Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252020v33n405rc

GENETIC SIMILARITY OF *Macrophomina pseudophaseolina* ISOLATES ASSOCIATED WITH WEEDS IN THE BRAZILIAN SEMIARID REGION[1]

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ABSTRACT - *Macrophomina pseudophaseolina* has recently been reported in association with weeds in melon producing areas in Northeastern Brazil. Species from this genus are the causal agents of root rot and vine decline (RRVD) in melon, reducing its productivity. It is needed to know the genetic variability of the pathogen to develop effective control methods. Thus, this work aimed to assess the genetic diversity among *M. pseudophaseolina* isolates collected from the weeds *Trianthema portulacastrum* L. and *Boerhavia diffusa* L. using ISSR and RAPD markers. For this, 41 *M. pseudophaseolina* isolates were submitted to amplification with five ISSR and ten RAPD primers. Genetic similarity was analyzed using the Jaccard's coefficient and cluster analysis was performed by the UPGMA method. Combining data from both markers, the 41 isolates were separated into eight groups. Most groups were not arranged according to geographical origin and host of the pathogen. The genetic similarity among isolates ranged from 0.15 to 0.87. On the other hand, the highest genetic dissimilarity (85%) was observed between the isolate MpBr11, collected from *T. portulacastrum* in Icapuí (CE), and MpBr65, collected from *B. diffusa* in Assú (RN). Results obtained herein can assist breeding programs for the selection of resistance sources and the development of effective control methods against RRVD in melon.

Keywords: Genetic variability. RAPD. ISSR. Melon.

SIMILARIDADE GENÉTICA DE ISOLADOS DE *Macrophomina pseudophaseolina* ASSOCIADOS A PLANTAS DANINHAS NO SEMI-ÁRIDO BRASILEIRO

RESUMO – *Macrophomina pseudophaseolina* foi recentemente relatada em áreas produtoras de melão em associação com plantas daninhas na região Nordeste. Espécies do gênero são agentes da podridão radicular e declínio das ramas (PRDR) do meloeiro, doença que reduz sua produtividade. O conhecimento da diversidade genética do patógeno é importante no desenvolvimento de métodos de controle eficientes. Nesse contexto, o objetivo desse trabalho foi analisar a variabilidade genética de isolados de *M. pseudophaseolina* coletados das plantas daninhas *Trianthema portulacastrum* L. e *Boerhavia diffusa* L. por meio dos marcadores moleculares ISSR e RAPD. Para isto, 41 isolados de *M. pseudophaseolina* foram submetidos à amplificação com cinco iniciadores ISSR e dez RAPD. A similaridade genética foi analisada através do coeficiente de Jaccard e o agrupamento obtido com o método UPGMA. Com a combinação dos dados dos marcadores os 41 isolados foram agrupados em 8 grupos principais. Não foi observado, para grande maioria dos grupos gerados, a relação do agrupamento de acordo com hospedeiro ou local de coleta. A similaridade genética entre os isolados variou de 0,15 a 0,87. A maior dissimilaridade genética (85%) foi observada entre o isolado MpBr11, coletado de *T. portulacastrum* no município de Icapuí/CE, do isolado MpBr65, coletado de *B. diffusa* em Assú/RN. Estas informações podem ser úteis para auxiliar programas de melhoramento genético na seleção de fontes de resistência e/ou em testes de métodos de controle contra PRDR do melão.

Palavras-chave: Variabilidade genética. RAPD. ISSR. Melão.

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[1]Received for publication in 04/20/2020; accepted in 07/15/2020.
Paper extracted from the dissertation of the first author.
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INTRODUCTION

Melon (*Cucumis melo* L.) is the second most produced cucurbit in the world (27.35 million tons) and Brazil ranks 11th among producing countries (FAO, 2020). Most of the national production is destined for export, generating an income of US$ 160 million (ABRAFRUTAS, 2019; ANUÁRIO, 2019).

The Northeast region accounts for 95% of the national production, mainly from the states of Ceará (CE) and Rio Grande do Norte (RN) (IBGE, 2020). Melon is adapted to the edaphoclimatic conditions of the region, such as high temperatures and radiation (MAIA; LIMA; LIMA, 2013), and the high technological level employed in the cultivation allowed its production to be intensified.

