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Effect of temperature on disease severity of charcoal rot of melons caused by *Macrophomina phaseolina*: Implications for selection of resistance sources.

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Abstract

Macrophomina phaseolina is the causal agent of charcoal rot disease of melons and causes significant losses worldwide and causes significant losses worldwide The use of resistant cultivars is a desirable method for controlling this disease, but there is no information about the influence of temperature on the resistant behavior found in melon accessions. The purpose of the present study was to assess the effect of temperature on the reaction of six melon accessions selected previously for their resistant response to *M. phaseolina*. They were inoculated with the *M. phaseolina* isolate CMM-1531 and grown under accurately controlled environmental conditions at different temperature regimes (25, 28, 31, and 34 ºC) in a replicate experiment. The increase in temperature increased the severity of symptoms in most genotypes, but this effect was less pronounced in the highly susceptible control, the cultivar 'Piel de sapo', and in the most resistant accession, the wild African *agrestis* Ag-15591Ghana

that remained resistant even at 34ºC. The use of several screening temperatures allowed a better characterization of accessions that behaved similarly as highly resistant at 25ºC (Con-Pat81Ko, Dud-QMPAfg, Can-NYIsr and Ag-C38Nig), but in which resistance breaking was observed with temperature rise. Temperatures of 28ºC and 31ºC were sufficient to make Dud-QMPAfg, Ag-C38Nig and Can-NYIsr moderately resistant, whereas Con-Pat81Ko remained highly resistant. All these genotypes were susceptible at 34ºC, which suggest that are not suitable for hot-climate growing areas. The most promising accession was Ag-15591Ghana, whose resistance was confirmed in two greenhouse experiments under stressful temperatures (>34ºC). The behavior of these sources should be confirmed in naturally infested fields, but the controlled screening methods presented here are essential to characterize new resistance sources and to conduct genetic studies when a high number of plants must be managed under controlled environmental conditions.

Keywords: soil borne fungus, *Cucumis melo* germplasm, heat stress, host resistance

Declarations

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Compliance with ethical standards

Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Ethics approval. Human participants and /or animals

The present research did not involve any experimentation on humans or animals.

Consent to participate

Not applicable

Consent for publication

All authors approved the version to be published.

Availability of data and material and code availability

All authors asure that all data and materials as well as software application or custom code support the published claims and comply with field standards.

Authors' contributions

Glauber Henrique de Sousa Nunes, Márcia Michelle Queiroz Ambrósio and Belén Picó contributed to the study conception and design. Material preparation, data collection and analysis were performed by Glauber Henrique de Sousa Nunes, Cheyla Magdala de Sousa Linhares, Márcia Michelle Queiroz Ambrósio and Salvador Barros Torres. The first draft of the manuscript was written by Belén Picó, Glauber Henrique de Sousa Nunes and Cristina Esteras. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Introduction

Brazil is the main producer and exporter of melons in South America. In 2017, it produced 540,229 tons in a cultivated area of 23,390 ha (FAO 2019). The Brazilian production is concentrated in the semiarid region (> 95 %), especially in the states of Ceará and Rio Grande do Norte, which have excellent environmental conditions for crop development such as high temperatures (> 28° C), low rainfall rate (approx. 600 mm per year), and high luminosity (Nunes et al. 2016).

Melon is usually cultivated all year long, without crop rotation, or rotating with another cucurbit, such as watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai). This intensive use of producing fields increases problems with soil borne pathogens. The generalist Ascomycete *Macrophomina phaseolina* (Tassi) Goidanich is one of the most important soil borne fungus affecting melon crop (Cohen et al. 2016a; Nascimento et al. 2018). This species presents high variability (Groenewald and Crous 2014), likely due to its heterokaryotic character, and affects a wide range of hosts species, including more than 500 crops and some weeds that can constitute a problem as important sources of inoculum (USDA 2019). To date, other three *Macrophomina* species, *M. pseudophaseolina* Crous, Sarr and Ndiaye, *M. euphorbiicola* A.R. Machado, D.J. Soares and O.L. Pereira, and *M. vaccinii* Y. Zhang ter and L. Zhao, sp. nov. have been reported affecting a few non-cucurbit species in specific regions such as Brazil, Senegal and China (Sarr et al. 2014; Machado et al. 2018; Zhao et al. 2019).

