±4 h. Images of the gel were recorded in a photo-documenter under white light, and protein bands were analyzed by the CLIQS 1D software (Version 1.1, TotalLab) to estimate the molecular weight and detect the presence (+) and/or absence (-) of each band in the samples.

RESULTS

Pathogenicity and virulence in melon. Pathogenicity analysis of the *M. pseudophaseolina* isolates revealed that CMM-4771 and CMM-4801 with 100% incidence caused root rot and vine decline in both melon cultivars evaluated (Table 1). Stem rot and leaf wilting and goldening were symptoms presented in the seedlings inoculated, whereas control seedlings showed no symptoms. CMM-4801 was the most aggressive with severity of 3.40 according to the Ambrósio scale, for both melon cultivars, while CMM-4771 caused a severity degree of 2.60 in Golden and 2.70 in Cantaloupe. Cultivars did not show tolerance or resistance to the isolates tested in this study.

Table 1. Root rot severity and incidence in Golden and Cantaloupe melons inoculated with *M. pseudophaseolina* isolates

M. pseudophaseolina isolates	Severity				Incidence			
	Golden		Cantaloupe		Golden		Cantaloupe	
	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean
CMM-4771	187.5	2.60	189.5	2.70	205.0	100	205.0	100
CMM-4801	222.5	3.40	220.5	3.40	205.0	100	205.0	100
Control	55.0	0.00	55.0	0.00	55.0	0	55.0	0
$\chi 2$	21.3		21.3		29.0		29.0	

$\chi 2$: Chi-squared value was significant at 5% probability by the Kruskal-Wallis test

In both cultivars, shoot height (SH), root length (RL), shoot fresh weight (SFW), and root fresh weight (RFW) were significantly different between inoculated and control seedlings (Table 2). In Golden melon, RL was 13.9 cm, and 12.6 cm in seedlings inoculated with isolates CMM-4771 and CMM-4801, respectively, inferior to the 20.5 cm observed in control seedlings. Similarly, SFW was 0.9 g and 1.0 g, and RFW was 0.09 g and 0.11 g in seedlings inoculated with CMM-4771 and CMM-4801, respectively, against 1.5 g and 0.21 g in control seedlings (Table 2).

Table 2. Mean for shoot height (SH, cm), root length (RL, cm), shoot fresh weight (SFW, g), root fresh weight (RFW, g) in Golden and Cantaloupe melon seedlings inoculated with *M. pseudophaseolina* isolates

M. pseudophaseolina	Golden cultivar				Cantaloupe cultivar			
	SH	RL	SFW	RFW	SH	RL	SFW	RFW
CMM-4771	16.015 a	13.905 b	0.940 b	0.09851 b	11.865 a	14.26 b	0.75729 b	0.12571 b
CMM-4801	15.140 a	12.640 b	1.000b	0.11731 b	11.548 a	13.89 b	0.87506 b	0.20513 b
Control	11.800 b	20.560 a	1.586 a	0.21570 a	8.424 b	18.98 a	1.28645 a	0.47045 a
CV (%)	18.03	31.78	21.74	56.95	19.92	25.28	18.83	76. 40

Means followed by same letter in each column are not significantly different by Tukey's test at 5 % probability
CV: coefficient of variation

