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ORIGINAL PAPER

Pathogenic Variation and Molecular Characterization of *Macrophomina phaseolina*, the Cause of Sesame Charcoal Rot

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ABSTRACT

Macrophomina phaseolina (Tassi) Goid is one of the most important soil-borne fungi that affects sesame and causes charcoal rot disease, with a great economic challenge to sesame growers worldwide. Pathogenic and molecular characterizations of eleven *M. phaseolina* isolates collected from different geographical regions in Egypt were carried out to determine the pathogenicity and genetic diversity. Pathogenicity tests showed a pathogenic variability among the isolates. The identification of *M. phaseolina* isolates was confirmed by a specific primer and analyzed for genetic diversity using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. The correlation between RAPD analysis of *M. phaseolina* isolates and their pathogenicity were estimated. Cluster analysis placed the isolates in two distinct clusters and exhibited clear correlation with their pathogenicity. Analysis of ISSR profiles revealed distinct genetic diversity among isolates and showed different clusters according to the geographical regions, in which close geographic origins tend to group nearly. The present study clearly demonstrated that *M. phaseolina* isolates which were obtained from different geographical regions were highly variable. RAPD and ISSR markers were suitable to reflect the genetic diversity among the studied isolates and could help in DNA finger printing which can be used in future breeding programs of sesame.

Keywords: Sesame, *Sesamum indicum*, *Macrophomina phaseolina*, genetic diversity, DNA, RAPD, ISSR, cluster analysis, molecular markers.

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INTRODUCTION

Sesame (*Sesamum indicum L.*) is grown as an oilseed crop in tropical and subtropical parts of the world (Premalatha *et al*., 2020). Sesame plants get attacked with several diseases during their growing stages causing significant quantitative and qualitative losses in seed and oil yield (Deepthi *et al*., 2014).

Macrophomina phaseolina (Tassi) Goid. causes charcoal rot disease on more than 500 plant species worldwide (Ghosh *et al*., 2018 and Hussien *et al*., 2018). The disease has caused economically critical losses on oilseed plants, particularly on corn, cotton, sesame, and sunflower (Machado *et al*., 2019). Charcoal rot is a destructive soil borne disease that favors high temperatures and dry weather or when plants are stressed by unfavorable environmental conditions (Etebarian, 2006 and Negreiros *et al*., 2019). Hence, a diagnostic tool for identifying the different isolates of this pathogen is critically needed. Variations in microsclerotia size, cultural characteristics, and pathogenicity were used to identify and classify *M. phaseolina* isolates (Aboshosha *et al*., 2007 and Saleh *et al*., 2010).

Molecular markers have been proven to be useful in characterizing and assessing genetic diversity within and across species and populations (Almeida *et al*., 2003; Al-Dhabaan et al., 2018; Mezzomo *et al*., 2018). Random Amplified Polymorphic DNA (RAPD) analysis is one of the most common and convenient molecular marker techniques. It is usually utilized in genetic mapping, taxonomy, and polygenic research (Jana *et al*., 2003; Fuhlbohm *et al*., 2013). In RAPD analysis, genomic DNA is amplified by PCR using short primers with an arbitrary sequence causing multiple amplicons of various lengths which are analyzed by gel electrophoresis, resulting in characteristic fingerprints of genomic DNA. As a result, it detects nucleotide sequence polymorphisms that occur at random throughout the genome (Sucher and Carles, 2008). RAPDs have many benefits, including speed, low cost, and the ability to identify variation in the genome with little genomic DNA as a template (Gajera *et al*., 2010).

Inter simple sequence repeat (ISSR) technique is a PCR-based technique that involves amplification of DNA segment located at an amplifiable distance in between two identical microsatellites repeat regions aligned in opposite

directions. The technique utilizes microsatellites, usually 16–25 bp long. ISSR uses a synthetic nucleotide primer to determine genetic variability and DNA fingerprints. This technique has been used successfully in a variety of taxonomic and genetic diversity studies (Hamza *et al*., 2013). ISSRs are extremely reliable because of high annealing temperature and longer sequence (Yao *et al*., 2008).