Macrophomina phaseolina has been detected infecting melons worldwide, in Mediterranean countries like Spain, Egypt, Israel and Turkey, in American countries like Brazil, Chile, and Honduras, in Near East countries like Iran and Oman, in African countries like Nigeria, and in islands like Australia (Reuveni et al. 1982; Apablaza 1993; Walker 1994; Bruton and Miller 1997; Bankole et al. 1999; García-Jiménez et al. 2000; Edraki and Banihashemi 2010; Cohen et al. 2012; Salari et al. 2012; Al-Mawaali et al. 2013; El-Kolaly and Abdel-Sattar 2013; Jacob et al. 2013; Ambrósio et al. 2015; Tok et al. 2018; Negreiros et al. 2019). However, it is most problematic in hot, arid regions. In Brazil it is usually isolated from diseased melon plants (Marinho et al. 2002; Andrade et al. 2005; Dantas et al. 2013) and rotative crops, like watermelon, and their associated weeds (Sales-Júnior et al. 2012; Negreiros et al. 2019). It causes a disease known as charcoal rot (Salari et al. 2012). Symptoms of charcoal rot start as depressed dark lesions in the stem. Affected plants show sometimes chlorosis, vine wilt and stem and root rot (Bianchini et al. 2005). Severely infected plants die early due to the effect of toxins produced by the fungus and to the interruption of the xylem flow (Islam et al. 2012).

Although several studies have focused on testing different strategies of soil management (Nascimento et al. 2018) and on biocontrol using antagonistic bacteria, such as strains of *Bacillus amyloliquefaciens* (Bakhshi et al. 2018), to control the incidence of root rot in melon fields, one of the most efficient most efficient method to control soil borne pathogens is the use of resistant cultivars. However, there are not commercial varieties resistant to *M. phaseolina*, and few scientific studies report efforts to identify sources of resistance. Salari et al. (2012) evaluated the reaction of seven Iranian cultivars and identified two melon sources with partial resistance. More recently, Ambrósio et al. (2015), studying a collection of melons representing the diversity of the species, found different levels of resistance in five accessions belonging to different infraspecific taxa (Pitrat 2017) and from different origins: two African accessions of wild agrestis type, from Ghana and Nigeria, one Korean accession of the Asian conomon group, one dudaim accession from Afghanistan, and the cantaloupe cultivar 'Noy Israel'. In this study, they used the toothpick method to inoculate the pathogen, the experiment was conducted at an average air temperature of 28 °C and average humidity 65%, and the response was evaluated after 30 days. The response of all these genotypes was determined later in a similar assay, using the same inoculation method in Mossoró (State Rio Grande do Norte, Brazil), with temperatures higher than 35 ºC and excellent epidemiological conditions for the development of *M. phaseolina*. In this assay, many plants of most accessions died in the first two weeks after transplanting, except from those of one wild African agrestis. Other studies have focused on the effects and progress of *M. phaseolina* infection on cucurbits like melon and watermelon, testing different inoculation methods such as toothpick or drenching, and growing conditions such as in a naturally infested field or greenhouse (Cohen

et al. 2016a). Germplasm screening for watermelon has been also carried out searching for resistant accessions useful as rootstocks for grafting, as an alternative to disease control (Cohen et al. 2016b).

It is well known the environmental factors influence host-pathogen interactions. Temperature stands out as the one with major effect on resistance of plants to different pathogens, such as fungi, viruses, bacteria and nematodes (Garrett et al. 2006; Akhtar et al. 2011; Wosula 2017). A classic example that illustrates temperature interference in resistance-breaking are *Mi* genes of tomato. These genes confer resistance to *Meloidogyne incognita* that breaks down at high temperatures (El-Sappah et al. 2019). Also resistance to *tomato spotted wilt virus* conferred by the *Tsw* gene is overcome at high temperatures in pepper (Chung et al. 2018). Previous studies reported an effect of soil temperature on disease severity in melon plants infected by soil borne melon pathogens associated to *Monosporascus* root rot/vine decline (Pivonia et al. 2002). There is no information about the effect of temperature on the reaction of melon accessions to *M. phaseolina*, although previous studies reported 30 to 35 º C as the optimum temperature range for *in vitro* growing of different *M. phaseolina* isolates (Manici et al. 1995; Akhtar et al. 2011; Tok et al. 2018). Under the current global warming scenario, this thermophilic fungal pathogen is expected to spread to new regions and to cause more severe outbreaks (Bashir 2017). Therefore, there is an urgent need to evaluate the effect of temperature on the reaction of melon accessions with different resistance levels to this pathogen.