Continuous cropping and expansion of planted areas without effective disease management increased the incidence and severity of numerous pathologies in producing fields in the Northeast region (SALES JÚNIOR et al., 2019). Root rot and vine declining (RRVD), caused by soilborne fungi species, limits melon production in the region (PORTO et al., 2019; SALES JÚNIOR et al., 2012). Species from the genus *Macrophomina* are commonly reported as the causal agents of RRVD in producing areas in the Northeastern Brazil (NEGREIROS et al., 2019).

Macrophomina pseudophaseolina Crous, Sarr & Ndiaye, belonging to the phylum *Ascomycota*, family *Botryosphaeriaceae*, was reported by Sarr et al. (2014) as a new species from the genus *Macrophomina* that shares distribution characteristics and host range with *Macrophomina phaseolina*.

Moreover, species from the genus *Macrophomina* have been found in association with weed species in off-season cultivation of cucurbits in Northeastern Brazil (SALES JÚNIOR et al., 2012; 2019). Negreiros et al. (2019) reported *M. pseudophaseolina* associated with weed species such as *Trianthema portulacastrum* and *Boerhavia diffusa* in melon producing fields in states of CE and RN.

Associating with weeds and producing resistance structures, the pathogen can remain in producing areas for long periods (KAUR et al., 2012). It is aggravated by the lack of tolerant cultivars and effective chemical control (GARCÍA et al., 2019), which hampers the disease management by producers.

In this context, using resistant cultivars is the first strategy for integrated and sustainable disease management (ALMEIDA et al., 2014). For the development of resistant cultivars, in addition to knowing the genetic diversity of the plant, it is needed to consider the pathogen's degree of variability. High intraspecific diversity of the pathogen can influence the degree of resistance developed by plants (ŽIVANOV et al., 2019).

Studying genetic variability using PCR (Polymerase Chain Reaction)-based molecular markers is an efficient and practical method to assess the genetic diversity among phytopathogenic fungi species (PAPLOMATAS, 2004). Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) are dominant markers that do not require prior knowledge of the genome to detect genetic variability among organisms. In addition, they are low-cost and time-saving methods for obtaining results (SOUZA, 2015; SINGH; SINGH; PANDEY, 2013).

To generate knowledge for assisting in the development of control methods against RRVD, this study aimed to assess the genetic variability among *M. pseudophaseolina* isolates associated with weed species using RAPD and ISSR markers and evaluate the effectiveness of each primer for detecting the genetic diversity of this species.´

MATERIAL AND METHODS

Description of fungal material

The 41 *M. pseudophaseolina* isolates used in this study belong to the mycological collection of the Phytopathology Laboratory II, Department of Agronomic and Forest Sciences, Federal Rural University of the Semiarid Region (UFERSA). Description of hosts and collection sites of the isolates are shown in Table 1.

Table 1. Name, identifier code in the Culture Collection of Phytopathogenic Fungi Prof. Maria Menezes (CMM) and GenBank, hosts, and collection sites of the M. pseudophaseolina isolates used in this study.

