Identification and pathogenicity of *Macrophomina* species collected from weeds in melon fields in Northeastern Brazil

Andréia Mitsa Paiva Negreiros[1]; Rui Sales Júnior[1*]; Maela León[2]; Naama Jéssica de Assis Melo[1], Sami J. Michereff[3]; Márcia Michelle de Queiroz Ambrósio[1]; Hohana Lissa de Sousa Medeiros[1]; Josep Armengol[2]

[1]Universidade Federal Rural do Semi-Árido - UFERSA, Mossoró-RN, Brazil

[2]Universidade Federal Rural de Pernambuco - UFRPE, Recife-PE, Brazil

[3]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Valencia, Spain.

Correspondence

*R. Sales Júnior, Center of Agrarian Sciences, Department of Agricultural and Forestry Sciences, Universidade Federal Rural do Semi-Árido - UFERSA, Mossoró-RN, Brazil. jrrui@hotmail.com

Abstract

In this work, a collection of 94 *Macrophomina* isolates obtained from roots of two weed species, *Trianthema portulacastrum* and *Boerhavia diffusa,* collected during surveys conducted during 2015 and 2016 in melon production fields in Northeastern Brazil, were characterized by using phenotypical and molecular techniques. Phylogenetic analysis of the EF1-α gene, allowed the identification of 32 isolates as *M. phaseolina* and 62 isolates as *M. pseudophaseolina*. Results of a pathogenicity test performed on melon seedlings of the cv. 'Gladial' revealed that all *M. phaseolina* isolates inoculated were able to cause disease to melon seedlings, but only some *M. pseudophaseolina* isolates were able to infect them. This study represents the first report of *M. pseudophaseolina* in both *T. portulacastrum* and *B. diffusa* weeds, which are

prevalent in the main Brazilian melon producing and exporting regions. Information about the biology and epidemiology of *M. pseudophaseolina* is scarce because of its recent description, thus further research is needed for a better understanding of this fungus as a potentially emerging pathogen of melon and other crops.

KEYWORDS: *Boerhavia diffusa*, *Macrophomina phaseolina*, *Macrophomina pseudophaseolina*, *Trianthema portulacastrum*, soilborne pathogen.

1 INTRODUCTION

Brazil is the 11th largest world producer of melon (*Cucumis melo*), with a production of 596,000 t in 2016 (FAOSTAT, 2018). Melon is currently the second most exported fruit in Brazil, generating an income of US$ 162.9 million (Anuário, 2018). The main melon producing states are Rio Grande do Norte (RN) and Ceará (CE) located in Northeastern Brazil, which account for 79.4% of the total production (IBGE, 2018).

One of the main diseases of melon and watermelon (*Citrullus lanatus*) crops in Northeastern Brazil is root rot and vine decline (RRVD) caused by a complex of different soilborne pathogens such as *Monosporascus cannonballus* Pollack & Uecker, *Rhizoctonia solani* Kühn and *Macrophomina phaseolina* (Tassi) Goid. (Andrade et al., 2005). *Macrophomina phaseolina* has been also reported as an important cucurbit pathogen in other countries of the world such as Iran (Salari, Panjehkeh, Nasirpoor, & Abkhoo, 2012), Israel (Cohen, Omari, Porat, & Edelstein, 2012; Reuveni, Krikun, Nachmias, & Schlevin, 1982), Chile (Jacob, Krarup, Díaz, & Latorre, 2013) and Egypt (El-Kolaly & Abdel-Sattar, 2013).

Fungi of the genus *Macrophomina* are members of the family Botryosphaeriaceae, belonging to the class Dothideomycetes. Currently, there are three species of *Macrophomina* reported in the world: *M. phaseolina*, *M. pseudophaseolina* Crous, Sarr & Ndiaye (Sarr, Ndiaye,

Groenewald, & Crous, 2014), and *M. euphorbiicola* A.R. Machado, D.J. Soares & O.L. Pereira (Machado, Pinho, Soares, Medeiros-Gomes, & Pereira, 2018). These species are soilborne fungi but, *M. phaseolina* has a wider host range, being pathogenic to more than 500 crops and in non-cultivated species (Farr & Rossman, 2018), including economically important hosts, such as common bean, cotton, sorghum and soybean (Baird & Brock, 1999; Baird, Watson, & Scruggs, 2003; Cruciol & Costa, 2017; Funnell-Harris, O'neill, Sattler, & Yerka, 2016; Rusuku, Buruchara, Gatabazi, & Pastor-Corrales, 1997). *Macrophomina phaseolina* has a worldwide distribution, but it is considered economically more important in subtropical and tropical countries with semi-arid climate (Wrather et al., 1997; Wrather et al., 2001). On the contrary, *M. euphorbiicola* has been described affecting only *Jatropha gossypifolia* and *Ricinus communis* in Brazil (Machado, Pinho, Soares, Medeiros-Gomes, & Pereira, 2018), and *M. pseudophaseolina* affecting *Abelmoschus esculentus*, *Arachis hypogaea*, *Hibiscus sabdarifa* and *Vigna unguiculata* in Senegal (Sarr, Ndiaye, Groenewald, & Crous, 2014) and *A. hypogaea*, *Gossypium hirsutum* and *R. communis* in Brazil (Machado, Pinho, Soares, Medeiros-Gomes, & Pereira, 2018).

According to Agustí-Brisach, Gramaje, León, García-Jiménez, & Armengol (2011) & Chaves, Braun, Eiras, Colariccio, & Galleti (2003), weeds can act as secondary hosts of phytopathogens, serving as potential sources of inoculum. Fuhlbohm, Ryley, & Aitken (2012) isolated *M. phaseolina* from the roots of symptomless plants of 23 weed species found in Australian mung bean (*V. radiata*) fields, and all isolates were pathogenic on mung bean seedlings. In similar studies, Sales Júnior et al. (2012) & Rodrigues (2013) confirmed the occurrence of *M. cannonballus*, *M. phaseolina* and *Rhizoctonia solani*, causal agents of RRVD, on melon and on roots of several weed species prevalent in melon cultivation areas in Northeastern Brazil. More specifically, Rodrigues (2013) isolated *M. phaseolina* from 85.7%

of the analyzed weed species. Among these, *Trianthema portulacastrum* L. and *Boerhavia diffusa* L. were confirmed as hosts of *M. phaseolina*.