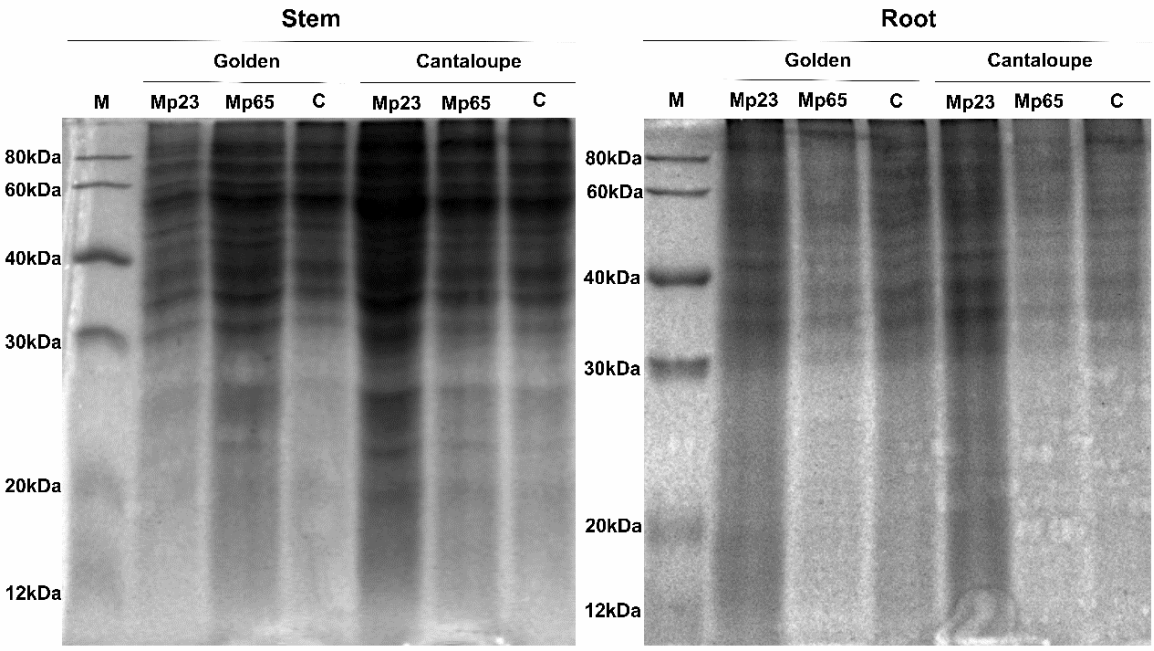
In Cantaloupe, SFW was 0.75 g in seedlings inoculated with CMM-4771 and 0.87 g with CMM-4801, while control seedlings had 1.28 g (Table 2). RFW was 0.12 g and 0.20 g in seedlings inoculated with CMM-4771 and CMM-4801, respectively, while it was 1.28 g in control seedlings (Table 2). RL in inoculated seedlings was 14.2 cm (CMM-4771) and 13.8 cm (CMM-4801), and it was 18.9 cm in control seedlings (Table 2).

On the other hand, SH was higher in inoculated seedlings than in control ones in both cultivars. In Golden melon, SH was 16 cm and 15.1 cm in seedlings inoculated with CMM-4771 and CMM-4801, respectively, against 11.8 cm observed in control seedlings. In Cantaloupe cultivar, it was 11.8 cm (CMM-4771) and 11.5 cm (CMM-4801) against 8.4 cm in the control (Table 2).

Protein profile analysis. In stem tissue, 17 bands were identified in Golden melon and 18 in

Cantaloupe (Table 3). The main bands were in the range of 88 to 19 kDa (Figure 1). From the 17 bands detected in Golden melon, seven were differential between inoculated and control plants. Seedlings inoculated with isolate CMM-4801 showed six different bands relative to control; when inoculated with CMM-4771, only one band was in the 24 kDa range (Table 3). In Cantaloupe melons, nine of the 18 bands were different from control. Seedlings inoculated with CMM-4771 showed six different bands from control, while seedlings inoculated with CMM-4801 showed five bands (Table 3).

Figure 1. Protein profile submitted to denaturing polyacrylamide gel electrophoresis (SDS-PAGE) of the stem and root melon tissues and interaction with *M. pseudophaseolina* isolates. C = Control. M = Protein molecular weight marker (Transgen Biotech, China).



The protein profile in melon root tissues revealed 20 polypeptide bands for Golden and 21 for Cantaloupe (Table 4). Bands were in the range of 88 to 13 kDa (Figure 2). Of the 20 bands detected in Golden melon, seven of them in inoculated seedlings were different from control (Table 4). Seedlings inoculated with isolate CMM-4801 showed six different bands from control seedlings, whereas, with CMM-4771, only three bands were different (Table 4). The decrease in the number of polypeptide bands between inoculated and control seedlings was five for both isolates (Table 4). In Cantaloupe, three of the 21 bands showed different profile in inoculated seedlings relative to control. Those inoculated with CMM-4801 showed three different bands from control, whereas CMM-4801 presented two different bands (Table 4).