Both RAPD and ISSR markers have been successfully used to differentiate several fungi (Bridge, 1998). Furthermore, these techniques are useful for grouping rather than identification. Therefore, a rapid diagnostic test that can exactly identify and detect *M. phaseolina* isolates is required. In fungi, the internal transcribed spacer (ITS) region is an excellent target for phylogenetic study (Ghosh *et al*., 2018). Nevertheless, ITS amplification has indicated that isolates belonged to one single species (Almomani *et al*., 2013).

In plant disease control, accurate diagnosis and early detection of pathogens are critical steps. Species-specific oligonucleotide primers can be used to quickly detect and identify *M. phaseolina* by polymerase chain reaction (PCR) (Babu *et al*., 2007 and Santos *et al*., 2020).

Both morphological examination and molecular techniques were used to characterize isolates of *M. phaseolina* isolated from sesames in different geographical distributions (Babu *et al*., 2007 and Sharma *et al*., 2013). Even though only one species of the genus *Macrophomina* was identified, its different isolates vary in their morphology, such as colony morphology, microscopic examination of microsclerotia, pycnidia, and pathogenicity (Saleh *et al*., 2010). Molecular markers succeeded in assessing the genetic diversity and phylogenetic relationships between various isolates to differentiate and account for their variation in pathogenicity (Purkayastha *et al*., 2004).

The objectives of this study were to detect and confirm the identification of *M. phaseolina* using specific primers and evaluate the relationship between the genetic diversity of *M. phaseolina* isolates and both the geographic origin and pathogenicity testing by using RAPD and ISSR markers techniques.

MATERIALS AND METHODS

Fungal Isolates:

Infected sesame plants, showing charcoal-rot symptoms, were collected from different governorates of Egypt (Table 1). To isolate *M. phaseolina*, five small (0.5 cm) epidermal sections were excised from diseased tissues then sterilized in 75% ethanol for 30 sec, transferred to 2.5% sodium hypochlorite for 30 sec and washed in sterile water for 1 min. Surface disinfested tissue samples were placed on potato dextrose agar (PDA) containing chloramphenicol (0.1 mg/ml). Plates were incubated at 28ºC in the dark for 5 days. The isolated fungi were purified using the hyphal tip techniques then they were identified according to Dhingra and Sinclair (1973).

Table (1): List of *M. phaseolina* isolates from different geographical regions in Egypt.

Isolate's code	Geographical origin	Host
MP-1	Qalyubia	Sesame
MP-2	Beheira	Sesame
MP-3	Sharqia	Sesame
MP-4	Dakahlia	Sesame
MP-5	Giza	Sesame
MP-6	Kafr El Sheikh	Sesame
MP-7	Faiyum	Sesame
MP-8	Beni Suef	Sesame
MP-9	Minya	Sesame
MP-10	Asyut	Sesame
MP-11	Sohag	Sesame

Morphological and cultural characteristics of *M. phaseolina* isolates:

Eleven *M. phaseolina* isolates were grown on PDA medium to study their morphological and cultural characteristics. Agar discs (5 mm) were plugged from 7 days old pure culture and transferred to the center of sterilized Petri dishes containing PDA medium. The plates were incubated at 27°C for 7 days. The experiment was repeated three times, each one consisting of 3 plates. On PDA cultures, morphological properties of pure cultures such as mycelial growth diameter, colony color, and sclerotial development and size were investigated (Abd El-Ghany, 1998). Sclerotia size were measured using a digital camera light microscope after culture slides were prepared with a 25% glycerol solution. For each isolate, the sizes of 10 randomly selected sclerotia were measured, and their means were calculated.

Pathogenicity tests of *M. phaseolina* isolates under greenhouse conditions:

Inoculum of *Macrophomina phaseolina* was prepared using sorghum - coarse sand - water (2:1:2 v/v) medium according to Ibrahim (2006).