Name	Isolate ID	GenBank access number	Host	Collection site
MpBr11	CMM-4765	MH373511	*T. portulacastrum*	Icapuí/CE
MpBr23	CMM-4771	MH373471	*T. portulacastrum*	Assú/RN
MpBr25	CMM-4773	MH373472	*T. portulacastrum*	Assú/RN
MpBr26	CMM-4774	MH373512	*T. portulacastrum*	Assú/RN
MpBr27	CMM-4775	MH373473	*T. portulacastrum*	Assú/RN
MpBr28	CMM-4776	MH373508	*T. portulacastrum*	Assú/RN
MpBr30	CMM-4778	MH373509	*T. portulacastrum*	Assú/RN
MpBr32	CMM-4780	MH373515	*T. portulacastrum*	Mossoró/RN
MpBr33	CMM-4781	MH373476	*T. portulacastrum*	Mossoró/RN
MpBr40	CMM-4784	MH373479	*T. portulacastrum*	Mossoró/RN
MpBr43	CMM-4786	MH373481	*T. portulacastrum*	Mossoró/RN
MpBr44	CMM-4787	MH373482	*T. portulacastrum*	Mossoró/RN
MpBr46	CMM-4788	MH373483	*T. portulacastrum*	Mossoró/RN
MpBr51	CMM-4789	MH373484	*B. diffusa*	Assú/RN
MpBr53	CMM-4791	MH373486	*B. diffusa*	Assú/RN
MpBr54	CMM-4792	MH373487	*B. diffusa*	Assú/RN
MpBr55	CMM-4793	MH373488	*B. diffusa*	Assú/RN
MpBr57	CMM-4795	MH373490	*B. diffusa*	Assú/RN
MpBr58	CMM-4796	MH373491	*B. diffusa*	Assú/RN
MpBr59	CMM-4797	MH373492	*B. diffusa*	Assú/RN
MpBr63	CMM-4800	MH373516	*B. diffusa*	Assú/RN
MpBr65	CMM-4801	MH373517	*B. diffusa*	Assú/RN
MpBr66	CMM-4802	MH373495	*B. diffusa*	Assú/RN
MpBr67	CMM-4803	MH373496	*B. diffusa*	Assú/RN
MpBr71	CMM-4805	MH373497	*B. diffusa*	Assú/RN
MpBr72	CMM-4806	MH373498	*B. diffusa*	Assú/RN
MpBr73	CMM-4807	MH373518	*B. diffusa*	Assú/RN
MpBr74	CMM-4808	MH373499	*B. diffusa*	Assú/RN
MpBr75	CMM-4809	MH373519	*B. diffusa*	Assú/RN
MpBr76	CMM-4810	MH373520	*B. diffusa*	Assú/RN
MpBr77	CMM-4811	MH373501	*B. diffusa*	Assú/RN
MpBr80	CMM-4813	MH373510	*B. diffusa*	Assú/RN
MpBr81	CMM-4814	MH373500	*B. diffusa*	Mossoró/RN
MpBr83	CMM-4815	MH373522	*B. diffusa*	Mossoró/RN
MpBr89	CMM-4817	MH373524	*B. diffusa*	Mossoró/RN
MpBr92	CMM-4818	MH373504	*B. diffusa*	Mossoró/RN
MpBr94	CMM-4820	MH373526	*B. diffusa*	Mossoró/RN
MpBr95	CMM-4821	MH373502	*B. diffusa*	Mossoró/RN
MpBr97	CMM-4823	MH373505	*B. diffusa*	Mossoró/RN
MpBr99	CMM-4825	MH373528	*B. diffusa*	Mossoró/RN
MpBr100	CMM-4826	MH373529	*B. diffusa*	Mossoró/RN

Fungal cultivation and DNA extraction

The isolates were cultivated in PDA (potato dextrose agar) medium for ±5 days at 28 °C in darkness to promote enough mycelium growth for DNA isolation. DNA was extracted using the method of Smith et al. (2001) with modifications. The mycelium frozen in liquid nitrogen was ground to a fine powder inside 2.0 ml microtubes. Then, SDS extraction buffer (2% and 3% SDS, 100 mM Tris-HCl pH 8.0, 500 mM NaCl, 50 mM EDTA, 0.6% β-mercaptoethanol, and q.s. ultrapure water) was mixed with the mycelial powder, and the tubes were incubated in a water bath at 60 °C for 40 min.

Afterward, the mixture phases were separated with chloroform:isoamyl alcohol (24:1). The supernatant layer was transferred to a new tube, added to isopropanol and kept at -20 °C for ±12 h. Then, the tubes were washed with 70% and 90% alcohol, sequentially, and the pellet was resuspended in RNase solution (10 μg ml^{-1}) (Ludwig Biotec, Brazil). Subsequently, the DNA was quantified on 1% agarose gel stained with ethidium bromide (0.5 μg ml^{-1}) by electrophoresis at 110 V for 30 min.

ISSR analysis

The genomic DNA of the 41 *M. pseudophaseolina* isolates was amplified via PCR using the ISSR primers listed in Table 2. Reactions were carried out in 12 μl volumes containing 1x $MgCl_2$ buffer, 0.16 mM of dNTP, 1 U Taq DNA polymerase (Cellco), 0.33 μM of each primer, BSA (bovine serum albumin) (0.25 mg ml^{-1}), and 30 ng of DNA. Amplification was performed in an automatic thermocycler (Amplitherm) programmed to operate an initial denaturation at 94 °C for 4 min, followed by 40 cycles of denaturation at 94 °C for 40 s, annealing at the specific temperature of each primer (Table 2) for 40 s, and extension at 72 °C for 2 min, followed by an extension at 72 °C for 7 min.

The amplified products were separated on 2.5% agarose gel stained with ethidium bromide (0.5 μg ml^{-1}) by electrophoresis at 110 V for ± 2 h in 1x TBE buffer (0.89 M Tris-base, 0.02 M boric acid, 0.89 M EDTA). A 1 kbp DNA ladder (BioLabs, New England) was used as a molecular weight standard.