Claudino & Soares (2014) hypothesized that in addition to *M. phaseolina*, other species of *Macrophomina* could be present in Brazil. This was recently confirmed by the report of *M. euphorbiicola* and *M. pseudophaseolina* associated with charcoal rot of oilseed crops in this country (Machado, Pinho, Soares, Medeiros-Gomes, & Pereira, 2018). In this context, the increasing economic importance of RRVD of melons associated with *M. phaseolina* in Northeastern Brazil, as well as the existing reports of weeds as hosts of this fungus (Fuhlbohm, Ryley, & Aitken, 2012; Rodrigues, 2013; Sales Júnior et al., 2012), suggest that more than one species of *Macrophomina* may be also present on weeds growing in melon fields in this region. Thus, the objective of this work was to characterize a wide collection of *Macrophomina* isolates obtained from roots of *T. portulacastrum* and *B. diffusa* weeds growing in melon production fields in Northeastern Brazil by using phenotypical and molecular techniques, as well as to evaluate its pathogenicity to melon seedlings.

2 MATERIALS AND METHODS

2.1 Sampling and fungal isolation

Field surveys were conducted during 2015 and 2016 in eight major commercial melon cropping areas, located in the agricultural centers of Mossoró and Assú (RN state) and Icapuí (CE state), Northeastern Brazil (Figure 1). Symptomless *T. portulacastrum* and *B. diffusa* weed species were selected based on their prevalence in commercial melon fields in RN and CE states and previous reports confirming its role as alternative hosts of *M. phaseolina* (Rodrigues, 2013). Two fields (2 ha each) were surveyed per area and thirty plants of each weed species were collected per field.

For fungal isolation, roots of weeds were washed under running tap water, immersed for 1 min in 1.5% sodium hypochlorite solution, and washed twice with distilled water for 1 min. Subsequently, small pieces of roots (4–5 mm) were dried on sterilized paper towels, and plated in Petri plates with Potato Dextrose Agar (PDA; Merck KGaA, Darmstadt, Germany) supplemented with 0.5 g L^{-1} streptomycin sulphate (PDAS) (seven pieces per plate). Plates were incubated at 30±1°C in the dark for 3–4 days. Fungal colonies emerging from roots pieces, which were morphologically similar to *Macrophomina* (Sarr, Ndiaye, Groenewald, & Crous, 2014) were transferred to PDA plates and incubated at 30±1°C in the dark.

For the identification of the colonies at the genus level, slides were prepared for microscopy containing fungal structures (mycelium and sclerotia), stained with lactophenol cotton blue, observed under an optical microscope and compared to the typical morphological characteristics of the genus *Macrophomina* (Sarr, Ndiaye, Groenewald, & Crous, 2014). Based on this, a total of 94 isolates were tentatively identified as *Macrophomina*. All isolates were hyphal-tipped and, then, they were stored on sandy-organic substrate and Castellani's method with distilled water (Alfenas & Mafia, 2016; Medeiros, Melo, Ambrósio, Nunes, & Costa, 2015).

2.2 DNA isolation, PCR amplification and sequencing

Molecular analysis was used to identify 94 isolates of *Macrophomina* at the species level (Table 1). Total genomic DNA was extracted from mycelium and sclerotia of pure cultures grown on PDA for two weeks at 30±1°C in the dark, using the E.Z.N.A. Plant Miniprep Kit (Omega Bio-tek, USA) following the manufacturer's short protocol instructions with some modifications in the samples preparation step. Briefly, lysis buffer P1 (650 µl) was added to the mycelium and sclerotia in a 2 ml screw-capped conical tubes (Thermo Scientific) containing four metal 2.38 mm beads (Qiagen) and two tungsten carbide 3 mm beads (Qiagen) and

124 homogenized twice at speed 5 m s^{-1} for 20 sec using FastPrep-24™5G homogenizer (MP
125 Biomedicals, Santa Ana, CA, USA).

126 The translation elongation factor-1*alpha* (*tef-1α*) was used as the *Macrophomina* species
127 marker (Machado, Pinho, Soares, Medeiros-Gomes, & Pereira, 2018; Sarr, Ndiaye,
128 Groenewald, & Crous, 2014). Polymerase Chain Reaction (PCR) amplifications were
129 performed using Horse-Power™ Taq DNA Polymerase (Canvax Biotech SL, Córdoba, Spain)
130 and the primers EF728F and EF986R (Carbone & Kohn, 1999). The amplification program
131 consisted of an initial step of 3 min at 94°C, followed by 35 cycles of denaturation at 94°C for
132 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 45 sec. A final extension was
133 performed at 72°C for 10 min. The PCR products were separated by electrophoresis in 1%
134 agarose gel (agarose D-1 Low EEO, Conda, Madrid, Spain), stained with Realsafe (Real,
135 Paterna Valencia, Spain), and visualized under UV light. Gene-ruler 100-bp DNA ladder plus
136 was used as a molecular weight marker (Fermentas, St. Leon-Rot, Germany). The resulting
137 products were sequenced by Macrogen Inc. (Madrid, Spain). Consensus sequences were
138 assembled using Sequencher software package version 5.0 (Gene Codes Corp., Ann Arbor,
139 MI).
140

141 **2.3 Phylogenetic analyses**

142 The DNA sequences generated in this study together with representative sequences for
143 the genus *Macrophomina* (Machado, Pinho, Soares, Medeiros-Gomes, & Pereira, 2018; Sarr,
144 Ndiaye, Groenewald, & Crous, 2014) from GenBank (Table 1) were aligned using the ClustalW
145 (Thompson, Higgins, & Gibson, 1994) contained within MEGA7 software package (Kumar,
146 Stecher, & Tamura, 2016). The alignments were inspected and manual adjustments were made
147 when necessary. Incomplete portions at either end of the alignments were excluded prior to
148 analyses. All sequences from this study were deposited on GenBank. The tree was rooted to

149 *Botryosphaeria dothidea* CMW8000 (Table 1). Sequence alignments were deposited in
150 TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S23031).

151 The sequences of all isolates were analyzed through Bayesian inference (BI), Maximum
152 Likelihood (ML) and Maximum Parsimony (MP) generating phylogenetic trees that enabled
153 their identifications. For BI analysis, the optimal substitution model was determined using
154 MrModeltest software v. 2.2. (Nylander, 2004), computed using MrBayes v3.2 (Ronquist et al.,
155 2012) with four simultaneous Markov Chain Monte Carlo from random trees over 100 million
156 generations with trees sampled every 1000th generation were run, resulting in 100,000 total
157 trees. The first 25% of saved trees were discarded as the "burn-in" phase and posterior
158 probabilities determined from the remaining. The ML analysis was performed with RAxML-
159 HPC2 on XSEDE v. 8.2.10 (Stamatakis, 2014) using a GTR+GAMMA substitution model with
160 1000 bootstrap iterations. Both BI and ML were run on the CIPRES Science Gateway portal
161 (Miller, Pfeiffer, & Schwartz, 2012), and the trees were visualized by FigTree
162 (http://tree.bio.ed.ac.uk/software/figtree/).The MP genealogies was estimated in MEGA7
163 software package (Kumar, Stecher, & Tamura, 2016), using the Tree-Bisection-Regrafting
164 (TBR) algorithm, and the tree was visualized in the same software.
165

166 **2.4 Pathogenicity and virulence on melon**

167 Ten representative isolates of each *Macrophomina* species were used for this experiment
168 (Table 1). In addition one isolate of *M. phaseolina* obtained from melon plants (CMM-1531)
169 was included as positive control. Melon seeds of the cv. 'Gladial' were germinated in a
170 'Tropstrato HT®' commercial substrate previously autoclaved. The plants were irrigated daily
171 to drainage with tap water and were not fertilized during the experiment.