Material and Methods

Germplasm

Six accessions were evaluated: two wild African accessions belonging to *Cucumis melo* subspecies *agrestis*, PI 185111 (Ag-15591Ghana) and CUM 287 (Ag-C38Nig), two Asian melons belonging to the dudaim, PI 273438 (Dud-QMPAfg), and chinensis, Pat 81 (Con-Pat81Ko), groups of *C. melo* subspecies *agrestis*, and two commercial melons belonging to the two main groups of *C. melo* subspecies *melo*, a Piel de sapo type of the ibericus group (In-PsPiñSp), and the cultivar 'Noy Israel' of the cantalupensis group (Can-NYIsr). All these accessions were reported to have different levels of resistance to *M. phaseolina* by Ambrósio et al. (2015), except from the 'Piel de sapo' cultivar that was used as susceptible control. PI and CUM accessions were kindly provided by the USDA-NPGS and IPK genebanks, respectively. The other accessions were provided by the COMAV-UPV Genebank.

Inoculum preparation

The CMM-1531 pathogenic isolate (deposited in the Coleção de Culturas de Fungos Fitopatogênicos at the Universidade Federal Rural de Pernambuco, Brazil) was obtained in 2012 from roots and stems of symptomatic muskmelon plants grown in Icapuí-RN, Brazil. The confirmation of the identity of this strain was done by partially

sequencing the translation elongation factor 1-alpha gene (GenBank number MN136199), that had 98 to 100% sequence identity with several *Macrophomina phaseolina* isolates from Brazil and China. This isolate was used due to its aggressiveness (Medeiros et al. 2015).

Tests were made to prove fungi pathogenicity. The isolate was sown in potato dextrose agar (PDA) + antibiotic (tetracycline 0.05 g/L). Four dishes (5 mm-diameter) of PDA medium with fungal structures were transferred into Petri dishes containing toothpicks in PDA medium. Plates were stored in BOD (Biochemical Oxygen Demand) incubators at 28 ± 2°C for seven days.

Assay conditions

Selected seeds were disinfested in NaClO (1.5 %) and placed in Petri dish with filter paper and damped cotton. Plates were wrapped in aluminum foil and stored in incubator for 24 hours at 37 °C. Pots of 0.4 kg of capacity were filled with commercial substrate (Tropstrato® HT Hortaliças). Substrate was sterilized in autoclave at 121 °C.

Two successive experiments were carried out consisting each one in four trials that were conducted simultaneously at the Laboratório de Sementes da UFERSA in Mossoró (State Rio Grande do Norte, Brazil), in four BOD incubators for ten days at constant temperatures of 25, 28, 31, and 34 °C, and relative humidity of 60 %, with light-period of 12 hours. Temperatures were chosen according to Ambrósio et al. (2015), which reported the average temperature in Mossoró (Brazil) and Valencia (Spain), throughout the study. The four trials were conducted in a randomized block design with 14 replicates in the first experiment, and 10 replicates in the second one. In both cases, the experimental unit was formed by one pot of 0.4 kg of capacity with one plant.

Apart from these assays in BOD incubators, two experiments under greenhouse conditions were also carried out in Mossoró (Brazil) (average air temperature of 34.5 and 35.7 ºC, and average relative humidity of 55.4 and 50.4% in experiment 1 and 2, respectively). Seeds of the six accessions germinated in commercial substrate previously autoclaved, and plants were manually irrigated to drainage daily and were not fertilized during the experiment. The experiment was carried out with a total of 15 plants per accession and a completely randomized design with the replication corresponding to one plant.