RAPD analysis

RAPD analysis was performed using ten arbitrary primers (Table 2). Amplification reactions were carried out in 12 μl volume under the same conditions as described for the ISSR markers, except for primer concentration, which was 0.82 μM, and without bovine serum albumin.

Amplifications were performed under the following conditions: initial denaturation at 95 ºC for 5 min, followed by 40 cycles of denaturation at 92 ºC for 1 min, annealing at 40 ºC for 1 min, and extension at 72 ºC for 2 min, followed by an extension at 72 ºC for 5 min. The amplified products were separated on 1.5% agarose gel stained with ethidium bromide (0.5 μg ml^{-1}) by electrophoresis at 100V for ± 2 h in 1x TBE buffer. A 1 kbp DNA ladder (BioLabs, New England) was used as a molecular weight standard.

Table 2. Identification, sequence, and annealing temperature (AT) of the molecular markers used in this study.

Marker	Sequence	AT (°C)
ISSR		
ISSR 03	CYCACACACACACACACA	50
ISSR 09	GTGGTGGTGGTGGTG	50
ISSR 10	GTGGTGGTGGTGGTGYC	52
ISSR 11	CYGTGGTGGTGGTGGTG	52
ISSR 16	ATGATGATGATGATGRC	40
RAPD		
OPM 3	GGGGGATGAG	40
OPM 4	GGCGGTTGTC	40
OPM 5	GGGAACGTGT	40
OPM 6	CTGGGCAACT	40
OPM 11	GTCCACTGTG	40
OPM 12	GGGACGTTGG	40
OPM 13	GGTGGTCAAG	40
OPM 16	GTAACCAGCC	40
OPM 18	CACCATCCGT	40
OPH 18	GAATCGGCCA	40

Statistical analysis

The fragments generated by both markers were converted into a binary matrix, attributing number 1 to the presence and 0 to the absence of bands. Then, a genetic similarity matrix was calculated using the Jaccard's coefficient and cluster analysis was performed using the UPGMA (Unweighted Pair Group Method with Arithmetic Average) hierarchical method in NTSYSpc software. The cut-off line position on the dendrogram was calculated as the mean of genetic similarity (JAMSHIDI; JAMSHIDI, 2011). Also, polymorphism information content (PIC), effective multiplex ratio (EMR), and marker index (MI) were calculated according to Chesnokov and Artemyeva (2015). Principal Coordinates Analysis (PCoA) was performed in NTSYSPc using the Eigen mode and

the graph was generated in Mod3D mode.

RESULTS AND DISCUSSION

In this study, the genetic diversity among 41 *Macrophomina pseudophaseolina* isolates from two weed species collected in melon producing areas of Ceará and Rio Grande do Norte states were evaluated using ISSR and RAPD markers. Five ISSR markers with reproducible and polymorphic bands were used to detect the genetic diversity among the 41 isolates. The selected ISSR markers generated 108 loci, of which 79 (73.1%) were polymorphic (Table 3). The genetic similarity coefficient ranged from 0.13 to 0.91. By the UPGMA method, the dendrogram arranged isolates into 11 groups, at 58% similarity (Figure 1).

Table 3. Resolving power of the ISSR and RAPD markers used in this study. Number of amplified fragments (NAF), number of polymorphic fragments (NPF), percentage of polymorphism (%P), polymorphism information content (PIC), effective multiplex ratio (EMR) and marker index (MI).

Marker	NAF	NPF	%P		PIC	EMR	MI
ISSR							
ISSR 03	12	7	58.3		0.25	4.1	1.0
ISSR 09	25	21	84.0		0.24	17.6	4.2
ISSR 10	28	20	71.4		0.22	14.3	3.1
ISSR 11	23	17	73.9		0.21	12.6	2.6
ISSR 16	20	14	70.0		0.32	9.8	3.1
Total	108	79	73.1	Mean	0.25	11.7	2.8
RAPD							
OPM 3	13	9	69.2		0.33	6.2	2.1
OPM 4	14	9	64.3		0.40	5.8	2.3
OPM 5	12	10	83.3		0.40	8.3	3.3
OPM 6	15	13	86.7		0.26	11.3	3.0
OPM 11	10	8	80.0		0.28	6.4	1.8
OPM 12	12	5	41.7		0.24	2	0.5
OPM 13	13	8	61.5		0.11	4.9	0.5
OPM 16	21	12	57.1		0.26	6.9	1.8
OPM 18	19	11	57.9		0.26	6.4	1.6
OPH 18	19	13	68.4		0.34	8.9	3.0
Total	148	98	66.2	Mean	0.29	6.7	2.0

