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Resistance of *Cucurbita* spp. germplasm to the fungus *Macrophomina phaseolina*[1]

Resistência de germoplasma de *Cucurbita* spp. para o fungo *Macrophomina phaseolina*

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ABSTRACT - The objective of this study was to evaluate genotypes of *Cucurbita moschata* and *Cucurbita maxima* for resistance to the fungus *Macrophomina phaseolina* (Taissi) Goid. The experiment evaluated 17 accessions of *Cucurbita moschata* Duchesne and 20 accessions of *Cucurbita maxima* Duchesne preserved in the Cucurbit Germplasm Collection of the Federal Rural University of the Semi-Arid Region (UFERSA). Four commercial controls were also used, two for each species. In both studies, three isolates of *M. phaseolina* were inoculated, which are kept at the Fungal Culture Collection of UFERSA: MM1531 (GenBank identificatio: MM1531 (GenBank identification: MN136199), ME249 and ME250. Plants received inoculum of the pathogen in pieces of colonized toothpick and were evaluated for the incidence and severity of the disease. Both species showed resistant plants, but with varied frequencies for the accessions. Considering the species, the frequencies observed for *C. moschata* were higher than those observed for *C. maxima.* The accessions of *C. moschata* ABPUN 206 F1, P114-1, P160-2, P11-2, P114-6, P14-02 and P97-1 show different levels of resistance to the *Macrophomina phaseolina* isolates MM1531, ME-249 and ME-250.Thus, for an efficient selection in a breeding program for the characteristic, the selection strategies adopted must consider each source of resistance in isolationand not each species.

Key words: *Cucurbita moschata.Cucurbita maxima.*Soil-borne pathogen. Variability. Plant Genetic Resources.

RESUMO - O objetivo deste trabalho foi avaliar genótipos de abóbora de leite e jerimum caboclo para resistência ao fungo *Macrophomina phaseolina* (Taissi) Goid. Foram utilizados 17acessos de *Cucurbita moschata* Duchesne and 20 de *Cucurbita máxima* Duchese. Também foram utilizadas quatro testemunhas comerciais, duas para cada espécie. Em ambos os trabalhos, inoculou-se três isolados de *M. phaseolina* que estão mantidos na Micoteca da UFERSA: MM1531 (GenBank: MN136199), ME249 e ME250. As plantas receberam inóculo do patógeno em pedaços de palito de dente colonizado, e foram avaliadas quanto a incidência e severidade da doença. Ambas as espécies apresentaram plantas resistentes, porém com frequências variadas para os acessos. Quando consideradas as espécies, as frequências verificadas para *C. Moschata* foram superiores às constatadas para *C. maxima.* Os acessos de *C. moschata* ABPUN 206 F1, P114-1, P160-2, P11-2, P114-6, P114-02 e P97-1 apresentam diferentes níveis de resistência a *Macrophomina phaseolina* para os isolados MM1531, ME-249 e ME-250. Assim, para a seleção eficiente em programa de melhoramento para a característica, as estratégias de seleção adotadas devem considerar cada fonte de resistência isoladamente e não cada espécie.

Palavras-chave: *Cucurbita moschata.Cucurbita maxima*. Patógeno habitante do solo. Variabilidade. Recursos genéticos vegetais.

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INTRODUCTION

The Cucurbitaceae family includes pumpkins, and melons among others, being economically important regarding its use as food (SALEHI *et al*., 2019). Among its genera, *Cucurbita* stands out for participating of the diet of almost all America with 20 to 27 species, five domesticated: *C. argyrosperma* Huber, *C. ficifolia* Bouché, *C. maxima* Duchesne, *C. moschata* Duchesne and *C. pepo* Linneaus (RODRÍGUEZ; VALDÉS; ORTIZ, 2018).

Cucurbites in general have morpho-agronomic characteristics that make it possible to use them in a variety of ways, whether in feeding or culture management when used as a rootstock (CHAUDHARI *et al*., 2017; PINHEIRO *et al*., 2019; SANTOS *et al*., 2020; ZHOU *et al*., 2014).Grafting has been very sought after for cucurbits in the management of diseases, including charcoal rot (COHEN; ELKABETZ; EDELSTEIN, 2016), caused by *Macrophomina phaseolina* (Tassi) Goid. (1947), which affects several economically important crops of the family, such as melon and watermelon (MEDEIROS *et al*., 2015). The pathogen occurs in more than 680 plant species, with great geographical distribution, surviving under the most adverse conditions due to the formation of resistance structures called microsclerotia (LINHARES *et al*., 2016), being able to affect from roots to leaves, pods, and fruits of plant species (ISHIKAWA *et al*., 2018; SALES JÚNIOR *et al*., 2020).

The rot of the coal infects roots in periods of drought and the infected or dead roots become substrate for the fungus that enters activity in the humid period (BROETTO *et al*., 2014), causing the reduction of yield and quality of several fruits, such like melon (AMBROSIO *et al*., 2015). The ideal environmental conditions for its occurrence are high temperatures (25 to 35 °C) and soils with a low level of humidity (LINHARES *et al*., 2016).

Although the species *C. maxima* and *C. moschata* have been used as rootstocks of several other cucurbit species,such as melon (ZHOU *et al*., 2014), watermelon (SMITH *et al.*, 2019) and cucumber (GORETA BAN *et al*., 2014), it is important to emphasize that, for being allogamous, the genetic variability of the species is favored (PRIORI *et al*., 2018), so if adequate rootstocks are not selected, there may be adverse effects as inefficiency regarding the control of the pathogen.

In addition to the genetic variability of the plant, it is also necessary to consider that dwelling pathogens may show variability in their various isolates and within the isolate, with respect to the severity of the interaction with their hosts, as demonstrated by Ambrósio *et al*. (2015).

Therefore, for selection it is important to consider the variability of the resistant species, the pathogen and the eminimizing the environment the risks of production losses.

Among the known species of *M. phaseolina* has the largest known range of hosts, in addition to the genetic variability of the pathogen itself, making it difficultto control (ISHIKAWA *et al*., 2018), which emphasizes the need for seeking sources of resistance because, up to the present time, there are no records of these sources for either *C. moschata* or *C. maxima* in the literature.

The genetic variability of *Macrophomina* spp., as well as the limited amount of fungicides capable of controlling the disease, make the use of resistant cultivars the most efficient and ecologically correct way for its management (LIMA *et al*., 2017).

Thus, the objective of this study was to evaluate genotypes of *C. moschata* and *C. maxima* for resistance to *Macrophomina phaseolina*.