DISCUSSION

In the present study, we analyzed the pathogenicity of genetically distinct *M. pseudophaseolina* isolates. A high disease incidence (100%) caused by the isolates was detected, evidencing their high virulence. However, this incidence differs from the results reported in previous studies. Negreiros et al. (2019) reported a low incidence of RRVD caused by *M. pseudophaseolina* (10%) in melon when compared to *M. phaseolina* isolates (86%). In cowpea and peanut varieties, Ndiaye et al. (2015) reported a higher incidence (64%) in the temperature range of 36/26 °C compared to 34/24 °C (22%).

Species from the genus *Macrophomina* have an advantage over hosts under water stress conditions and in hot and dry environments such

as in the Brazilian Semiarid Region (Cohen et al., 2016). Under these conditions, a high incidence of RRVD caused by *M. pseudophaseolina* was observed in cowpea seedlings (Sales Júnior et al., 2020).

Table 3. Number of protein bands detected and their presence (+) and absence (-) in melon stem samples.

Bands	MW	Golden				Bands	MW	Cantaloupe			
		CMM 4771	CMM 4801	C	PL			CMM 4771	CMM 4801	C	PL
1	88	+	-	+	P	1	91	-	+	+	P
2	85	-	+	-	P	2	88	+	-	-	P
3	75	+	-	+	P	3	75	-	+	+	P
4	73	-	+	+	P	4	72	+	-	-	P
5	60	-	+	+	P	5	60	+	+	+	M
6	53	-	+	+	P	6	52	-	+	+	P
7	45	-	-	+	P	7	50	+	-	-	P
8	44	+	+	-	P	8	44	+	+	+	M
9	41	+	+	+	M	9	41	+	+	-	P
10	38	+	+	-	P	10	38	+	+	+	M
11	36	-	-	+	P	11	34	+	+	+	M
12	34	+	+	-	P	12	30	+	+	+	M
13	32	-	-	+	P	13	27	+	+	-	P
14	31	+	+	-	P	14	25	+	+	+	M
15	25	-	+	+	P	15	23	-	+	-	P
16	24	+	-	-	P	16	22	-	+	-	P
17	22	-	+	-	P	17	21	+	-	-	P
						18	19	+	+	-	P
Total of bands		8	11	10				13	14	9	
		Polymorphism rate (%)			94						66

C= Control. MW = Molecular weight of bands as kilodalton (kDa). PL= Polymorphism: polymorphic (P); monomorphic (M)

The genetic variability of *M. pseudophaseolina* isolates can explain the difference in their aggressiveness to melon cultivars. Similarly, Purkayastha et al. (2006) observed that some genetically distinct *M. phaseolina* isolates were more aggressive to cluster bean. Moreover, high genetic diversity in the genus *Macrophomina* can reflect on its degree of pathogenicity since changes in their physiological characteristics guarantee adaptability to different agroecological conditions (Sánchez et al., 2019; Rafiei et al., 2013).

Infection caused by species from the genus *Macrophomina* mainly affects roots, limiting water and nutrient uptake by plants, consequently impairing their development (Sánchez et al., 2019). Such behavior was observed in both melon types studied, with an intense reduction in length and fresh weight on this organ. Other studies have shown a significant reduction in root growth in other cultivated species. For instance Sharma et al. (2018) reported that root and stem length and root fresh weight were significantly affected by inoculation with *M. phaseolina*. In mung bean, Morales and Hernández (2021) found that affected roots caused a serious reduction of in soybean yield.