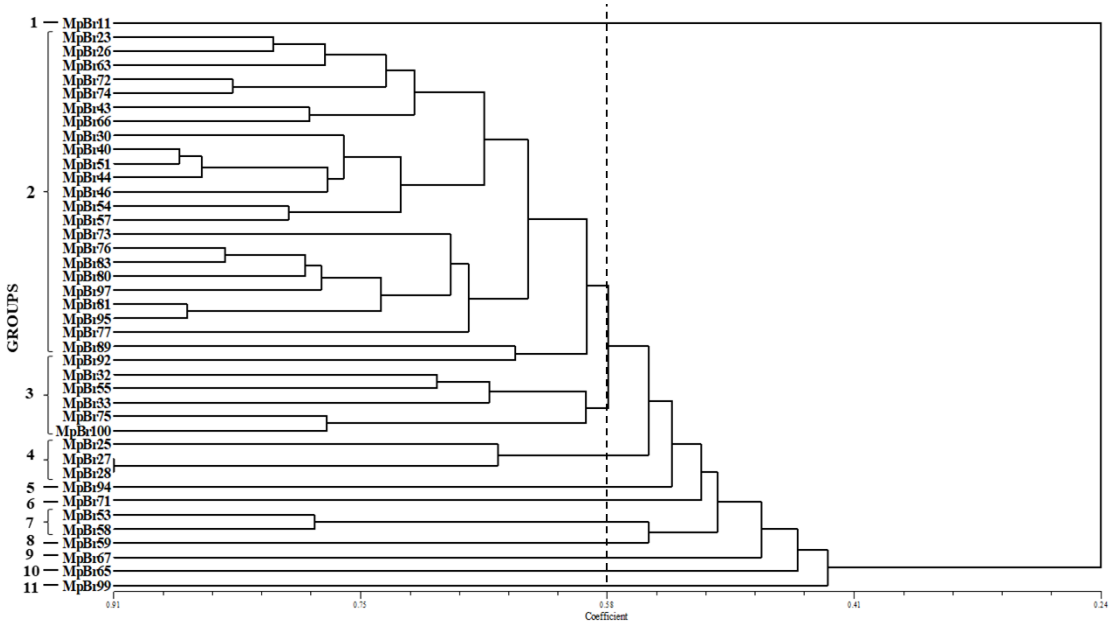

Figure 1. Dendrogram obtained by the UPGMA method using the Jaccard coefficient derived from the ISSR analysis of 41 M. pseudophaseolina isolates.

In turn, the RAPD analysis with ten markers produced well-defined and polymorphic fragments for genetic diversity analysis among *M. pseudophaseolina* isolates. In total, 148 loci were amplified, of which 98 (66.2%) were polymorphic (Table 3). The genetic similarity coefficient ranged from 0.07 to 0.84. The dendrogram by the UPGMA method using Jaccard's coefficient arranged the 41 isolates into nine groups at 55.5% similarity (Figure 2).