Inocula of *M. phaseolina* were mixed thoroughly with sterilized soil of each sterilized pot (30 cm diameter × 27 cm height) at the rate of 3% w/w and covered with a thin layer of sterilized soil. Infested pots were irrigated and kept for 7 days until sowing; non inoculated pots were used as a control. Ten sterilized sesame seeds Giza 32 cv. were sown per pot; five replicates (pots) were used for each treatment. Disease assessment was made 15 and 45 days after planting for pre- and post-emergence damping-off, respectively. The percentage of charcoal rot was estimated, after 90 days from planting and calculations were processed according to Manici *et al*. (1992) as follows:

$$\% \text{ Pre-emergence} = \frac{\text{Number of non - germinated seeds}}{\text{Number of sown seeds}} \times 100$$

$$\% \text{ Post-emergence} = \frac{\text{Number of dead seedlings}}{\text{Number of germinated seeds}} \times 100$$

$$\% \text{ Charcoal rot incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

Genomic DNA extraction:

A 5mm culture plug from a 2-day-old culture of each isolate was grown in the dark at 28°C for 5 days in 250 ml glass bottles containing 50 ml potato dextrose broth (PDB). Mycelia were filtered through Whatman No. 1 filter paper, then frozen in liquid nitrogen, and ground with mortar and pestle. Genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Santa Clarita, CA) according to the manufacturer's instructions. Quantification of the DNA concentration in different samples was done by measuring the optical density at 260 nm and stored at -20°C for further use.

PCR amplification and gel electrophoresis:

DNA samples of each isolate were subjected to molecular analysis by amplifying the genomic DNA in a total volume of 25 µl using 18 RAPD random primers and 15 ISSR primers (Table 2). PCR amplifications were performed using 7 µl of GoTaq® Green Master Mix (2X) (Promega Corporation, Madison, USA), 14 µl of nuclease-free water, 2 µl of primer and 2 µl of DNA template (25-35 ng/ µl). The DNA amplification was carried out using a thermal cycler. Amplification was carried out using initial denaturation at 95 °C for 4 min followed by 45 cycles at 94 °C for 1 min, 35 °C for 1 min, 72 °C for 2 min and final extension step at 72 °C for 5 min.

The amplification product was separated in a 1.5% agarose gel using 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid and 2 mM EDTA pH 8.0). The gel was stained with ethidium bromide (0.50 µg/ml) and visualized under UV to confirm DNA amplification. The amplified fragments were photographed using a UV trans-illuminator and analyzed with a gel documentation system. DNA ladder (100 bp) (GeneDireX, USA) was used as standard DNA.

Table (2): List of used primers and their base sequences according to Purkayastha *et al.*, 2006 and Moulin et al., 2012.

primers	Sequences
RAPD-1	(5´- GGTGCGGGAA-3´)
RAPD-2	(5´- GTTTCGCTCC-3´)
RAPD-3	(5´- GTAGACCCGT-3´)
RAPD-4	(5´- AAGAGCCCGT-3´)
RAPD-5	(5´- AACGCGCAAC-3´)
RAPD-6	(5´- CCCGTCAGCA-3´)
RAPD-7	(5´- CAGGCCCTTC-3´)
RAPD-8	(5´-AGGTGACCGT-3´)
RAPD-9	(5´-AGTCAGCCAC-3´)
RAPD-10	(5´-GTGATCGCAG-3´)
RAPD-11	(5´-CAATCGCCGT-3´)
RAPD -12	(5´-TCGGCGATAG-3´)
RAPD-13	(5´-TCTGTGCTGG-3´)
RAPD-14	(5´-TTCCGAACCC-3´)
RAPD-15	(5´-CCGCATCTAC-3´)
RAPD-16	(5´-GAACGGACTC-3´)
RAPD-17	(5´-AGGGGTCTTG-3´)
RAPD-18	(5´-GAAACGGGTG-3´)
ISSR-1	$TC(GACA)_4$
ISSR-2	$(AG)_8T$
ISSR-3	$(CT)_8T$
ISSR-4	$(CA)_8G$
ISSR-5	$(TG)_8A$
ISSR-6	$(CAG)_5AT$
ISSR-7	$(CAC)_3GC$
ISSR-8	(GT)6CC
ISSR -9	$(AG)_8C$
ISSR-10	$(AC)_8CT$
ISSR-11	$(CT)_8G$
ISSR-12	$(GGAT)_3AG$
ISSR -13	$(ATG)_6$
ISSR-14	$(AC)_8T$
ISSR-15	$(GAA)_6AA$