172 The inoculation technique used was the toothpick method, because of the easy
173 multiplication of inoculum and fast inoculation (Ambrósio et al., 2015; Medeiros, Melo,

Ambrósio, Nunes, & Costa, 2015; Mir et al., 2018). Twelve mm long toothpicks were placed, with the sharpened end up, in holes made in a 90 mm diameter filter paper. The toothpicks were then placed in a Petri plate and autoclaved for 30 min, for 2 days with an interval of 24 h, at 121ºC. Twenty ml of melted PDA + streptomycin sulfate was added to each toothpick-containing Petri plate. Once solidified, the PDAS plates were inoculated with five mycelial plugs (8 mm in diameter) of one isolate of *Macrophomina* and then were incubated at 28 ± 2ºC in the dark for 8 days.

Melon seedlings were inoculated 10 days after sowing (DAS) by inserting the toothpicks colonized with mycelia and microsclerotia of the corresponding isolate in each hypocotyl, 1 cm above the soil. Non colonized toothpicks were used as negative controls. The inoculated plants were maintained in a greenhouse at an average temperature of 35°C for 30 days, under natural daylight conditions.

Thirty days after inoculation, the virulence of the isolates was assessed as disease severity using a modified version of the rating 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 as the total number of infected plants from each *Macrophomina* species and expressed as percentage.

Seven small fragments (0.2 to 0.5 cm) of necrotic lesions from each symptomatic plant were cut and placed on PDAS in an attempt to recover the inoculated fungi and complete Koch's postulates. *Macrophomina* spp. were identified as described above.

The experiment was arranged in a completely randomized design with five replicates per treatment (isolate) and one plant per replicate. The experiment was conducted twice. For each species of *Macrophomina*, a preliminary ANOVA was performed to determine if there were significant differences between the two repetitions of the experiments, and if the data

could be combined. Severity results by isolates of *M. phaseolina* and *M. pseudophaseolina* were analysed with the nonparametric Kruskal-Wallis test at the probability level of 5% ($p < .05$) using the software Assistat, version 7.7 (Silva & Azevedo, 2016). Differences in virulence caused by *Macrophomina* species were determined using the mean of both bioassays by one-way ANOVA and compared by Mann-Whitney test at the 5% significance level using STATISTIX v. 9.0 (Analytical Software).

3 RESULTS

3.1 PCR, sequencing, and *tef-1α* phylogeny

All the isolates were identified based on the phylogenetic analysis of the *EF1-α* gene, which was amplified with the primers EF728F and EF986R. A PCR fragment ranging from 217–221 bp was obtained for them. The first approximation to the identification of the 94 isolates, putative belonging to *Macrophomina* genus, was based on the BLAST analysis of their *EF1-α* sequence.

Phylogenetic analysis on the *tef-1α* locus alignment contained a total of 106 taxa, from which 94 were of the studied isolates, six of *M. phaseolina*, two of *M. pseudophaseolina*, three of *M. euphorbiicola*, and *Botryosphaeria dothidea* CMW8000, which was used as outgroup, resulting in a dataset of 227 characters, including alignment gaps, of which 162 were constant, 22 parsimony-informative, and 43 parsimony-uninformative. Sequences of *M. phaseolina*, *M. pseudophaseolina*, *M. euphorbiicola* and *B. dothidea* were obtained from GenBank (Table 1).

The topology of the tree identified by MP analysis were similar to those obtained by the BI and ML analyses, therefore only the MP tree is presented, with ML and MP bootstrap support values and BI posterior probability scores at the nodes.

The *Macrophomina* group was divided into three well-supported clades (Figure 2). Each clade corresponded to previously described species. One clade (62 isolates) clustered together

224 with the species *M. pseudophaseolina* (KF952153, KF952148), strongly supported by bootstrap
225 values (ML/MP/BI: 100/99/1). The remaining 32 isolates clustered together with *M. phaseolina*
226 (KF951997, KU058910, KF952009, KF952013, KF952005, KF951998), with high bootstrap
227 support for ML and MP (ML/MP/BI: 98/94/0.99). These isolates were subdivided into three
228 sub-clades, with low support. None of our isolates clustered with *M. euphorbiicola*.
229
230 **3.2 Pathogenicity and virulence on melon**
231 All *M. phaseolina* isolates inoculated were pathogenic to melon, while only three *M.*
232 *pseudophaseolina* isolates (CMM-4780, CMM-4788 and CMM-4807) were able to infect
233 melon seedlings. Percent recovery of the inoculated isolates from the necrotic tissues of
234 symptomatic plants was higher than 95% and reisolated species were confirmed to be the same
235 inoculated previously. No isolates were obtained from the negative controls.
236 Disease severity and disease incidence presented significant differences ($P \leq 0.05$)
237 between *M. phaseolina* and *M. pseudophaseolina* isolates (Table 2). When results from all
238 isolates of each species were combined, disease severity was higher for *M. phaseolina* (3.84)
239 than *M. pseudophaseolina* (0.22) (Figure 3A). Disease incidence of *M. phaseolina* on melon
240 seedlings was also higher (86%) than *M. pseudophaseolina* (10%) (Figure 3B).
241
242 **4 DISCUSSION**
243 The characterization of a wide collection of *Macrophomina* isolates obtained from
244 asymptomatic *T. portulacastrum* and *B. diffusa* plants collected in melon growing fields in
245 Northeastern Brazil, confirmed the identification of two *Macrophomina* species, *M. phaseolina*
246 and *M. pseudophaseolina*, associated with the roots of both species. Moreover, *T.*
247 *portulacastrum* and *B. diffusa* are reported for the first time as new hosts for *M.*
248 *pseudophaseolina*.