MATERIAL AND METHODS

The study was conducted at Universidade Federal Rural do Semi-Árido, in a greenhouse, geographically located at 5°12’27.92” S and 37°19’03.86” W, from January to August 2019.According to the Koppen climatic classification, Mossoró has a BSwh ’type climate, which is characterized by being dry, hot and with a rainy season in summer, ideal conditions for the development of the pathogen.

Two experiments were installed simultaneously, in a greenhouse, in a randomized block design, with six replicates; the first one was in a 19 x 3 factorial scheme, consisting respectively of 19 genotypes (17 accessions and two commercial controls) of *C. moschata*, Maranhão Pumpkin and Tetsukabuto Hibrid) of *C. moschata* (Table 1) and three isolates of *M. phaseolina*, and the second one in 22 x 3 factorial scheme, consisting respectively of 22 genotypes of *C. maxima* (20 accessions and two commercial controls), Hiroko Pumpkin and Tetsukabuto Hibrid) (Table 2) and threeisolates of *M. phaseolina.* Each plot was represented by one pot with capacity for one liter of the substrate containing one plant.

The germplasm used belongs to the Cucurbit Germplasm Collection of UFERSA and is preserved in a cold chamber at 10 ºC, with a variation of ± 2 ºC and relative humidity of 50%.

The seeds of the genotypes were disinfected in 1.5% sodium hypochlorite (NaClO) for two minutes, washed in distilled water and dried at room temperature under absorbent paper for 24 hours, according to methodology adapted by Michereff, Andrade and SalesJúnior (2008).

These genotypes were sown in 1-L plastic pots, containing the commercial substrate Tropstrato HT Hortaliças - Vida Verde®, previously sterilized in an autoclave for two hours, for one hour per day, with an

interval of 24 hours. The plants were irrigated daily throughout the research period.

According to the need of the crop, after an initial period of thirty dayssowing, fertilizations containing Nitrogen (N), Phosphorus (P) and Potassium (K) were carried out, applying the amounts recommended by the Brazilian Society of Soil Sciences (SBCS), since the substrate would not be sufficient to nourish the plant during the entire evaluation period (COSTA; FARIA; PEREIRA, 2008).

Three isolates of *M. phaseolina* were collected from roots of symptomatic melon plants: MM1531 (GenBank identification: MN136199), ME249 and ME250. The isolates MM1531 and ME250 were collected in fields of Rio Grande do Norte and Ceará, respectively. ME249 was collected in an experimental field of UFERSA. All isolates are preserved at the Fungal Culture Collectionof UFERSA.

These isolates were selected by initially performing a pathogenicity test, in which 10 isolates of the pathogen were inoculated in the studied cucurbit species and, among these, the three chosen for the study were those that were most aggressive based on time to cause injury and size Subsequently, the isolates were subcultured in potato-dextrose-agar (PDA) culture medium with tetracycline ($0.05\ gL^{-1}$), and then each isolate was kept in a Biological Oxygen Demand (BOD) incubator at 28 ± 2 ºC, for seven days, so that they could be used to prepare the inoculum (MEDEIROS *et al*., 2015).

The inoculum was prepared using the tips of toothpicks with approximately 1.0 cm, which were inserted vertically into a filter paper disc with a dimension equivalent to the inner diameter of the Petri dish and, after being placed in the dishes, with the pointed part of the toothpicks upwards, autoclave sterilization was performed for 30 minutes at 121 °C (MEDEIROS *et al*., 2015).

Then, the toothpicks were colonized with*M. phaseolina*, by pouring PDA culture medium, with exposure of 2 mm of the toothpick tip. After solidification, three discs with approximately 0.5 mm diameter containing mycelium and microsclerotia of the fungus were subcultured, with equidistant distribution, and incubated for one week in BOD incubatorat 28 ± 2 °C (MEDEIROS *et al*., 2015).

Finally, 15 days after the sowing of the genotypes, when the toothpicks were completely colonized by the pathogen isolates, inoculation was performed. In this process, the toothpicks colonized with the isolates were

Table 1 - Geographic origin of *C. moschata* genotypes belonging to the Cucurbit Germplasm Collection of UFERSA

Genotype	Origin	Location
ABTOU 805 F1	Touros (RN)	5° 11’ 56” S -35° 27’ 39” W
ABO 168	Macaíba (RN)	05º 56’ 31’’ S -35º 22’ 04’’ W
ABPUN 206 F4	Rio do Fogo (RN)	5° 16’ 22” S - 35° 22’ 59” W
ABPUN 201 F6	Rio do Fogo (RN)	5° 16’ 22” S - 35° 22’ 59” W
ABO 09	Touros (RN)	5°11’ 56” S - 35° 27’ 39” W
ABPUN 206 F6	Rio do Fogo (RN)	5° 16’ 22” S - 35° 22’ 59” W
ABPUN 206 F2	Rio do Fogo (RN)	5° 16’ 22” S - 35° 22’ 59” W
ABPUN 206 F1	Rio do Fogo (RN)	5° 16’ 22” S - 35° 22’ 59” W
ROÇA DE PAI	Unknown origin	-
ABTOU 805 F4	Touros (RN)	5°11’ 56” S - 35° 27’ 39” W
P114-1	Assú (RN)	5°34’ 56” S - 36° 56’ 40” W
P160-2	Unknown origin	-
P11-2	Mossoró (RN)	5° 12’ 12” S - 37° 21’ 08” W
P114-6	Assú (RN)	5°34’ 56” S - 36° 56’ 40” W
P131-21	Ipanguaçu (RN)	5° 28’ 31” S - 36° 51’ 58” W
P114-02	Assú (RN)	5°34’ 56” S - 36° 56’ 40” W
P97-1	Unknown origin	-
ABÓBORA MARANHÃO[1]	Feltrinsementes®	Local market in Mossoró (RN)
TETSUKABUTO HYBRID[1]	Topseed®	Local market in Mossoró (RN)

[1]Commercial controls

Table 2 - Geographic origin of *C. maxima* genotypes belonging to the Cucurbit Germplasm Collection of UFERSA