Plant inoculation and disease assessment

Twenty days after transplanting, the inoculation was conducted *via* direct insertion of a toothpick tip overgrown with mycelia and microsclerotia of the CMM-1531 isolate (method previously tested with good results in melon by Cohen et al. 2016a) at the base of the stem, 1 cm above the soil (Ambrósio et al. 2015), and stored in BOD. Ten days after inoculation, accessions were evaluated for disease severity using a score scale (0 to 5), where 0:

asymptomatic, 1: less than 3 % of infected stem tissue, 2: 3 to 10 % of infected stem tissue, 3: 11 to 25 % of infected stem tissue, 4: 26 to 50 % of infected stem tissue, and 5: more than 50 % of infected stem tissue (Ambrósio et al. 2015). The average disease severity was calculated for each accession and classified in five classes of reaction 0: immune (I); 0.1 to 1.0: highly resistant (HR); 1.1 to 2.0: moderately resistant (MR); 2.1 to 4.0: susceptible (S); and 4.1 to 5.0: highly susceptible (HS) (Salari et al. 2012).

In the greenhouse experiment, seedlings were inoculated 14 days after planting by the toothpick method described above. Non infested and autoclaved toothpicks were used as negative controls. Plants were kept in the greenhouse for 30 days. Disease severity was assessed as previously described.

Statistical analysis

As the variable response considered is not quantitative and the residuals do not present normal distribution, the original nonparametric values were transformed according to the methodology of the Aligned Rank Transform (ART) for Nonparametric Factorial Analyses. The aligned rank transformation allows non-parametric testing for interactions and main effects using standard ANOVA techniques. For the transformation of the original data the software (ARTool) was used (Wobbrock et al. 2011). The ANOVA to study the effects of genotype (accessions), temperature and experiment, and their interactions, was performed with the PROC GLM of SAS 9.2 (SAS Institute Inc., Cary, NC) (Durner 2019). We utilized the methodology described by Scott and Knott (1974) for grouping of accessions mean ranks. The correlation coefficients of Spearman and Gamma were calculated according to Siegel and Castellani Jr. (1988).

Results

Results of the statistical joint analyses performed with the data of the two BOD experiments to study the effect of genotype, temperature and experiment on the response to *M. phaseolina* are shown in Table 1. Genotype and temperature effects were significant ($p < 0.0001$), as well as their interaction ($p = 0.003$). The effect of the experiment was no significant ($p = 0.1068 > 0.05$), so data obtained in both BOD experiments could be pooled and analyzed together (Table 2).

Despite the significant temperature x genotype interaction, the main effects of the temperature and genotype were meaningful (Table 2 and Fig. 1). There was a clear effect of genotype. The wild *agrestis* accession from Ghana (Ag-15591Ghana) was the most resistant, and the cultivar 'Piel the sapo' (In-PsPiñSp) the most susceptible (with significantly different average disease severity scores: 0.45 (highly resistant) *versus* 4.46 (highly susceptible), respectively). The other accessions displayed an intermediate response (being moderately resistant with average scores between 1.30 and 1.76). According to their average scores they can be ranked as follows from

more to less resistant: Con-Pat81Ko≈Can-NYIsr≈Ag-C38Nig>Dud-QMPAfg. There was also a clear main effect of temperature. An increase in the severity of the disease was found with temperature rising (significantly different average scores of 1.09, 1.41, 1.91 and 2.87, at 25, 28, 31 and 34ºC, respectively). This relation observed between temperature and severity was also estimated with the Spearman's coefficient ($r_s = 0.53$; $p<0.01$). The Gamma coefficient between temperature and the resistance classes was positive as well ($\gamma = 0.79$; $p < 0.01$).

The temperature increase resulted in more severe symptoms in all genotypes. Fig 1 shows this trend with higher average scores at higher temperatures in both BOD experiments, but the effect was more or less pronounced depending on the genotype. The susceptible control and the most resistant accession were less affected by the temperature rise than the genotypes with intermediate responses (Table 2). The average disease severity scores of 'Piel de sapo' cultivar, used as susceptible control, increased from 4.00 (susceptible) to 4.42 (highly susceptible) at 25 and 28ºC, and remained highly susceptible at higher temperatures, whereas the resistant accession from Ghana was highly resistant till 31ºC (average scores from 0.08 to 0.42) and moderately resistant at 34ºC (1.08).