249 Phylogenetic analyses, supported by phenotypical studies, confirmed the identification
250 of *M. phaseolina* and *M. pseudophaseolina*. It was possible to distinguish both species using
251 the gene *tef-1α*. In recent studies, this gene demonstrated to have potential for use as a tool to
252 identify known species of *Macrophomina* and other Botryosphaeriaceae spp. in diagnostic
253 studies (Machado, Pinho, & Pereira, 2014; Machado, Pinho, Soares, Medeiros-Gomes, &
254 Pereira, 2018; Sarr, Ndiaye, Groenewald, & Crous, 2014).
255 In our research, *M. pseudophaseolina* was the most frequent species found among the
256 94 *Macrophomina* spp. isolates collected from *T. portulacastrum* and *B. diffusa* weeds in melon
257 production fields located in Northeastern Brazil. Nevertheless, Sarr, Ndiaye, Groenewald, &
258 Crous (2014) reported different results when determining the genetic vatiation of a global set
259 of 189 isolates of *Macrophomina* spp. obtained from 23 hosts and 30 soil samples in 15
260 countries, in which only 18 isolates were identified as *M. pseudophaseolina* and 171 isolates
261 were *M. phaseolina*. Recently, Machado, Pinho, Soares, Medeiros-Gomes, & Pereira (2018)
262 determined the identity of 35 *Macrophomina* spp. isolates obtained from diverse oilseed crops
263 in Brazil using phylogenetic analysis and morphological characteristics, from which only 11
264 were confirmed as *M. pseudophaseolina*.
265 Results of the pathogenicity test to melon seedlings with *M. phaseolina* and *M.*
266 *pseudophaseolina* conducted under greenhouse conditions revealed that both *Macrophomina*
267 species are able to infect this crop, but *M. phaseolina* presented higher disease incidence and
268 severity than *M. pseudophaseolina*. Similar differences in virulence of *Macrophomina* species
269 were also observed by Ndiaye, Sarr, Cisse, & Ndoye (2015), where the isolates of *M. phaseolina*
270 presented the highest values of incidence of charcoal rot when compared with *M.*
271 *pseudophaseolina* after inoculation of bean cultivars.
272 It is well known that *M. phaseolina* can be isolated from symptomless weed species
273 (Fuhlbohm, Ryley, & Aitken, 2012; Rodrigues, 2013; Sales Júnior et al., 2012), which can serve

as alternative hosts for the pathogen. This fact, together with the longevity of its microsclerotia in soil, enable *M. phaseolina* to survive for many years in the absence of a host crop (Short, Wyllie, & Bristow, 1980). Although the information about the host range of *M. pseudophaseolina* is limited due to its recent description (Machado, Pinho, & Pereira, 2014; Machado, Pinho, Soares, Medeiros-Gomes, & Pereira, 2018; Sarr, Ndiaye, Groenewald, & Crous, 2014), our results demonstrate that *T. portulacastrum* and *B. diffusa* can also be considered sources of inoculum for this fungus in cucurbits fields. Nevertheless, to date only *M. phaseolina* has been reported as causal agent of RRVD of melon in Northeastern Brazil (Andrade et al., 2005; Rodrigues, 2013).

This work reports for the first time the association of *M. pseudophaseolina* with asymptomatic roots of *T. portulacastrum* and *B. diffusa* weeds, which are common in the main Brazilian producing and exporting regions of melon. Although *M. pseudophaseolina* was the most frequent species and the pathogenicity tests showed that some isolates are able to infect melon seedlings, further research is needed for a better understanding of this fungus as a potentially emerging pathogen of melon and other crops (Machado, Pinho, Soares, Medeiros-Gomes, & Pereira, 2018; Sarr, Ndiaye, Groenewald, & Crous, 2014).

ACKNOWLEDGMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001 and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

REFERENCES

297 Agustí-Brisach, C., Gramaje, D., León, M., García-Jiménez, J., & Armengol, J. (2011).
298 Evaluation of vineyard weeds as potential hosts of black-foot and Petri disease pathogens.
299 *Plant Disease,* **95**, 803-810.
300 Alfenas, A. C., & Mafia, R. G. (2016). Métodos em fitopatologia (p. 516). Viçosa, Brasil: Ed.
301 UFV, Universidade Federal de Viçosa.
302 Ambrósio, M. M. Q., Dantas, A. C. A., Martínez-Perez, E., Medeiros, A. C., Nunes, G. H. S.,
303 & Picó, M. B. (2015). Screening a variable germplasm collection of *Cucumis melo* L. for
304 seedling resistance to *Macrophomina phaseolina*. *Euphytica*, **206**, 287-300.
305 Andrade, D. E. G. T., Michereff, S. J., Biondi, C. M., Nascimento, C. W. A., & Sales Júnior,
306 R. (2005). Frequência de fungos associados ao colapso do meloeiro e relação com
307 características físicas, químicas e microbiológicas dos solos. *Summa Phytopathologica*, **31**,
308 327-333.
309 Anuário - Anuário Brasileiro da Fruticultura 2018 (p. 88). (2018). Santa Cruz do Sul: Ed.
310 Gazeta Santa Cruz.
311 Baird, R. E., & Brock, J. H. (1999). First report of *Macrophomina phaseolina* on cotton
312 (*Gossypium hirsutum*) in Georgia. *Plant Disease*, **83**, 487-487.
313 Baird, R. E., Watson, C. E., & Scruggs, M. (2003). Relative longevity of *Macrophomina*
314 *phaseolina* and associated mycobiota on residual soybean roots in soil. *Plant Disease*, **87**,
315 563-566.
316 Carbone, I., & Kohn, L. M. (1999). A method for designing primer sets for speciation studies
317 in filamentous ascomycetes. *Mycologia*, **91**, 553-556.
318 Chaves, A. L. R., Braun, M. R., Eiras, M., Colariccio, A., & Galleti, S. R. (2003). *Erigon*
319 *bonariensis*: hospedeira alternativa do *Lettuce mosaic vírus* no Brasil. *Fitopatologia*
320 *Brasileira*, **28**, 307-311.