Genotype	Origin	Location
ABPUN-213	Rio do fogo (RN)	5° 16’ 22” S -35° 22’ 59” W
ABCRN-304	Currais Novos (RN)	6° 15’ 47” S - 36° 31’ 4” W
ABTOU-802 F4	Touros (RN)	5° 11’ 56” S - 35° 27’ 39” W
ABAPO-002	Apodi (RN)	5° 38’ 58” S - 37° 47’ 45” W
ABAPO-005	Apodi (RN)	5° 38’ 58” S - 37° 47’ 45” W
JERIMUM CABOCLO-COBAL 2014	Mossoró (RN)	5° 12’ 12” S - 37° 21’ 08” W
ABO-156	Unknown origin	-
ABPUN-212	Rio do fogo (RN)	5° 16’ 22” S - 35° 22’ 59” W
ABCRN-315	Currais Novos (RN)	6° 15’ 47” S - 36° 31’ 4” W
ABTOU-802 F1	Touros (RN)	5°11’ 56” S - 35° 27’ 39” W
ABAPO-007	Apodi (RN)	5° 38’ 58” S - 37° 47’ 45” W
JERIMUM LURUGADO	Unknown origin	-
ABAPO-024	Apodi (RN)	5° 38’ 58” S - 37° 47’ 45” W
ABPUN-211	Rio do fogo (RN)	5° 16’ 22” S - 35° 22’ 59” W
RGV 2011.1 (01)	Unknown origin	-
ABCRN-306	Currais Novos (RN)	6° 15’ 47” S - 36° 31’ 4” W
ABBAR-101	Baraúna (RN)	5° 4’ 14” S - 37° 37’ 2” W
ABCRN-302	CurraisNovos (RN)	6° 15’ 47” S - 36° 31’ 4” W
ABTOU-802-F3	Touros (RN)	5° 11’ 57” S - 35° 27’ 40” W
JERIMUM CABOCLO ALMINO AFONSO	AlminoAfonso (RN)	6° 9’ 8” S - 37° 45’ 58” W
ABÓBORA HIROKO[1]	Feltrinsementes®	Local market in Mossoró (RN)
TETSUKABUTOHYBRID[1]	Topseed®	Local market in Mossoró (RN)

[1]Commercial controls

Table 2 - Geographic origin of *C. maxima* genotypes belonging to the Cucurbit Germplasm Collection of UFERSA

Genotype	Origin	Location
ABPUN- 213	Rio do fogo	5° 16’ 22” S -35° 22’ 59” W
ABCRN-304	Currais Novos	6° 15’ 47” S - 36° 31’ 4” W
ABTOU-802 F4	Touros	5° 11’ 56” S - 35° 27’ 39” W
ABAPO-002	Apodi	5° 38’ 58” S - 37° 47’ 45” W
ABAPO-005	Apodi	5° 38’ 58” S - 37° 47’ 45” W
JERIMUM CABOCLO-COBAL 2014	Mossoró	5° 12’ 12” S - 37° 21’ 08” W
ABO-156	Unknown origin	-
ABPUN-212	Rio do fogo	5° 16’ 22” S - 35° 22’ 59” W
ABCRN-315	Currais Novos	6° 15’ 47” S - 36° 31’ 4” W
ABTOU-802 F1	Touros	5°11’ 56” S - 35° 27’ 39” W
ABAPO-007	Apodi	5° 38’ 58” S - 37° 47’ 45” W
JERIMUM LURUGADO	Unknown origin	-
ABAPO-024	Apodi	5° 38’ 58” S - 37° 47’ 45” W
ABPUN-211	Rio do fogo	5° 16’ 22” S - 35° 22’ 59” W
RGV 2011.1 (01)	Unknown origin	-

Continuation table 2

ABCRN-306	Currais Novos	6° 15’ 47” S - 36° 31’ 4” W
ABBAR-101	Baraúna	5° 4’ 14” S - 37° 37’ 2” W
ABCRN-302	CurraisNovos	6° 15’ 47” S - 36° 31’ 4” W
ABTOU-802-F3	Touros	5° 11’ 57” S - 35° 27’ 40” W
JERIMUM CABOCLO ALMINO AFONSO	AlminoAfonso	6° 9’ 8” S - 37° 45’ 58” W
ABÓBORA HIROKO[1]	Feltrinsementes®	Local market in Mossoró (RN)
TETSUKABUTOHYBRID[1]	Topseed®	Local market in Mossoró (RN)

[1]Commercial controls

inserted into the hypocotyls (0.05 mm from the soil) of the plants. All accessions were inoculated with the three isolates of *M. phaseolina*, but the inoculations were performed in different plants, and the same accession may show resistance to the three isolates, but not in the same plant.

The plants of each accession were observed for disease progress on a daily basis and were evaluated for incidence and severity of the disease, at 60 days after inoculation for *C. moschata* and at 90 days for *C. maxima*, since the first species had the disease progress stabilized in that period, while the second had its symptoms in constant progress even after the initial 60 days, stabilizing only over the 90 days evaluated. Therefore, Scores from 0 to 5 were assigned according to the visual severity of symptoms present in the hypocotyl of the plant, estimated through the descriptive scale,: 0 – absence of symptoms; 1 – less than 3% of infected tissues, with small lesions; 2 – between 3 and 10% of infected shoot tissues, with intermediate lesions;3 – between 11 and 25% of infected shoot tissues; 4 – between 26 and 50% of infected shoot tissues, with the possibility of plant lodging; 5 – more than 50% of infected shoot tissues or plant necrosis (AMBRÓSIO *et al*., 2015).

Disease severity data were usedto groupthe genotypesinto classesof resistance, according to the scale proposedby Salari *et al*. (2012), namely: 0 - immune; from 0.1 to 1 - highly resistant; from 1.1 to 2 - moderately resistant; from 2.1 to 4 – susceptible; and from 4.1 to 5 - highly susceptible.

After evaluation, the genotypes with scores higher than zero were taken to the Phytopathology Laboratory of Center of Agrarian Sciences/Department of Agricultural And Forest Sciences (CCA/DCAF) to confirm the presence of the fungus in the plant, by collecting fragments from the bordering part (between the symptomatic part and healthy part) of the lesion and disinfesting themin 70% alcohol for 30 seconds and in 2% NaClO for one minute, in a laminar flow chamber, followed by washing in distilled and sterilized water. The properly disinfested fragments were placed in PDA + tetracycline (0.05 gL^{-1}) medium to confirm the pathogen.

The data were tabulated in the Microsoft Office Excel 2019®program and, for better interpretation, subjected to descriptive analysis, where the relative frequency of each genotype was calculated for the different levels of reaction, making it possible to observe the effect of the isolates on each genotype separately and select the most resistant among them.

As the variable response has residuals that do not show normal distribution because the values come from a diagrammatic scale, the original values were transformed according to the Aligned Rank Transform (ART) methodology for nonparametric factor analysis. Aligned rank transformation enables nonparametric testing for interactions and main effects using standard ANOVA techniques. The ANOVA to study the effects of genotype (accessions), isolates and their interactions was performed using F test ($p<0,001$) with the R program (R CORE TEAM, 2020). The methodology described by Scott-Knott was used to group the average classifications of accessions.