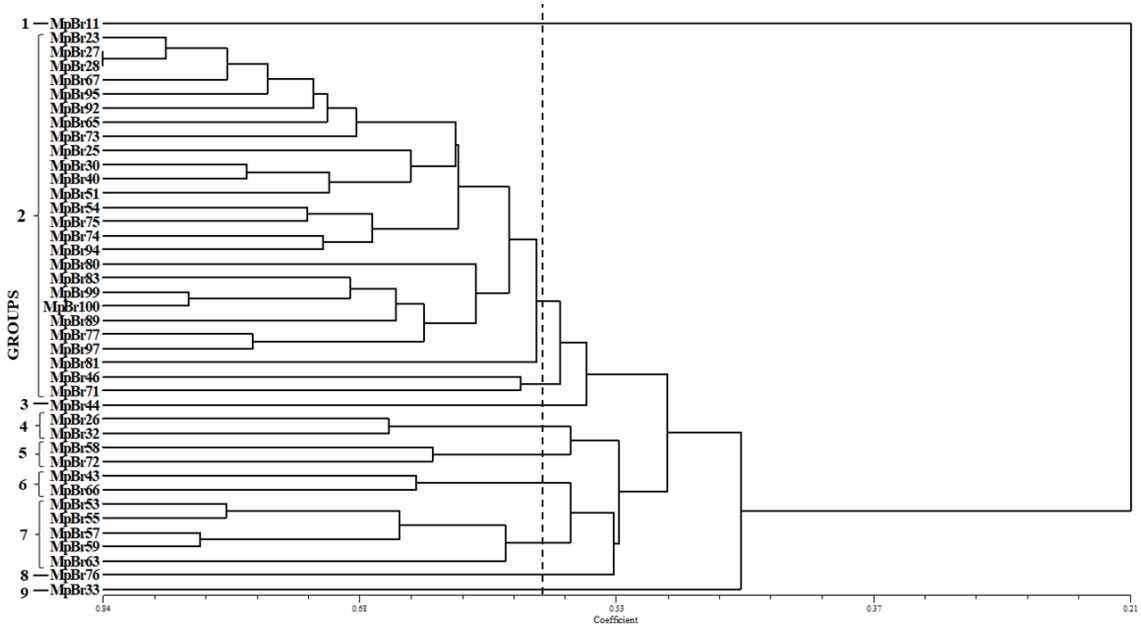

Figure 2. Dendrogram obtained by the UPGMA method using the Jaccard coefficient derived from the RAPD analysis of 41 M. pseudophaseolina isolates.

Moreover, the dendrogram generated by the UPGMA method using both ISSR and RAPD markers separated the 41 *M. pseudophaseolina* isolates into eight groups at 56.2% similarity level (Figure 3). The genetic similarity coefficient ranged from 0.15 to 0.87.

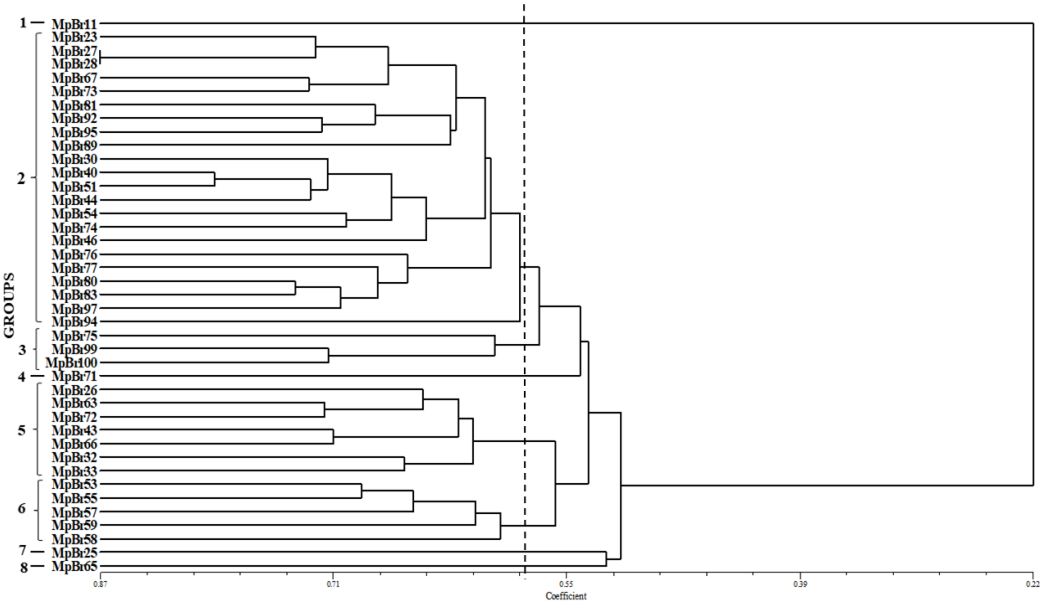

Figure 3. Dendrogram obtained by the UPGMA method using the Jaccard coefficient derived from combination of ISSR and RAPD analysis of 41 M. pseudophaseolina isolates.