321 Claudino, M. R., & Soares, D. J. (2014). Pathogenicity and aggressiveness of *Macrophomina*
322 *phaseolina* isolates to castor (*Ricinus communis*). *Tropical Plant Pathology,* **39**, 453-456.
323 Cohen, R., Omari, N., Porat, A., & Edelstein, M. (2012). Management of *Macrophomina* wilt
324 in melons using grafting or fungicide soil application: pathological, horticultural and
325 economical aspects. *Crop Protection*, **35**, 58-63.
326 Cruciol, G. C. D., & Costa, M. L. N. (2017). Influência de metodologias de inoculação de
327 *Macrophomina phaseolina* no desempenho de cultivares de soja. *Summa*
328 *phytopathologica*, **43**, 337-343.
329 El-Kolaly G. A. A., & Abdel-Sattar M. A. (2013). The etiology of sudden wilt disease syndrome
330 on melon in Egypt. *Nature and Science*, **11**, 79-87.
331 FAOSTAT (2018). FAO Statistical Databases, Food and Agriculture Organization of the
332 United Nations. http://www.fao.org/faostat/en/#home.
333 Farr, D. F., & Rossman, A. Y. (2018). Fungal Databases, U.S. National Fungus Collections,
334 ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/.
335 Fuhlbohm, M. J., Ryley, M. J., & Aitken, E. A. B. (2012). New weed hosts of *Macrophomina*
336 *phaseolina* in Australia. *Australasian Plant Disease Notes*, **7**, 193-195.
337 Funnell-Harris, D. L., O'neill, P. M., Sattler, S. E., & Yerka, M. K. (2016). Response of sweet
338 sorghum lines to stalk pathogens *Fusarium thapsinum* and *Macrophomina phaseolina*.
339 *Plant Disease*, **100**, 896-903.
340 IBGE (2018). Instituto Brasileiro de Geografia e Estatística.
341 https://sidra.ibge.gov.br/home/pms/brasil.
342 Jacob, C. J., Krarup, C., Díaz, G. A., & Latorre, B. A. (2013). A severe outbreak of charcoal
343 rot in cantaloupe melon caused by *Macrophomina phaseolina* in Chile. *Plant Disease,* **97**,
344 141.

345 Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics
346 Analysis Version 7.0 for Bigger Datasets. *Molecular Biology Evolution,* **33**, 1870-1874.
347 Machado, A. R., Pinho, D. B., & Pereira, O. L. (2014). Phylogeny, identification and
348 pathogenicity of the Botryosphaeriaceae associated with collar and root rot of the biofuel
349 plant *Jatropha curcas* in Brazil, with a description of new species of *Lasiodiplodia*. *Fungal*
350 *Diversity*, **67**, 231-247.
351 Machado, A. R., Pinho, D. B., Soares, D. J., Medeiros-Gomes, A. A., & Pereira, O. L. (2018).
352 Bayesian analyses of five gene regions reveal a new phylogenetic species of
353 *Macrophomina* associated with charcoal rot on oilseed crops in Brazil. *European Journal*
354 *of Plant Pathology* (in press). https://doi.org/10.1007/s10658-018-1545-1
355 Medeiros, A. C., Melo, D. R. M., Ambrósio, M. M. Q., Nunes, G. H. S., & Costa, J. M. (2015).
356 Methods of inoculation of *Rhizoctonia solani* and *Macrophomina phaseolina* in melon
357 (*Cucumis melo*). *Summa Phytopathologica*, **41**, 281-286.
358 Miller, M. A., Pfeiffer, W., & Schwartz, T. (2012). The CIPRES science gateway: enabling
359 high-impact science for phylogenetics researchers with limited resources. In: *Proceedings*
360 *of the 1st Conference of the Extreme Science and Engineering Discovery Environment:*
361 *Bridging from the extreme to the campus and beyond* (pp. 39). Chicago, Illinois, USA.
362 Mir, Z. R., Singh, P. K., Zaidi, P. H., Vinayan, M. T., Sharma, S. S., Krishna, M. K., … Nair,
363 S. K. (2018). Genetic analysis of resistance to post flowering stalk rot in tropical
364 germplasm of maize (*Zea mays* L.). *Crop Protection*, **106**, 42-49.
365 Ndiaye, M., Sarr, M. P., Cisse, N., & Ndoye, I. (2015). Is the recently described *Macrophomina*
366 *pseudophaseolina* pathogenically different from *Macrophomina phaseolina*? *African*
367 *Journal of Microbiology Research*, **9**, 2232-2238.
368 Nylander, J. A. A. (2004). MrModeltest V2. Program Distributed by the Author. Evolutionary
369 Biology Centre, Uppsala University, Sweden.

370 Reuveni, R., Krikun, J., Nachmias, A., & Schlevin, E. (1982). The role of *Macrophomina*
371 *phaseolina* in a collapse of melon plants in Israel. *Phytoparasitica*, **10**, 51-56.
372 Rodrigues, A. P. M. S. (2013). Ocorrência de plantas daninhas como hospedeiras alternativas
373 de fitopatógenos radiculares e avaliação da patogenicidade sobre as culturas do melão e da
374 melancia. Mossoró, Rio Grande do Norte, Brasil, Universidade Federal Rural do
375 Semiárido, Master's Dissertation.
376 Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., …
377 Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and
378 model choice across a large model space. *Systematic Biology*, **61**, 539-542.
379 Rusuku, G., Buruchara, R. A., Gatabazi, M., & Pastor-Corrales, M. A. (1997). Occurrence and
380 distribution in Rwanda of soilborne fungi pathogenic to the common bean. *Plant disease*,
381 **81**, 445-449.
382 Salari, M., Panjehkeh, N., Nasirpoor, Z., & Abkhoo, J. (2012). Reaction of melon (*Cucumis*
383 *melo* L.) cultivars to soil-borne plant pathogenic fungi in Iran. *African Journal of*
384 *Biotecnology*, **11**, 15324-15329.
385 Sales Júnior, R., Oliveira, O. F., Medeiros, E. V., Guimarães, I. M., Correia, K. C., & Michereff,
386 S. J. (2012). Ervas daninhas como hospedeiras alternativas de patógenos causadores do
387 colapso do meloeiro. *Revista Ciência Agronômica*, **43**, 195-198.
388 Sarr, M. P., Ndiaye, M., Groenewald, J. Z., & Crous, P. W. (2014). Genetic diversity in
389 *Macrophomina phaseolina*, the causal agent of charcoal rot. *Phytopathologia*
390 *Mediterranea*, **53**, 250-268.
391 Short, G. E., Wyllie, T. D., & Bristow, P. R. (1980). Survival of *Macrophomina phaseolina* in
392 soil and in residue of soybean. *Phytopathology,* **70**, 13-17.
393 Silva, F. A. Z., & Azevedo, C. A. V. (2016). The Assistat Software Version 7.7 and its use in
394 the analysis of experimental data. *African Journal Agricultural Research*, **11**, 3733–3740.

395 Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of
396 large phylogenies. *Bioinformatics*, **30**, 1312-1313.
397 Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). Clustal W: improving the sensitivity
398 of progressive multiple sequence alignment through sequence weighting, position- specific
399 gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673-4680.
400 Wrather, J. A., Anderson, T. R., Arsyad, D. M., Gai, J., Ploper, L. D., Porta-Puglia, A., …
401 Yorinori, J. T. (1997). Soybean disease loss estimates for the top 10 soybean producing
402 countries in 1994. *Plant Disease*, **81**, 107-110.
403 Wrather, J. A., Anderson, T. R., Arsyad, D. M., Tan, Y., Ploper, L. D., Porta-Puglia, A., …
404 Yorinori, J. T. (2001). Soybean disease loss estimates for the top 10 soybean producing
405 countries in 1998. *Canadian Journal of Plant Pathology*, **23**, 115-121.
406
407

TABLE 1 List of isolates used in phylogeny of *Macrophomina* species.