RESULTS AND DISCUSSION

Six days after inoculation it was already possible to visualize the first symptoms of stem rot by *M. phaseolina*in *C. maxima*, while it was only possible to make this observation in *C. moschata* 20 days after inoculation. The symptoms evolved continuously until the end of the evaluations, with visible differences in severity, which presented themselves in the form of yellowing, cracking, wilting and lodging of the plant. In some plants the disease did not manifest itself at the site of toothpick insertion, but the symptom arose in another part of the stem.

In terms of absolute frequency, *C. moschata* obtained a greater number of immune plants, equal to 67, 53 and 60 plants for the isolates MM1531, ME250and ME249, respectively

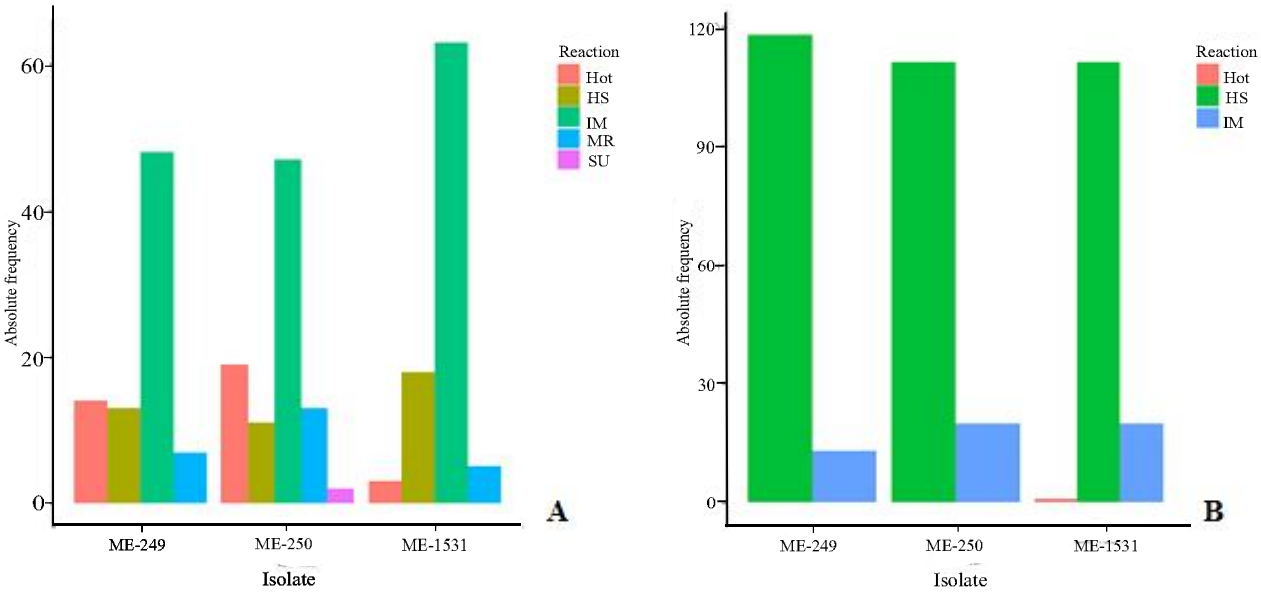
(Figure 2A). There was also a considerable number of highly resistant plants, equal to 5, 17 and 34, when inoculated with isolates MM1531, ME249 and ME250, respectively, in addition to 1 plant moderately resistant to isolate MM1531 and 1 to ME249 (Figure 2A).

The opposite was observed in *C. maxima*, which had a higher number of highly susceptible plants, equal to 110, 119 and 111 plants for isolates MM1531, ME249 and ME250, respectively (Figure 1B). Immune plants were also classified in each accession, being 19 plants inoculated with the isolate MM1531, 13 with the isolate ME249 and 21 with the isolate ME250. The isolate MM1531also had one highly resistant plant. Moderately resistant or susceptible plants were not classified (Figure 2B).

The diversity of the reaction of the plant genotype to the pathogen, even when within the same access and isolated, is mainly due to the variability already described for both species of pumpkins and the pathogen, so that the plant-to-plant evaluation was extremely important.

Figure 1 - Surgimento dos primeiros sintomas de podridão de carvão por *Macrophomina. Phaseolina*em *Cucurbita. Máxima* (A) e *Cucurbita. Moschata* (B)

Figure 2 - Absolute frequency of reaction classes per isolate from accessions of *Cucubita moschata* (A) 60 days after inoculation and accessions of *Cucubita maxima* (B) 90 days after inoculation with *Macrophomina phaseolina* using the toothpick method. HR - highly resistant, HS - highly susceptible, IM - immune, MR - moderately resistant, SU- susceptible



Among the 19 genotypes of *C. moschata*, the accessions ABTOU 805 F1, ABO 168, ABO 09, ABPUN 206 F6, ABPUN 206 F2, ABPUN 206 F1, ROÇA DE PAI, ABTOU 805 F4, P114-1, P160-2, P11-2, P114-6, P131-21, P114-02, P97-1 and the commercial cultivars were resistant to the isolate MM1531, and all genotypes showed resistance in at least one plant to the isolates ME249 and ME250. However, when considering the reaction of genotypes to the three isolates, a varied frequency was observed in terms of resistance classification (Figure 3).

To the isolate MM1531 (Figure 3), the accessions ABPUN 206 F2, ABPUN 206 F1, ROÇA DE PAI, ABTOU 805 F4, P114-6, P114-02 and P97-1 were the most resistant, while ABPUN 201 F6 was the most susceptible, with death in 100% of plants. The accessions ABTOU 805 F4, ABPUN 206 F6, P114-1, P160-2, P131-21, the commercial cultivar of open pollination and the hybrid commercial cultivar showed immunity in 50% or more of the evaluated plants, which is a relevant result in the use for selection in breeding programs.

Regarding the isolate ME249 (Figure 3), the accessions ABO 168, P11-2, P97-1 and the hybrid commercial cultivar showed 100% immunity, the commercial cultivar showed87% of immune plants and 17% of highly resistant plants. For the accession P114-1, there was 80% of immunity and 20% of high resistance, P114-6 and P160-2 had 87% of immune plants and 33% of highly resistant plants.