Molecular characterization:

Genomic DNA of the tested isolates of *M. phaseolina* were extracted and used for molecular identification. Then, PCRs were performed using the universal fungal primers ITS4/ITS5 to amplify the ITS1–5.8S–ITS2 region and specific primers for *M. phaseolina* MpKF1/MpKR1 (5′-CCGCCAGAGGACTATCAAAC-3′/5′-CGTCCGAAGCGAGGTGTATT-3′) located in ITS1 and ITS2 regions, respectively. PCRs were conducted as follows: 5 min at 95°C; followed by 35 cycles of 45 sec at 94°C, 45 sec at 55 or 60°C (depending on the primer pair), and 1 min at 72°C; and a final extension of 10 min at 72°C (White *et al*., 1990). A negative control with all reaction reagents except the DNA template was included with each set of PCR amplification reactions.

Data analysis:

The data obtained from molecular analyses of *M. phaseolina* isolates were analyzed with a gel documentation system. Relative relatedness among isolates was determined using CLIQS v1.1 software. A dendrogram was constructed from the similarity coefficient data by the unweighted pair-group method with arithmetic mean (UPGMA) clustering algorithm.

Statistical analysis:

Comparison means were performed using Duncan's at $p < 0.05$ and the standard error was calculated using the Statistical analysis software "COStat 6.4" (CoStat, 2005).

RESULTS

Morphological and cultural characteristics of *M. phaseolina* isolates:

By using morphological characterization, all the used isolates of M. *phaseolina* showed different phenotypes when grown on PDA medium. Appressed mycelium was observed on the culture plate with microsclerotia imbedded within the hyphae. The color of colonies was white to gray and black (Fig. 1). Mycelial growth diameter of isolates ranged from 65.79 to 95.12 mm.

Some of the isolates had larger sclerotia with higher rates of mycelial growth. The color of the sclerotia was light brown in the beginning which became darker with age and finally brown to black, microsclerotia varied in shape: either irregular, spherical, or oval. Variations were also observed among these isolates regarding the size of their sclerotia. The individual average sclerotia sizes of isolates ranged from 12.68 to 36.80 μm (Table 3). The isolate having the biggest sclerotia size was MP-3 with 36.80 μm, followed by MP-11 with 33.56 μm, MP-9 with 31.45 μm, and MP-10 with 30.87 μm. On the other hand, MP-4 had the smallest sclerotia size with 12.68 μm, followed by MP-5 with 13.01 μm.

Table (3): Morphological variations among isolates of *M. phaseolina*.

Isolate code	Mycelial growth diameter (mm)	Sclerotia size (μm)	Phenotype	Mycelial growth color
MP-1	85.17^{ef*}	13.74^{ef}	Appressed	Gray
MP-2	87.23^{cd}	15.23^{def}	Feathery	Gray
MP-3	95.12a	36.80^{a}	Appressed	black
MP-4	57.98^{j}	12.68^{f}	Feathery	white
MP-5	65.79^{i}	13.01^{f}	Appressed	white
MP-6	84.23^{f}	18.28^{d}	Feathery	Gray
MP-7	86.45^{de}	16.94^{de}	Appressed	Gray
MP-8	79.86^{h}	24.12^{c}	Feathery	Gray
MP-9	88.43^{c}	31.45^{b}	Appressed	black
MP-10	82.51^{g}	30.87^{b}	Feathery	Gray
MP-11	92.08^{b}	33.56^{b}	Appressed	black

*Means in each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan`s multiple range test; **Each value represents the mean of three replicates.