Species	Strain number	Host	Collected by/year	Location	GenBank Accession Numbers
M. phaseolina	CMM 4733[a]	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373464
M. phaseolina	CMM 4734	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373440
M. phaseolina	CMM 4735	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373441
M. phaseolina	CMM 4736	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373436
M. phaseolina	CMM 4737	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373442
M. phaseolina	CMM 4738	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373461
M. phaseolina	CMM 4739	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373457
M. phaseolina	CMM 4740	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373465
M. phaseolina	CMM 4741	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373443
M. phaseolina	CMM 4742[a]	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373466
M. phaseolina	CMM 4743	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373453
M. phaseolina	CMM 4744	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Ceará, Icapuí	MH373458
M. phaseolina	CMM 4745	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Ceará, Icapuí	MH373467
M. phaseolina	CMM 4746	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Ceará, Icapuí	MH373462
M. phaseolina	CMM 4747	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373437
M. phaseolina	CMM 4748[a]	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373438
M. phaseolina	CMM 4749	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373444
M. phaseolina	CMM 4750[a]	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373445
M. phaseolina	CMM 4751	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373446
M. phaseolina	CMM 4752	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373454
M. phaseolina	CMM 4753	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373459
M. phaseolina	CMM 4754	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373447
M. phaseolina	CMM 4755[a]	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373463
M. phaseolina	CMM 4756	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373460
M. phaseolina	CMM 4757	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373448
M. phaseolina	CMM 4758[a]	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373450
M. phaseolina	CMM 4759	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373449
M. phaseolina	CMM 4760[a]	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373439

Species	Strain number	Host	Collected by/year	Location	GenBank Accession Numbers
M. phaseolina	CMM 4761[a]	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373452
M. phaseolina	CMM 4762[a]	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373456
M. phaseolina	CMM 4763	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373451
M. phaseolina	CMM 4764[a]	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373455
M. phaseolina	CDA 1100	*Ricinus communis*	-	Brazil, Bahia	KU058910
M. phaseolina	CBS 457.70	*Phaseolus aureus*	-	Denmark	KF952009
M. phaseolina	CBS 461.70	*Phaseolus vulgaris*	-	Denmark	KF952013
M. phaseolina	CBS 270.34	*Vigna sinensis*	-	USA, Missouri	KF952005
M. phaseolina	CBS 205.47	*Phaseolus vulgaris*	-	Italy	KF951997
M. phaseolina	CBS 224.33	*Sesamum indicum*	-	Uganda	KF951998
M. pseudophaseolina	CMM 4765[a]	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373511
M. pseudophaseolina	CMM 4766	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Ceará, Icapuí	MH373507
M. pseudophaseolina	CMM 4767	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Ceará, Icapuí	MH373513
M. pseudophaseolina	CMM 4768	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Ceará, Icapuí	MH373468
M. pseudophaseolina	CMM 4769	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373469
M. pseudophaseolina	CMM 4770[a]	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373470
M. pseudophaseolina	CMM 4771	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373471
M. pseudophaseolina	CMM 4772	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373514
M. pseudophaseolina	CMM 4773	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373472
M. pseudophaseolina	CMM 4774	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373512
M. pseudophaseolina	CMM 4775	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373473
M. pseudophaseolina	CMM 4776	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373508
M. pseudophaseolina	CMM 4777	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373474
M. pseudophaseolina	CMM 4778	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373509
M. pseudophaseolina	CMM 4779	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373475
M. pseudophaseolina	CMM 4780[a]	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373515
M. pseudophaseolina	CMM 4781	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373476
M. pseudophaseolina	CMM 4782	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373477
M. pseudophaseolina	CMM 4783	*Trianthema portulacastrum*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373478
M. pseudophaseolina	CMM 4784	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373479

Species	Strain number	Host	Collected by/year	Location	GenBank Accession Numbers
M. pseudophaseolina	CMM 4785	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373480
M. pseudophaseolina	CMM 4786[a]	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373481
M. pseudophaseolina	CMM 4787	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373482
M. pseudophaseolina	CMM 4788[a]	*Trianthema portulacastrum*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373483
M. pseudophaseolina	CMM 4789	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373484
M. pseudophaseolina	CMM 4790[a]	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373485
M. pseudophaseolina	CMM 4791	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373486
M. pseudophaseolina	CMM 4792	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373487
M. pseudophaseolina	CMM 4793	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373488
M. pseudophaseolina	CMM 4794	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373489
M. pseudophaseolina	CMM 4795	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373490
M. pseudophaseolina	CMM 4796	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373491
M. pseudophaseolina	CMM 4797	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373492
M. pseudophaseolina	CMM 4798	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373493
M. pseudophaseolina	CMM 4799	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373494
M. pseudophaseolina	CMM 4800[a]	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373516
M. pseudophaseolina	CMM 4801	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373517
M. pseudophaseolina	CMM 4802	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373495
M. pseudophaseolina	CMM 4803	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373496
M. pseudophaseolina	CMM 4804	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Assú	MH373527
M. pseudophaseolina	CMM 4805	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373497
M. pseudophaseolina	CMM 4806	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373498
M. pseudophaseolina	CMM 4807[a]	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373518
M. pseudophaseolina	CMM 4808	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373499
M. pseudophaseolina	CMM 4809	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373519
M. pseudophaseolina	CMM 4810	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373520
M. pseudophaseolina	CMM 4811	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373501
M. pseudophaseolina	CMM 4812	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373521
M. pseudophaseolina	CMM 4813	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Assú	MH373510
M. pseudophaseolina	CMM 4814[a]	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373500

Species	Strain number	Host	Collected by/year	Location	GenBank Accession Numbers
M. pseudophaseolina	CMM 4815	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373522
M. pseudophaseolina	CMM 4816	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373523
M. pseudophaseolina	CMM 4817	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373524
M. pseudophaseolina	CMM 4818	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373504
M. pseudophaseolina	CMM 4819	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373525
M. pseudophaseolina	CMM 4820	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373526
M. pseudophaseolina	CMM 4821[a]	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373502
M. pseudophaseolina	CMM 4822	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373503
M. pseudophaseolina	CMM 4823	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373505
M. pseudophaseolina	CMM 4824	*Boerhavia diffusa*	A.M.P. Negreiros, 2015	Brazil, Rio Grande do Norte, Mossoró	MH373506
M. pseudophaseolina	CMM 4825	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373528
M. pseudophaseolina	CMM 4826	*Boerhavia diffusa*	A.M.P. Negreiros, 2016	Brazil, Rio Grande do Norte, Mossoró	MH373529
M. pseudophaseolina	CPC 21394	*Vigna unguiculata*		Senegal, Thiès	KF952148
M. pseudophaseolina	CPC 21417	*Arachis hypogaea*	-	Senegal, Louga	KF952153
Botryosphaeria dothidea	CMW 8000	*Prunus* sp.	B. Slippers, 2000	Switzerland, Crocifisso	AY236898

[a] Isolates used in the pathogenicity test.