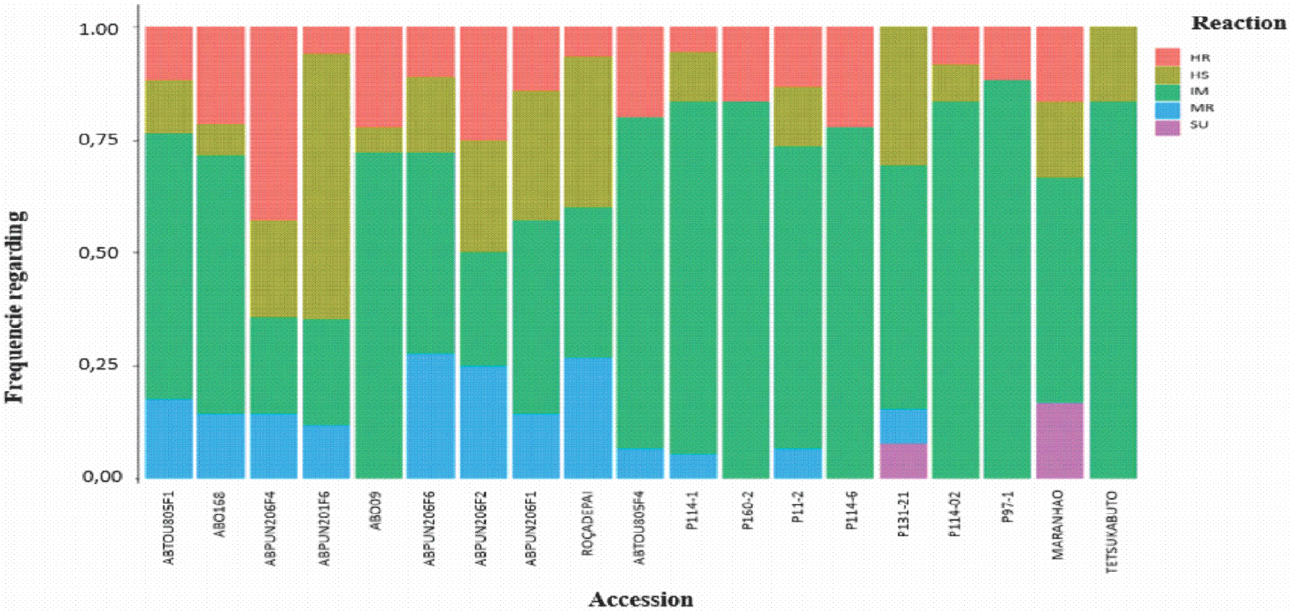
The isolate ME250 caused the highest number of plant deaths in all genotypes (Figure 3) and, despite that, the accession P160-2 was immune to all replicate, and ABO 168 was highly resistant. The accessions ABTOU 805 F1, ABTOU 805 F4, ABPUN 206 F1, ROÇA DE PAI, ABTOU 805 F4, P114-1, P11-2, P114-6, P131-21, P114-02, P97-1 and the Tetsukabuto hybrids showed immunity frequencies equal to or greater than 50% (Figure 3).

Regarding the genotypes of *C. maxima*, the highest frequencies regarding immunity were observed in the hybrid commercial cultivar, for isolates ME249 and ME250, with 50 and 66.67%, respectively. As for the isolate MM1531 (Figure 4), among the accessions, ABAPO-007 and JERIMUM LU showed resistance equal to or greater than 50%, at different levels. JERIMUM LU had 50% immunity, ABAPO-007 and ABTOU-802 F3 had 33.33% immunity and the accessions ABCRN-304, JCCM-2014, ABCRN-315, ABCRN-306, ABCRN-302 and the commercial cultivars obtained 16.67% immunity (Figure 4).

Regarding the isolate ME249 (Figure 4), the accession ABCRN-315 showed 33.33 % of immunity and the accessions ABTOU-802 F4, ABTOU-802 F1, JERIMUM LU, ABAPO-024 and ABCRN-302 obtained 16.67%. The commercial cultivars, hybrid and of open pollination, reached immunity of 50 and 33%, respectively. In addition to these, the accession ABAPO-007 showed moderate resistance at a frequency of 16.67% (Figure 4).

For the isolate ME250 (Figure 3), there was one genotype with higher percentage of immunity, the hybrid commercial cultivar, with frequency > 60%. Following this, the accessions ABAPO-007 and JCAA showed an immunity

Figure 3 - Relative frequency of reaction classes of *Cucubita moschata* accessions 60 days after inoculation with *Macrophomina phaseolina* isolates using the colonized toothpick method. HR - highly resistant, HS - highly susceptible, IM - immune, MR - moderately resistant, SU - susceptible



frequency of 33.33%. The accessions ABAPO-005, JCCM-2014, ABCRN-315, ABTOU 802 F1, ABPUN 211, RGV 2011.1(01), ABCRN-306, ABCRN-302, ABTOU-802 F3 and the cultivar Hiroko obtained 16.67% immunity. The genotypes ABCRN-315 and RGV2011.1 (01) also had a frequency of 16.67% of high resistance.

The classe sof the reaction of the *C. maxima* and *C. moschata*accessions to the isolates of *M. phaseolina* can be observed in Figure 4, which clearly shows the contrast in the resistance between the two species.

Significant differences were found by the Snedecor's F test ($p < 0.001$) between accessions of both species and between isolates of *M. phaseolina* in *C. maxima*, but there was no difference between isolates in *C. moschata* (Table 3). There was interaction between accessions and isolates in *C. moschata*, with no significant difference regarding this interaction in *C. maxima* (Table 3).

Figure 4 - Relative frequency of reaction classes of *Cucubita maxima* accessions 90 days after inoculation with *Macrophomina phaseolina* isolates using the colonized toothpick method. HR - highly resistant, HS - highly susceptible, IM – immune