Of the 41 isolates, 22 were clustered in Group 2, with 60% similarity on average. Of these 22, seven isolates were obtained from *T. portulacastrum* (four from Assú-RN and three from Mossoró-RN) and 15 from *B. diffusa* (eight from Mossoró-RN and seven from Assú-RN). The second largest group (Group 5) contained four isolates obtained from *T. portulacastrum* (three from Mossoró-RN and one from Assú-RN) and three from *B. diffusa* (all from Assú). Group 6 combined five isolates from Assú-RN, and Group 3 comprised two isolates from Mossoró-RN and one from Assú-RN, all obtained from *B. diffusa*. Groups 1, 4, 7, and 8 contained one isolate each, MpBr11 (from *T. portulacastrum*, Icapuí-CE), MpBr71, MpBr25 (from *T. portulacastrum*, Assú-RN) and MpBr65 (from *B. diffusa*, Assú-RN), respectively. Principal Coordinates Analysis (Figure 4) confirmed clustering in the dendrogram, showing that isolates MpBr11 and MpBr65 are the most genetically distant.

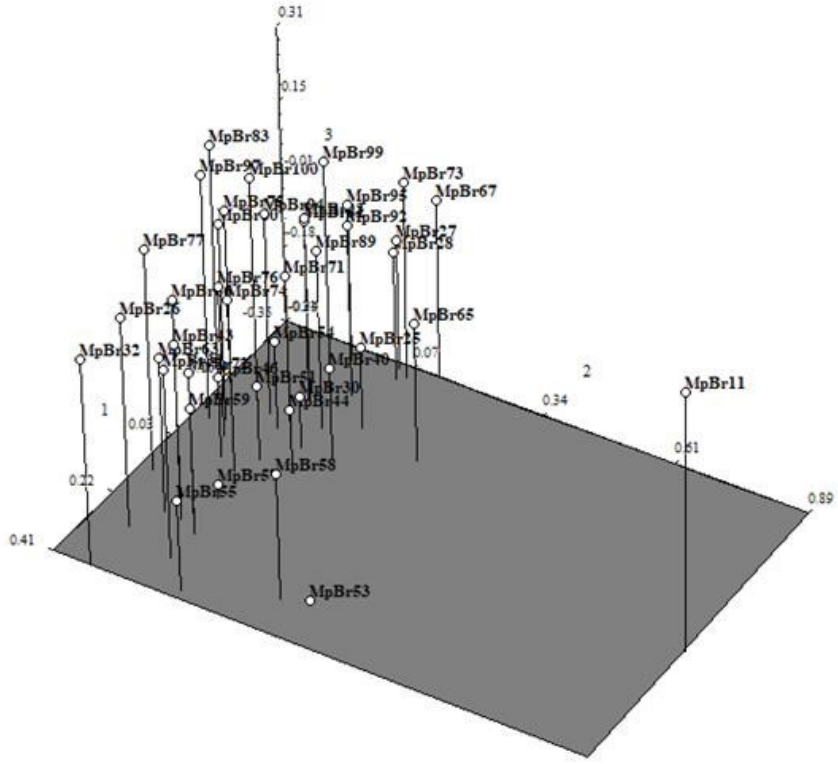

Figure 4. Genetic relationships among 41 M. pseudophaseolina isolates according to the Principal Coordinates Analysis of data from ISSR and RAPD markers.

The high dissimilarity found among the *M. pseudophaseolina* isolates may be related to species specialization to the cultivation conditions of each region (IQBAL; MUKHTAR, 2014). It was evident by the high genetic diversity between the isolate MpBr11 collected from Icapuí-CE and isolates collected from other sites (Mossoró-RN and Assú-RN), despite being obtained from the same weed species (*T. portulacastrum*) (Figure 3).

This ability allows the pathogen to have greater adaptation and survival, which hampers disease management through generalist strategies (GARCÍA et al., 2019; COSER et al., 2017). Also, the high genetic diversity among *M. pseudophaseolina* isolates makes it difficult to develop resistant melon varieties to all isolates (ALMEIDA et al., 2014).

Furthermore, the high genetic diversity among *M. pseudophaseolina* isolates we observed may explain its pathogenic variability in plants (BABU et al., 2010), as well as its ability to adapt to different agroecological conditions. Csöndes et al. (2012) found that the genetic variability of *M. phaseolina* isolates collected from different regions was correlated with the pathogen's ability to grow and develop under different temperature and pH conditions, characteristics that allow it to adapt to different environments and hosts (IQBAL; MUKHTAR, 2014).

In other molecular studies, high genetic diversity among species from the genus *Macrophomina* was found considering different hosts and geographical origins. By PCR-RFLP, Purkayastha et al. (2006) found highly aggressive isolates for beans grouped separately from less aggressive ones. Živanov et al. (2019), using RAPD

markers, observed high variability among *M. phaseolina* isolates collected from different hosts in different countries in Asia and Europe.