The effect of temperature on disease severity was more pronounced in the four genotypes with intermediate resistance (Fig.1), as all started being highly or moderately resistant at 25 and 28ºC and became susceptible at higher temperatures, with significantly higher average scores (Table 2). Among these four genotypes, the reaction of Dud-QMPAfg changed from moderately resistant to susceptible at 31ºC, whereas this change occurred at 34ºC in Can-NYIsr and Ag-C38Nig. Con-Pat81Ko remained highly resistant till 31ºC (0.21 to 0.88), occurring the resistance break at 34 ºC.

An illustration of the resistant/susceptible response of the six accessions at the different temperatures assayed is showed in Fig. 2. The susceptible and resistant responses of 'Piel the sapo' and the wild *agrestis* Ag-15591Ghana, respectively, are evident, as well as the effect of temperature on the response of the genotypes with intermediate resistance.

The experiments conducted under greenhouse conditions confirmed the response at the highest temperature (Table 3). Greenhouse experiments were performed at an average temperature of 34.5 and 35.7ºC respectively and results show that plants of most accessions died or had disease severity scores higher than 4 (average scores from 4.1-5.0). All genotypes behaved as highly susceptible except Ag-15591Ghana that remained moderately resistant 30 days after inoculation (average disease score of 1.1) in both assays.

Discussion

This study evaluated for the first time the response of melon accessions with different resistance levels against the charcoal rot under different temperature regimes. The use of several BOD incubators made it possible to set

different temperature regimes, with an accurate temperature control maintaining constant relative humidity and photoperiod, and to replicate the assays to obtain reliable results.

The resistance response to *M. phaseolina* found in the genotypes studied was the expected, as the accessions evaluated here and the susceptible control were selected for their differential response to this pathogen in a previous study (Ambrósio et al. 2015). The significant influence of temperature on the reaction of these accessions to this fungus is here described for the first time and is consistent with what was suggested for this pathogen in other crops (Akhtar et al. 2011). Akhtar et al. (2011) carried out a screening for resistance to *M. phaseolina* in sesame germplasm and observed differences in the severity of symptoms likely related to different temperature and rain conditions. The influence of the temperature in the *in vitro* growth rate has been reported in some works using isolates of *M. phaseolina* from different crops, and the optimum temperature for this pathogen has been established between 30-35 ºC (Manici et al. 1995; Akhtar et al. 2011). However, to date no study had been focused on the effect of temperature in *M. phaseolina* resistant melon germplasm.

Our results show the general effect of the temperature increase on the severity of symptoms caused by *M. phaseolina* in seedlings of both susceptible and resistant genotypes, but the occurrence of a significant temperature x genotype interaction evidences the differential effect of the temperature increase on the different genotypes. In fact this effect was less pronounced in the most susceptible and the most resistant genotypes. The high susceptibility of the "Piel de sapo' cultivar was evident at all the temperatures, indicating that all the inoculation assays successfully resulted in a severe *M. phaseolina* infection, and suggesting that this pathogen can be damaging for this type of susceptible melon cultivars even at moderate temperatures. Under these infection conditions that caused severe damage to the susceptible control, Ag-15591Ghana behaved as resistant at all the temperatures, although its response change from highly to moderately resistant at 34 ºC. Resistance breaking due to the temperature rise was observed in all the genotypes with intermediate resistance, with Dud-QMPAfg and Pat81Ko showing the resistance breaking at the lowest and at the highest temperature, respectively.

According to their response to *M. phaseolina* at different temperature regimes, accessions ranked as follows from more to less resistant: Ag-15591Ghana>Con-Pat81Ko>Can-NYIsr≈Ag-C38Nig>Dud-QMPAfg>In-PsPiñSp. These results partially agree with those reported by Ambrósio et al. (2015) who conducted the screening assay at an average temperature of 28 °C and relative humidity of 65%. In this study, 'Piel de sapo' behaved as a susceptible cultivar and the other genotypes displayed all a highly resistant response. Ambrósio et al. (2015) results are similar to those obtained in the two assays of our study performed at 25 and 28 ºC, although disease severity was higher in the present study, and Dud-QMPAfg and Ag-C38Nig were classified as moderately resistant at 28

°C, and Can-NYIsr, was moderately resistant at 31ºC. The use of a wide range of temperatures allowed a much better characterization of the resistant sources, suggesting that these moderately resistant accessions could not be a good option under high temperature conditions. We detected differences among these genotypes with intermediate resistance, and, for example, the *chinensis* genotype Con-Pat81Ko, which behaved as the three previous accessions under moderate temperature, was highly resistant even at 31 ºC.