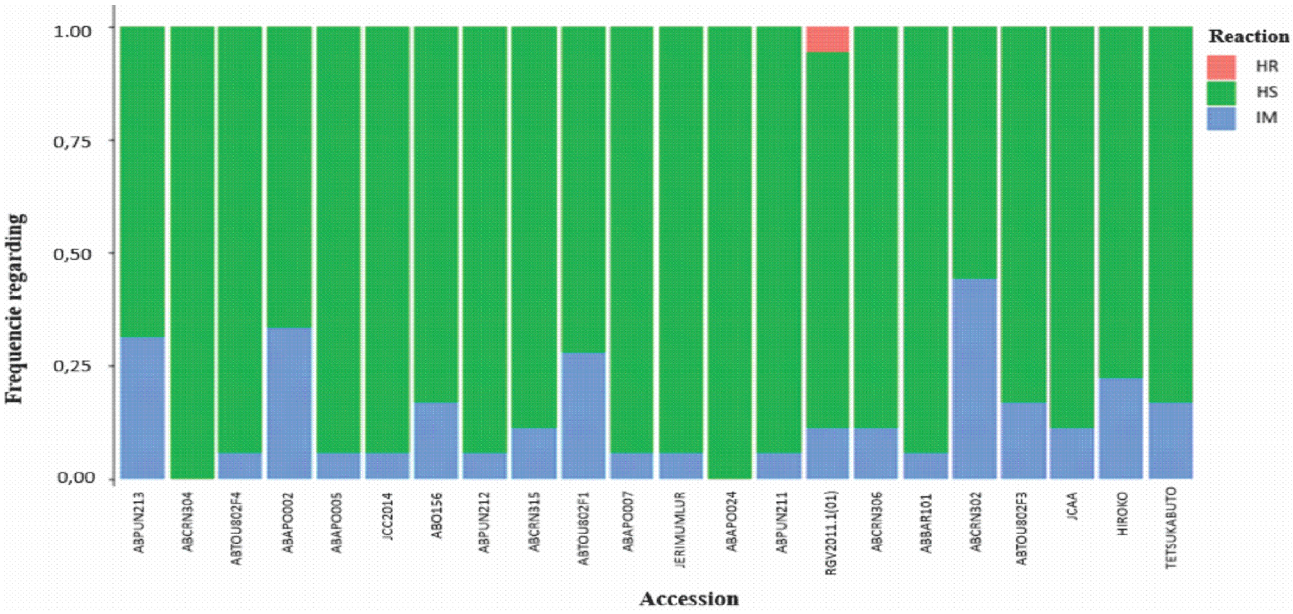
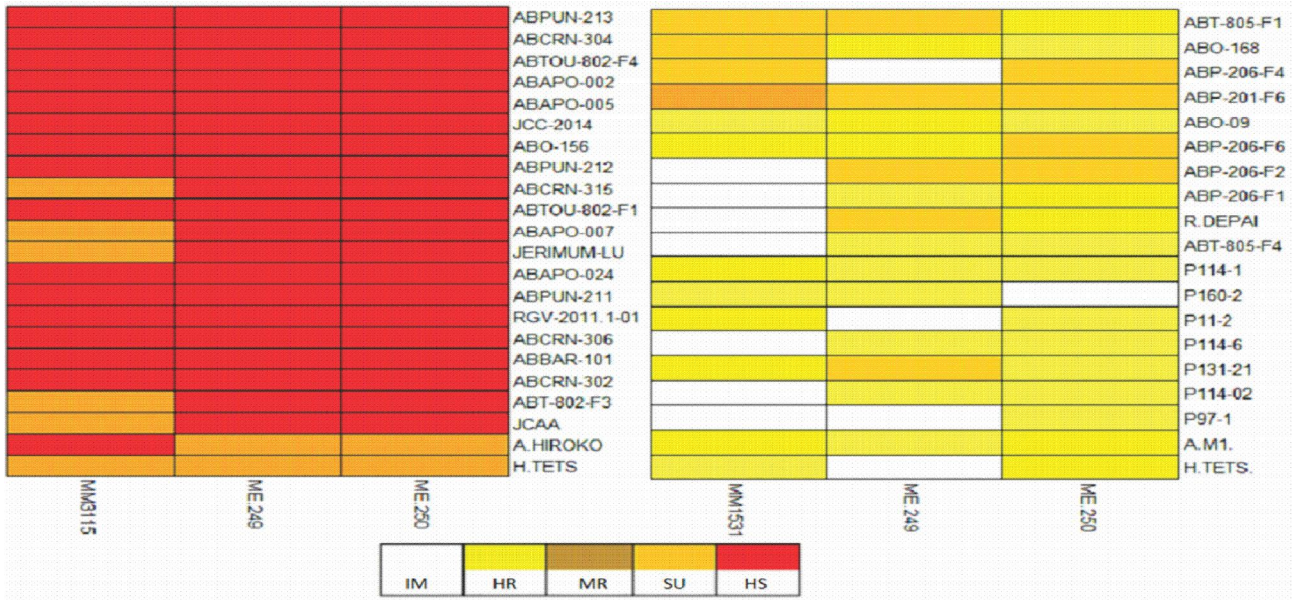


Figure 5 - Reaction class of *Cucurbita maxima* (left) and *Cucurbita moschata* (righ) accessions to three isolates of with *Macrophomina* spp. using the toothpick method. IM – immune, HR - highly resistant, MR - moderately resistant, SU – susceptible, HS - highly susceptible



When inoculated with the isolate MM1531, the *C. moschata* accessions ABPUN 206 F2, ABPUN 206 F1, ROÇA DE PAI, ABTOU 805 F4, P114-6, P114-02, P97-1, ABO 09, ABPUN 206 F6, P114-1, P160-2, P11-2, P131-21 andthecommercialcultivarsoccupiedthelastplaceintherank (Table 4), being the most resistant. In relation to the isolate ME-249, the accessions ABPUN 206 F4, P11-2, P97-1 and the hybrid commercial cultivar Tetsukabutooccupied the last position, with no statistical difference between ABPUN 206 F1, P114-1, P160-2, P114-6, P114-02 and the commercial cultivar ABÓBORA MARANHÃO, classified as the most resistant to this isolate. The accessions ABTOU 805 F1, ABO 09, ABPUN 206 F1, ROÇA DE PAI, ABTOU 805 F4, P114-1, P160-2, P11-2, P114-6, P131-21, P114-02, P97-1 and the hybrid commercial cultivar Tetsukabuto occupied the last positions in the rank when inoculated with the isolate ME-250, showing resistance.

It can also be noted that, according to the average rank, the accessions ABPUN 206 F1, P114-1, P160-2, P11-2, P114-6, P114-02 and P97-1were resistant regardless of the inoculated isolate, i.e., they were resistant to MM1531, ME-249 and ME-250, although this resistance occurs at different levels (immune, highly resistant and moderately resistant).Thus, it can be inferred that the accesses can be used in programs that aim to select the characteristic. Those with a higher level of resistance may provide faster results or even be indicated for direct use such as for use as rootstock for other cucurbitaces. However, even those who presented a lower level of resistance can be used, since through improvement they can be subjected to crosses that may increase the alleles that confer resistance.