Results showed that the RAPD and ISSR markers were effective in detecting genetic diversity among the 41 *M. pseudophaseolina* isolates. The dendrogram obtained with a combination of data showed a significant correlation (r = 0.86) between the genetic similarity matrix and cluster analysis. Furthermore, the EMR obtained with ISSR analysis (11.67) was greater than that obtained with RAPD (6.71) (Table 3), showing the more resolving power of the ISSR marker as compared to RAPD to assess the genetic variability among *M. pseudophaseolina* isolates.

The ISSR and RAPD markers, isolated or associated, could not differentiate the *M. pseudophaseolina* isolates according to their geographical origin and host plant, as observed by Babu et al. (2010) when evaluating the genetic diversity among 50 *M. phaseolina* isolates using RAPD markers. Despite the low correlation between host and geographical origin where the *M. pseudophaseolina* isolates were sampled, a pattern was observed in clusters of isolates from the same origin or host.

The ISSR markers grouped three isolates from *T. portulacastrum* (MpBr25, MpBr27, and MpBr28) and two from *B. diffusa* (MpBr53 and MpBr58) collected in Assú. Also, the same markers grouped eight isolates in Group 2, all from *B. diffusa*, indicating that a clustering pattern can be obtained by molecular markers. Sánchez et al. (2017) and Tarakanta, sharma and Singh (2005) using SSR markers reported that *M. phaseolina* isolates strongly tended to group according to their geographical origin. In contrast, using RAPD markers, clusters of *M. pseudophaseolina* isolates were not associated with the host or geographical origin where the pathogen was sampled (ŽIVANOV et al., 2019).

The greatest similarity (87%) was observed between isolates MpBr27 and MpBr28, both obtained from *T. portulacastrum* collected in Assú. Also, high similarity (79% and 75%, respectively) was observed between isolates MpBr40 (*T. portulacastrum*, Mossoró-RN) and MpBr51 (*T. portulacastrum*, Assú-RN), and between MpBr40 and MpBr30 (both from *T. portulacastrum*, Mossoró -RN). The high similarity between isolates from different geographical origins suggests they share a common ancestor (ALMEIDA et al., 2003), or they were propagated by contaminated soil and seeds among cultivation areas (AGHAKHANI; DUBEY, 2009).

On the other hand, high genetic distances were found between MpBr11 (*T. portulacastrum*, Icapuí-CE) and MpBr65 (*B. diffusa*, Assú-RN) as compared to the other isolates, by the combination of the ISSR and RAPD markers (Figure 3). Such high intraspecific variability in the species complex of the genus *Macrophomina* can be explained by the high mutation rate during the parasexual reproduction of the species (PURKAYASTHA et al., 2006) and high amount of transposable elements and repetitive DNA in the genome (ISLAM et al., 2012).

Search for sources of disease resistance in melon accessions must consider the genetic heterogeneity of the pathogen species, which allows using a uniform population in resistance tests (MUNDT, 2014). Sources of genetic variability directly influence the pathogen adaptation to host resistance genes (CASELA, 2005). Therefore, integrated control methods should be used to avoid selection pressure in the pathogen population.

High genetic variability among *M. pseudophaseolina* isolates, as we observed in the present study, hinders breeding for disease resistance. It directly affects the effective strategy for disease management, since using resistant cultivars is the most efficient and economically viable control method against root rot and vine decline in melon (ALMEIDA et al., 2014). In this study, the isolates MpBr11 and MpB65 stood out for being more genetically distant from the others.

Therefore, for melon cultivars highly susceptible to isolates MpBr11 and MpBr65, developing resistant cultivars is crucial for sustainable management of the disease. Moreover, ISSR and RAPD markers were effective in detecting genetic variability among isolates, assisting in decision making in sustainable management systems. Thus, we suggest that pathogenicity tests be carried out to identify isolates or groups of isolates that cause high productivity losses for the crop.

CONCLUSION

Genetic variability among 41 *M. pseudophaseolina* isolates could be detected using ISSR and RAPD markers, and high intraspecific variability was detected among isolates from the same host and origin or not. Both markers were satisfactory in detecting genetic diversity among the isolates, but ISSR markers showed greater resolving power. Results obtained herein can assist in developing effective control methods against root rot and vine decline in melon.

ACKNOWLEDGMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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