TABLE 2 Reaction of *Cucumis melo* seedlings cv. Gladial to isolates of *Macrophomina phaseolina* and *M. pseudophaseolina*.

Isolates	*Macrophomina phaseolina*				Isolates	*Macrophomina pseudophaseolina*			
	Disease Severity		Disease Incidence			Disease Severity		Disease Incidence	
	Rank	Mean	Rank	Mean (%)		Rank	Mean	Rank	Mean (%)
CMM-4733	34.9	4.0 ab	30.5	80.0 ab	**CMM-4765**	25.5	0.0 a	25.5	0.0 a
CMM-4742	42.0	5.0 b	36.5	100.0 b	**CMM-4770**	25.5	0.0 a	25.5	0.0 a
CMM-4748	19.7	3.4 ab	36.5	100.0 b	**CMM-4780**	36.5	0.8 ab	37.5	40.0 ab
CMM-4750	8.8	0.6 a	12.5	20.0 ab	**CMM-4786**	25.5	0.0 a	25.5	0.0 a
CMM-4755	42.0	5.0 b	36.5	100.0 b	**CMM-4788**	36.9	1.2 ab	37.5	40.0 ab
CMM-4761	24.6	3.0 ab	30.5	80.0 ab	**CMM-4790**	25.5	0.0 a	25.5	0.0 a
CMM-4762	42.0	5.0 b	36.5	100.0 b	**CMM-4800**	25.5	0.0 a	25.5	0.0 a
CMM-4758	24.3	2.8 ab	30.5	80.0 ab	**CMM-4807**	30.6	0.2 a	31.5	20.0 ab
CMM-4760	37.2	4.6 ab	36.5	100.0 b	**CMM-4814**	25.5	0.0 a	25.5	0.0 a
CMM-4764	42.0	5.0 b	36.5	100.0 b	**CMM-4821**	25.5	0.0 a	25.5	0.0 a
CMM-1531	42.0	5.0 b	36.5	100.0 b	**CMM-1531**	58.0	5.0 b	55.5	100.0 b
CONTROL	6.5	0.0 a	6.5	0.0 a	**CONTROL**	25.5	0.0 a	25.5	0.0 a
***c*χ2**	42.49		39.33			40.26			

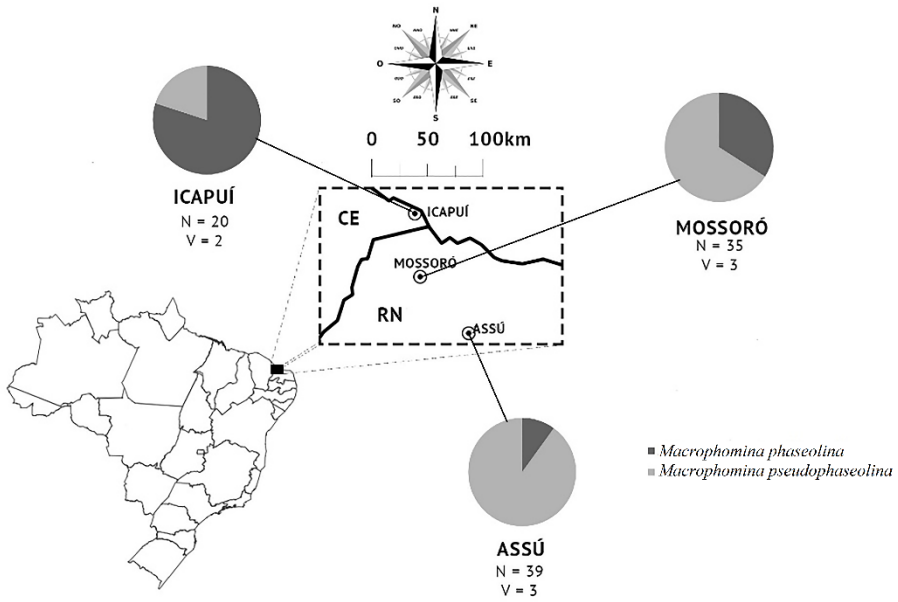
cχ^2, chi-squared value significant at 5% by Kruskal–Wallis test. Letters are for comparison of means in the same column.