The accessions of *C. maxima* were noticeably less resistant than those of *C. moschata*; when inoculated with the isolate MM1531, ABCRN-315, ABAPO 007, JERIMUM LURUGADO, ABTOU 802 F3 and the commercial cultivars occupied the last places in the ranking, differing from the others (Tables 4 and 5). These data denote the importance of evaluating the two species separately when it comes to reacting to pathogens in general. Considering that hybrids between the two species are already used in order to control soil pathogens, the data and results presented in the present work show that this technique can be inefficient, since the genotypes involved in obtaining the hybrid will not always be resistant to the pathogen you want to control. Regarding isolate ME-249, there was no significant difference between accessions and none of them showed resistance means. For accessions inoculated with ME-250, ABCRN 315 and RGV 2011.1 (01) occupied the last place in the rank, differing from the others. In addition to considering the difference between species, it is important to consider the variability within each species, favored both by its mode of reproduction and by the way it is maintained by farmers. Even though no access has shown an average for resistance to the ME-249 isolate, in the results presented in figure 1B it is possible to obtain that positive results were observed for this characteristic. Although with low frequency, the result does not preclude its use in a selection process.

It should be noted that even the accessions that occupied the last positions in the rank did not show resistance means. Even so, some plants can be selected from these accessions for later recommendation in breeding programs that seek this characteristic. Both studied species are allogamous, that is, they reproduces preferably by cross-pollination, which favors genetic variability (PRIORI *et al.*, 2018). Considering this variability, already described for several traits, it can be suggested that this also occurs for resistance. The variation in the amplitude of the plants evaluated for resistance shows that there was a high variability within the genotype. Although we work with averages to rank the resistance level of the accessions, in the case of a selection, it is recommended to consider each plant, since the alleles for resistance may not be present in the assessed individual of the access.

With the different responses of interaction between the isolates of the pathogen and the various genotypes, the occurrence of different levels of virulence is also evident, indicating that there is variability within the species. On

Table 3 - Deviance analysis (Type III) for severity evaluated in accessions of *Cucurbita moschata* and *Cucurbita maxima* in response to *Macrophomina phaseolina*

Effect	Test (Type III – Wald)							
	C. moschata				C. maxima			
	df_1	df_2	F	Pr(> F)	df_1	df_2	F	Pr (> F)
Accessions (A)	18	206	4.67	< 0.001	21	328	1.88	0.001
Isolate (I)	2	206	0.09	0.064	2	328	12.61	< 0.0001
A x I	36	206	1.79	0.012	42	328	1.07	< 0.355

df_1: degrees of freedom of numerator; df_2: degrees of freedom of denominator

Table 4 - Mean rank and mean severity for accessions of *Cucurbita maxima* inoculated with three isolates of *Macrophomina phaseolina*

Accession	Isolate					
	MM1531		ME-249		ME-250	
	ME-250	ME-250	Rank	Severity	Rank	Severity
ABT 805 F1	189.00 a	2.6 (SU)	192.25 a	3.0 (SU)	148.25 b	1.5 (MR)
ABO 168	169.60 a	2.4 (SU)	175.70 a	1.8 (MR)	176.00 a	1.0 (HR)
ABP 206 F4	230.00 a	4.0 (SU)	79.00 b	0.0 (IM)	199.88 a	2.3 (MR)
ABP 201 F6	242.50 a	5.0 (HS)	181.75 a	2.8 (MR)	172.75 a	2.3 (MR)
ABO 09	103.25 b	0.3 (HR)	175.70 a	1.8 (MR)	123.60 b	0.6 (HR)
ABP 206 F6	119.88 b	1.3 (MR)	205.00 a	2.0 (MR)	209.25 a	3.0 (SU)
ABP 206 F2	79.00 b	0.0 (IM)	186.83 a	2.3 (SU)	192.33 a	2.3 (SU)
ABP 206 F1	79.00 b	0.0 (IM)	111.33 b	0.3 (HR)	138.58 b	1.2 (MR)
R. DE PAI	79.00 b	0.0 (IM)	209.25 a	3.0 (SU)	149.75 b	2.0 (MR)
ABT 805 F4	79.00 b	0.0 (IM)	153.33 a	1.0 (HR)	111.33 b	0.3 (HR)
P114-1	144.40 b	2.0 (MR)	98.40 b	0.2 (HR)	126.10 b	0.8 (HR)
P160-2	111.70 b	1.0 (HR)	111.33 b	0.3 (HR)	79.00 b	0.0 (IM)
P11-2	127.25 b	1.2 (MR)	79.00 b	0.0 (IM)	122.42 b	1.0 (HR)
P114-6	79.00 b	0.0 (IM)	111.33 b	0.3 (HR)	111.33 b	0.3 (HR)
P131-21	151.38 b	1.8 (MR)	177.10 a	3.0 (SU)	114.38 b	1.0 (HR)
P114-02	79.00 b	0.0 (IM)	127.50 b	0.5 (HR)	98.40 b	0.2 (HR)
P97-1	79.00 b	0.0 (IM)	79.00 b	0.0 (IM)	111.33 b	0.3 (HR)
A. MAR.	131.10 b	1.2 (MR)	95.17 b	0.2 (HR)	169.25 a	1.8 (MR)
TETS. H.	106.25 b	0.8 (HR)	79.00 b	0.0 (IM)	133.50 b	1.7 (MR)

Mean ranks followed by the same letter belong to the same group ($p>0.05$) according to Scott-Knott (1975). IM - immune, HR - highly resistant, MR - moderately resistant, SU - susceptible, HS - highly susceptible

Table 5 - Mean rank and mean severity for accessions of *Cucurbita moschata* inoculated with three isolates of *Macrophomina phaseolina*

Accession	Isolate					
	MM1531		ME-249		ME-250	
	Rank	Severity	Rank	Severity	Rank	Severity
ABPUN- 213	224.50 a	5.0 (HS)	224.50 a	5.0 (HS)	191.58 a	4.2 (HS)
ABCRN-304	191.58 a	4.2 (HS)	224.50 a	5.0 (HS)	224.50 a	5.0 (HS)
ABTOU-802 F4	224.50 a	5.0 (HS)	191.58 a	4.2 (HS)	224.50 a	5.0 (HS)
ABAPO-002	224.50 a	5.0 (HS)	224.50 a	5.0 (HS)	224.50 a	5.0 (HS)
ABAPO-005	224.50 a	5.0 (HS)	224.50 a	5.0 (HS)	191.58 a	4.2 (HS)
JCC 2014	191.58 a	4.2 (HS)	224.50 a	5.0 (HS)	191.58 a	4.2 (HS)
ABO-156	191.58 a	4.2 (HS)	224.50 a	5.0 (HS)	224.50 a	5.0 (HS)
ABPUN-212	224.50 a	5.0 (HS)	224.50 a	5.0 (HS)	224.50 a	5.0 (HS)
ABCRN-315	158.67 b	3.3 (SU)	191.58 a	4.2 (HS)	158.67 b	3.3 (SU)
ABTOU-802 F1	224.50 a	5.0 (HS)	191.58 a	4.2 (HS)	191.58 a	4.2 (HS)
ABAPO-007	125.75 b	2.5 (SU)	191.58 a	4.2 (HS)	158.67 b	3.3 (SU)
JERIMUM LU	125.75 b	2.5 (SU)	191.58 a	4.2 (HS)	224.50 a	5.0 (HS)
ABAPO-024	224.50 a	5.0 (HS)	191.58 a	4.2 (HS)	224.50 a	5.0 (HS)