The six accessions were also checked in two replicate assays under greenhouse conditions in Mossoró. These greenhouse conditions (with average temperatures > 34°) are similar to those of the open field cultivation of melons in this semiarid region, where temperatures often are over 40ºC. The behavior in both greenhouse assays agree with BOD results, being Ag-15591Ghana the only accession that remained moderately resistant under these stressful conditions. The symptoms of the remaining accessions where more severe in the greenhouse, where most of the plants died few days after inoculation. In BOD incubators at 34ºC these accessions were susceptible, but plant death was not observed. The higher severity in the greenhouse can be due to differences in relative humidity, greater in BOD incubators than in the greenhouse, and to differences in the thermal amplitude, higher in greenhouses, that make plants more prone to pathogen attacks. Cohen et al. (2016a) reported more severe charcoal rot symptoms in melon and watermelon in their experiment carried out in an infested field than in their greenhouse experiment, probably due to the less controlled growing conditions in field compared to the greenhouse.

These results agree with the general knowledge the *M. phaseolina* is more adapted and aggressive in hot and dry climates, with high temperatures and reduced humidity (Bruton and Wann 1996; Akhtar et al. 2011). In this sense, the water stress has also been reported to affect the development of many soil borne diseases (Blanco-López and Jiménez-Díaz 1983). In general, diseases caused by soil borne pathogens, like *M. phaseolina*, are said to be highly influenced by environmental conditions (Miyasaka 2008). For instance, Pivonia et al. (2002) associated the highest rates of Melon root rot and vine decline disease caused by M. cannonballus with the hottest season when studying the response of the melon plants grown in a field infested with this pathogen. However, Sales-Júnior et al. (2019) recently reported that high temperature is not the main factor affecting the severity of symptoms caused by *M. cannonballus*, suggesting other factors such as the level of inoculum in soil as more important for the infection, maybe due to the relatively similar high temperatures in the seasons tested.

Therefore, our results indicate that the use of different assay temperatures may result in a better characterization of the resistance levels, allowing the selection the sources most appropriated for different climatic conditions. For example, some of the sources with intermediate levels of resistance could be useful in countries with less stressful growing conditions for melon crop, such as Spain or other European countries, but more stressful

299 countries as Brazil, Israel, African or Asian regions would need the use of resistant sources with stable resistance
300 at higher temperatures. This kind of screening assays are very necessary as they allow an accurate control of
301 environmental conditions, highly variable in field, and of the source of inoculum, avoiding the occurrence of
302 different isolates and/or fungal species frequently mixed in naturally infested soils, and allow working with high
303 plant numbers and replicates. The variability of field conditions make impossible to use it for routine screenings
304 and genetic studies, although the behavior of the selected accession should be finally confirmed in in naturally
305 infested fields.

306 The Ag-15592Ghana accession was one of the first melon accessions reported with high level of resistance
307 to *M. phaseolina* (Ambrósio et al. 2015). The present study confirms the high level of resistance in this accession,
308 stable even at high temperature conditions and confirm its potential use as source of resistance in genetic breeding
309 programs with *M. phaseolina*. In order to include this source in breeding programs, the genetics of this resistance
310 should be studied. The effect of the temperature on disease severity should be taken into account during the genetic
311 studies. Also differences in the genetic background should be considered to recover the quality of commercial
312 melon and select against the wild traits of the *agrestis* background during the development of new resistant
313 varieties.

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Tables

Table 1. Mean square, F values and associated probability obtained in joint variance analysis after data alignment and ranking (Aligned Rank Transform, ART) for the response (score of disease severity) of six melon accessions inoculated with *M. phaseolina* and grown at four temperature regimes in the two BOD experiments.