Protein profile showed that inoculation with *M. pseudophaseolina* isolates changed the protein expression in melon seedlings. In both stem and root, differential bands were detected between inoculated and control seedlings, indicating that

the host responded to stress caused by the fungus infection. During pathogenesis, plant expresses several defense mechanisms against the pathogen, including cell wall lignification, release of oxidative enzymes, and biosynthesis of pathogenesis-related (PR) proteins (Kaur et al., 2017). These mechanisms alter some protein enzyme synthesis. Despite the change in protein expression, both melon types tested were highly susceptible to the isolates CMM-4771 and CMM-4801, suggesting that these proteins did not act as a defense system against the pathogen.

Table 4. Number of protein bands detected and their presence (+) and absence (-) in melon root samples.

Bands	MW	Golden				Bands	MW	Cantaloupe			
		CMM 4771	CMM 4801	C	PL			CMM 4771	CMM 4801	C	PL
1	86	+	-	+	P	1	88	+	+	+	M
2	70	-	+	+	P	2	78	-	+	-	P
3	59	-	-	+	P	3	73	+	-	+	P
4	54	+	+	-	P	4	65	-	-	+	P
5	51	-	-	+	P	5	56	+	+	+	M
6	48	+	-	-	P	6	54	-	+	+	P
7	46	-	+	-	P	7	46	-	+	+	P
8	44	+	-	+	P	8	43	+	+	-	P
9	40	+	+	+	M	9	38	+	+	+	M
10	37	+	+	+	M	10	35	+	+	+	M
11	34	+	+	+	M	11	31	+	+	+	M
12	32	-	-	+	P	12	28	+	-	+	P
13	30	-	+	+	P	13	26	+	+	+	M
14	27	-	+	-	P	14	25	-	-	+	P
15	25	+	+	+	M	15	23	+	+	-	P
16	22	+	+	+	M	16	22	+	+	+	M
17	20	-	+	-	P	17	21	-	+	+	P
18	18	+	+	+	M	18	19	+	+	+	M
19	15	-	+	-	P	19	17	-	+	+	P
20	14	-	+	-	P	20	15	-	+	+	P
						21	13	+	-	+	P
Total of bands		10	14	13				13	16	18	
			Polymorphism rate (%)		65						61

C= Control. MW = Molecular weight of bands as kilodalton (kDa). PL= Polymorphism: polymorphic (P); monomorphic (M).

Protein profile assessment in other crop species has allowed to identify susceptible cultivars. Aravind and Brahmbhatt (2018) reported that *M. phaseolina* infection induced the expression of three polypeptide bands in resistant cultivars that were not expressed in susceptible ones. Likewise, Aboshosha et al. (2008) observed that resistant sunflower cultivars expressed polypeptide bands, whereas susceptible cultivars did not. The overexpression of some proteins in cultivars resistant to pathogens may be associated with an increase in peroxidase activity, a pathogenesis-related enzyme that causes a hypersensitivity reaction in plant tissues (Nurcahyani et al., 2016).

Reduced protein expression, revealed by the lower number of bands in control relative to inoculated seedlings, mainly in root tissues, may

be associated with damage caused by the fungus in roots, such as decreased fresh weight.

Furthermore, differences in band pattern between seedlings inoculated with the isolates CMM-4771 and CMM-4801 indicate different host-pathogen interactions. Plant-pathogen interaction depends on pathogenicity and virulence capacity, and the existence of defense mechanisms in plants. Association between these interactions results in plant susceptibility or resistance (González-Fernández et al., 2010). Though, the different interactions between Golden and Cantaloupe melons and CMM-4771 and CMM-4801 isolates assessed by protein expression did not change the plant susceptibility.

Results herein reinforce that *M. pseudophaseolina* is a fungus species exhibiting severity with intraspecific variation related to its high degree of genetic heterogeneity. These factors should be considered by researchers searching for resistance sources for use in breeding programs and/or *in vitro* and *in vivo* tests for control methods against *M. pseudophaseolina*.

CONCLUSION

Pathogenicity analysis in *M. pseudophaseolina* isolates showed that genetic differences between individuals from the same species may influence their degree of aggressiveness. This influence was also observed by changes in protein expression in seedlings infected by the isolates. Elucidating these response mechanisms against the pathogen may be the key to the development of efficient control measures.

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