Continuation table 5

ABPUN-211	224.50 a	5.0 (HS)	224.50 a	5.0 (HS)	191.58 a	4.2 (HS)
RGV 2011.1 (01)	191.58 a	4.2 (HS)	224.50 a	5.0 (HS)	158.67 b	3.3 (SU)
ABCRN-306	191.58 a	4.2 (HS)	224.50 a	5.0 (HS)	191.58 a	4.2 (HS)
ABBAR-101	224.50 a	5.0 (HS)	191.58 a	4.2 (HS)	224.50 a	5.0 (HS)
ABCRN-302	191.58 a	4.2 (HS)	191.58 a	4.2 (HS)	191.58 a	4.2 (HS)
ABT 802 F3	163.17 b	3.5 (SU)	224.50 a	5.0 (HS)	191.58 a	4.2 (HS)
JCAA	158.68 a	4.2 (HS)	224.48 a	5.0 (HS)	191.58 a	4.2 (HS)
A. HIROKO	191.58 b	3.4 (SU)	145.48 a	3.0 (SU)	145.48 a	3.0 (SU)
TETS. H.	158.72 b	2.8 (SU)	125.78 a	2.5 (SU)	125.78 a	2.5 (SU)

Mean ranks followed by the same letter belong to the same group (p>0.05) according to Scott-Knott (1975). IM - immune, HR - highly resistant, MR - moderately resistant, SU - susceptible, HS - highly susceptible

the other hand, one cannot fail to consider that different isolates of *M. Phaseolina* may also have different levels of virulence when in association with different hosts, which highlights the importance of evaluating and selecting genotypes for specific conditions of use.

Such variability in the severity response, especially when related to the same genotype and isolate, may be associated with the heterogeneity of the species, due to the mode of pollination, which is cross-sectional, sometimes leading to distinct traits in a given character, in this case the resistance (PEREIRA *et al*., 2017). This characteristic, associated with the identification of genotypes with higher frequencies of resistance, enables the subsequent obtaining of homogeneous strains for resistance, through successive selections of individuals and studies on their genetic inheritance by breeding programs, as suggested by Padley *et al*. (2008).

It is important to note that even the genotypes that had higher resistances showed variation in this level of resistance when the isolates are considered separately, which highlights the need for considering each genotype individually. Thus, for selection purposes in breeding programs, accessions that showed levels of resistance have great potential. However, aiming at direct use as rootstock, it can be inferred that it would be early to make a recommendation, since the variability of reaction, when considering different isolates, as well as the variation in the levels of resistance observed, may result in economic losses if used in the field. Thus, it is necessary to make a selection for the resistance characteristic, even if the objective is to use genetic material as rootstock of other crops.

Most of the studies already conducted to evaluate the resistance of gray stem rot by *Macrophomina* spp. in different species were carried out in fields that had a history of the disease (ISHIKAWA *et al*., 2018). In the state of Paraíba, Northeastern Brazil, Soares *et al*. (2016) verified the occurrence of several soil-dwelling fungi causing diseases in pumpkin and watermelon cultivated in fields of several producing municipalities and, among the pathogens found, *Macrophomina* spp. can be found in watermelon, which proves its presence in the studied soils. However, this pathogen was not identified for pumpkin, suggesting a certain tolerance of the species to the pathogen for the conditions of the locality.

For the species *C. maxima*, there are few reports in the literature on the use as rootstock; however, when associated with *C. moschata*, inthe obtaining of hybrids through selected strains, several studies report its success for controlling diseases related to soil-dwelling pathogens, being widely used in the control of *Fusarium* spp. (ÁLVAREZ-HERNANDEZ *et al*., 2015; ZHOU *et al*., 2014) and other pathogens.

Studies with other soil-dwelling pathogens suggest that, among cucurbits, *C. maxima* is less tolerant than *C. moschata*, and the sources of resistance of this species are quite scarce (PEREIRA *et al*., 2017), which can also be observed in the present study (Figures 2 and 3). Considering the use of the hybrid as rootstock, the present study shows that not all cultivars can be used for controlling *Macrophomina* spp. because, with the observed frequency of resistance, its use may lead to problems in production.

There was high variability in the severity frequencies of the tested genotypes, with no stability regarding resistance, which makes it impossible to use them directly as rootstock. However, knowing that few sources of resistance to *Macrophomina* spp. are recorded for cucurbitaceous (AMBRÓSIO *et al*., 2015), genotypes that have shown some resistance to each or all isolates are valuable for the development of endogamous progenies more resistant to the pathogen.

Considering that, by the mean rank method, the *C. moschata* accessions ABPUN 206 F1, P114-1, P160-2, P11-2, P114-6, P114-02 and P97-1 were resistant to the three inoculated isolates, these can be used later for selection in breeding programs. From this same method, it is not possible to select *C. maxima* accessions (Table 3); however, in view of their resistance frequency (Figure 4), JERIMUM LU, ABAPO 007 and ABTOU 802 F3, inoculated with MM1531, ABCRN-315 inoculated with ME249 and ABAPO-007 and JCAA inoculated with ME250, obtained some plants that can be indicated for future selection, to obtain endogamous progenies.

It is important to emphasize that, in breeding programs, for the development of pathogen-resistant genotypes, it is necessary to previously conduct genetic studies to define the best method to be adopted, thus ensuring the efficiency of the program (LIMA *et al*., 2017).

CONCLUSIONS

1. There is variability in the germplasm of *C. moschata* and *C. maxima* for resistance to *M. phaseolina*, in the Cucurbit Germplasm Collection of UFERSA;
2. The accessions of *C. moschata*: ABPUN 206 F1, P114-1, P160-2, P11-2, P114-6, P114-02 and P97-1show different levels of resistance to the *Macrophomina phaseolina*.

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