Source of variation	df[a]	MS[b]	F	Prob
Experiment (E)	1	5.610973	2.61	0.1068
Genotype (G)	5	173.8963	80.86	<.0001
Temperature (T)	3	83.28676	38.73	<.0001
T x G	15	5.035124	2.34	0.003
E x G	5	0.112624	0.05	0.9983
E x T	3	0.183041	0.09	0.9682
E x T x G	15	0.285888	0.13	1
Error	528	2.150527		

df[a] = numerator degrees of freedom

MS[b]: Mean Square.

Table 2. Average and standard deviation for the response (score of disease severity) of six melon accessions inoculated with *Macrophomina phaseolina* and grown at four temperature regimes in the two BOD experiments. Joint analysis.

Accession	Temperature				Average
	25ºC	28ºC	31ºC	34ºC	
Ag-15591Ghana	0.08±0.28	0.21±0.40	0.42±0.71	1.10±0.65	0.45±0.66
	(139.63) cA	(160.06) cA	(185.33) cA	(282.58) cA	(191.90) d
Con-Pat81Ko	0.21±0.65	0.67±1.23	0.88±1.48	3.46±2.14	1.30±1.94
	(151.27) cB	(197.35) cB	(211.08) cB	(403.88) bA	(240.89) c
Can-NYIsr	0.63±1.37	0.88±1.65	2.00±1.96	2.21±1.50	1.44±1.76
	(183.88) bB	(205.73) bB	(315.23) bA	(353.19) bA	(264.51) c
Ag-C38Nig	0.75±1.42	1.13±1.62	1.54±1.89	2.67±1.81	1.52±1.84
	(208.58) bB	(232.25) bB	(268.65) bB	(361.92) bA	(267.85) c
Dud-QMPAfg	0.83±1.40	1.17±1.44	2.13±1.98	2.92±1.89(375.4	1.76±1.86
	(222.21) bB	(257.13) bA	(319.10) bA	4 bA	(293.47) b
In-PsPiñSp	4.00±1.57	4.42±1.31	4.57±1.08	4.80±0.65	4.46±1.21
	(443.85) aA	(470.71) aA	(476.35) aA	(497.72) aA	(472.38) a
Average	1.09±1.80 (224.90) D	1.41±1.93 (253.87) C	1.91±2.05 (294.70) B	2.87±1.91 (379.94) A	

Averages in a row followed by the same uppercase letter and in a column by the same lowercase letter do not differ ($p<0.05$) according by the Scott-Knott cluster (1974). Data in parentheses correspond to the average ranks. Each number is the average of 24 plants. The effect of the experiment was no significant (Table 1), so data obtained in both BOD experiments could be pooled and analyzed together.

Table 3. Average for the response (disease of disease severity) and reaction of melon accessions inoculated with *Macrophomina phaseolina* in two replicate greenhouse experiments conducted in Mossoró-RN (Brazil).

Accession	Mossoró (Brazil) (34.5 ºC, 55.4% RH)[a]		Mossoró (Brazil) (35.7 ºC, 50.4% RH)[a]	
	Disease score	Reaction[b]	Disease score	Reaction[b]
Ag-15591Ghana	1.10	MR	1.10	MR
Con-Pat81Ko	5.00	HS	4.80	HS
Can-NYIsr	5.00	HS	5.00	HS
Ag-C38Nig	5.00	HS	4.60	HS
Dud-QMPAfg	4.60	HS	4.10	HS
In-PsPiñSp	5.00	HS	5.00	HS

[a] RH: average relative humidity

[b] 0: immune (I); 0.1-1.0: highly resistant (HR); 1.1-2.0: moderately resistant (MR); 2.1-4.0: susceptible (S) and 4.1-5.0: highly susceptible (HS).

Figures

Fig. 1 Severity of charcoal rot as a function of temperature for the six accessions assayed in BOD incubators (experiments 1 and 2). Average scores of disease severity and genotype reaction: 0: immune (I); 0.1-1.0: highly resistant (HR); 1.1-2.0: moderately resistant (MR); 2.1-4.0: susceptible (S) and 4.1-5.0: highly susceptible (HS), are represented

Fig. 2 Effect of toothpick inoculation of *M. phaseolina* in plants of six accessions of melon assayed at four temperatures in BOD incubators