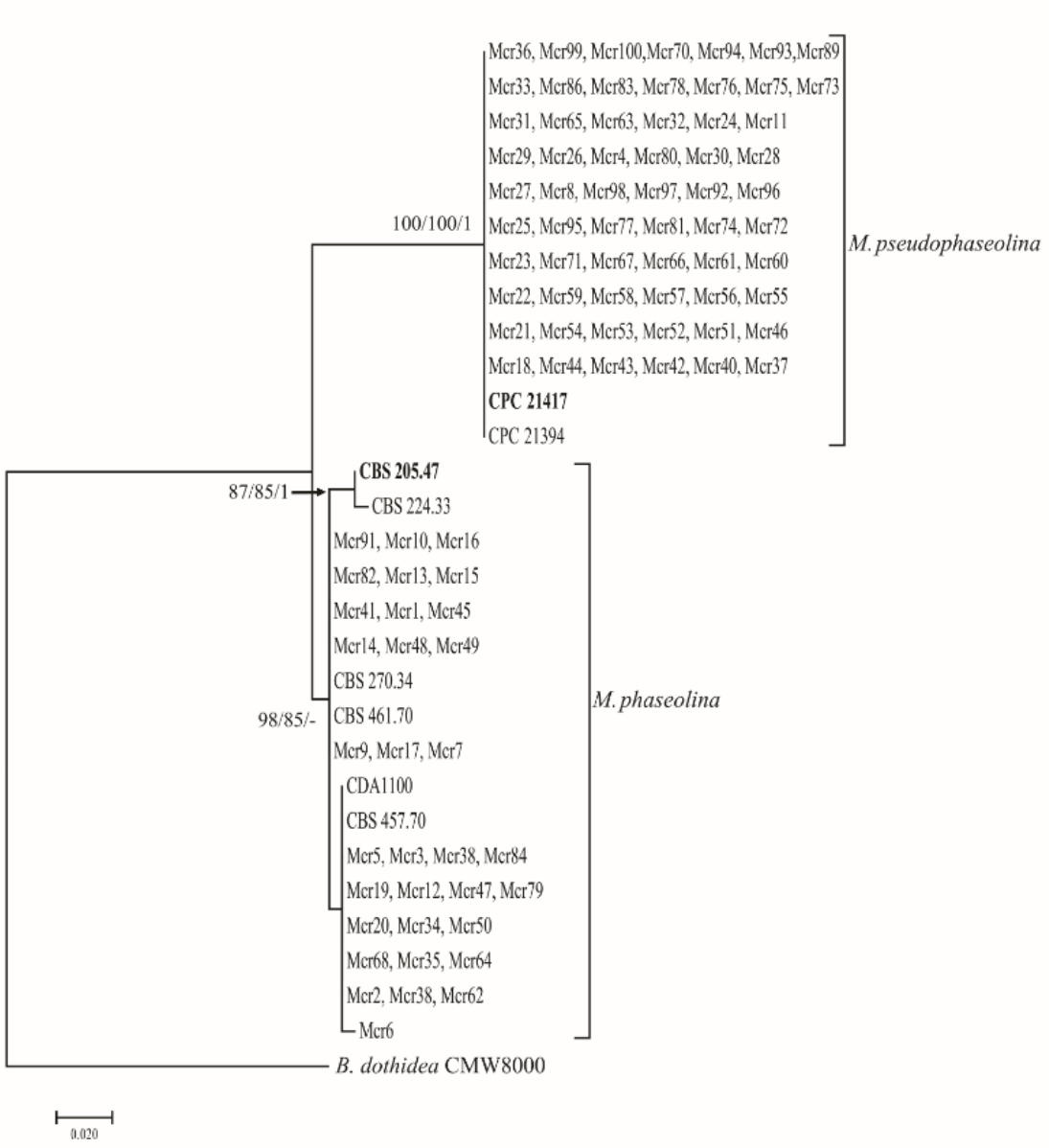
Figure Captions

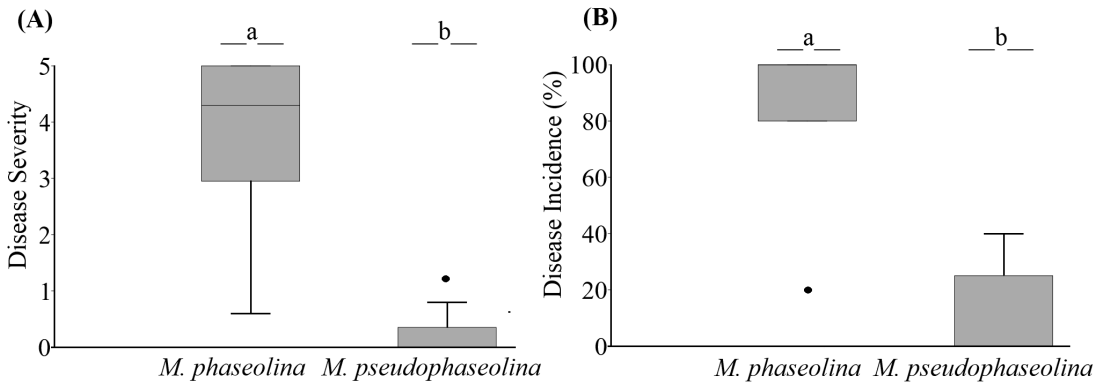
Figure 1 Collection sites of *Macrophomina* species obtained from the weeds *Trianthema portulacastrum* and *Boerhavia diffusa* in the melon growing areas of Mossoró and Assú (Rio Grande do Norte state) and Icapuí (Ceará state), located in the Northeast Region of Brazil. Circles represent association frequency of each *Macrophomina* species in each agricultural area sampled, N is the number of isolates analyzed in each agricultural area, and V is the number of commercial crops areas sampled in each agricultural center. CE, Ceará; RN, Rio Grande do Norte.

Figure 2 Phylogenetic relationships within the genus *Macrophomina*. Maximum parsimony (MP) phylogeny based on *tef-1α* sequence alignment. Nodes receiving Maximum Likelihood and MP bootstrap > 70% and Bayesian posterior probabilities > 0.9 are considered as supported. The tree was rooted to *Botryosphaeria dothidea* CMW8000.

Figure 3 Boxplots showing (A) Disease Severity and (B) Incidence of the *Macrophomina* species in melon plants. The boxes show the first and third quartiles. Bold horizontal line represents median of group. Lower and upper whiskers extend from the boxes to the extreme values, or outlying values are indicated by black dots. Different lowercase letters indicate significant differences according to Mann-Whitney test ($p \leq 0.05$).


N
O
E
S
0 50 100km
ICAPUÍ
N = 20
V = 2
CE
ICAPUÍ
MOSSORÓ
RN
ASSÚ
MOSSORÓ
N = 35
V = 3
ASSÚ
N = 39
V = 3
Macrophomina phaseolina
Macrophomina pseudophaseolina


Mcr36, Mcr99, Mcr100,Mcr70, Mcr94, Mcr93,Mcr89
Mcr33, Mcr86, Mcr83, Mcr78, Mcr76, Mcr75, Mcr73
Mcr31, Mcr65, Mcr63, Mcr32, Mcr24, Mcr11
Mcr29, Mcr26, Mcr4, Mcr80, Mcr30, Mcr28
Mcr27, Mcr8, Mcr98, Mcr97, Mcr92, Mcr96
100/100/1
Mcr25, Mcr95, Mcr77, Mcr81, Mcr74, Mcr72
Mcr23, Mcr71, Mcr67, Mcr66, Mcr61, Mcr60
Mcr22, Mcr59, Mcr58, Mcr57, Mcr56, Mcr55
Mcr21, Mcr54, Mcr53, Mcr52, Mcr51, Mcr46
Mcr18, Mcr44, Mcr43, Mcr42, Mcr40, Mcr37
CPC 21417
CPC 21394
M. pseudophaseolina
CBS 205.47
87/85/1
CBS 224.33
Mcr91, Mcr10, Mcr16
Mcr82, Mcr13, Mcr15
Mcr41, Mcr1, Mcr45
Mcr14, Mcr48, Mcr49
CBS 270.34
98/85/-
CBS 461.70
Mcr9, Mcr17, Mcr7
CDA1100
CBS 457.70
Mcr5, Mcr3, Mcr38, Mcr84
Mcr19, Mcr12, Mcr47, Mcr79
Mcr20, Mcr34, Mcr50
Mcr68, Mcr35, Mcr64
Mcr2, Mcr38, Mcr62
Mcr6
M. phaseolina
B. dothidea CMW8000
0.020


(A)
__a__
__b__
Disease Severity
5
4
3
2
1
0
M. phaseolina
M. pseudophaseolina
(B)
__a__
__b__
Disease Incidence (%)
100
80
60
40
20
0
M. phaseolina
M. pseudophaseolina