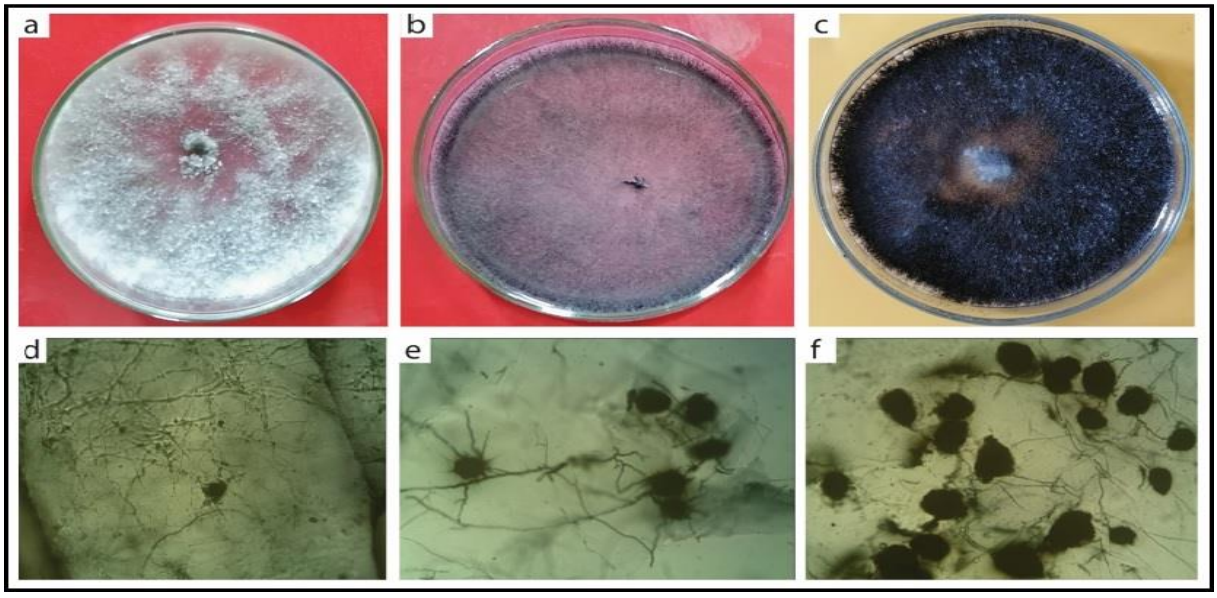

Figure (1): Cultural and morphological characteristics of *M. phaseolina*. (a, b, and c): Colony on the agar plate. (d, e, and f): Different sizes of microsclerotia.

Pathogenic variability of tested *M. phaseolina* isolates under greenhouse conditions:

Pathogenicity of different isolates of *M. phaseolina* showed that all isolates were pathogenic on sesame plants (Giza 32 cv.) and showed significant differences for incidence of pre- emergence, post- emergence damping off and charcoal rot under greenhouse conditions. Data represented in Table (4) indicate that all the tested isolates were divided into three groups according to the degree of pathogenicity. First group showed high pathogenicity including isolates MP-9, MP-3, MP-10 and MP-11 which recorded charcoal rot incidence 63.3, 60, 56.7 and 46.7% respectively. The second group showed moderate pathogenicity including MP-2, MP-5 and MP-7, while the third group showed low pathogenicity for isolates MP-1, MP-4, MP-6 and MP-8.

Table (4): Effect of soil infestation with different isolates of *M. phaseolina* on damping-off and charcoal rot diseases incidence on Giza 32 sesame cv. under greenhouse conditions.

Isolate code	Damping-off %			Standing plants %		Degree of pathogenicity
	Pre-emergence	Post-emergence	Total damping-off	Charcoal rot	Healthy	
MP-1	3.33^{d*}	13.33^{b}	16.76^{c}	10.00^{j}	73.34^{a}	Less pathogenic
MP-2	13.33^{a}	6.67^{d}	20.00^{b}	23.33^{f}	56.67^{c}	Moderately pathogenic
MP-3	6.67^{c}	13.33^{b}	20.00^{b}	60.00^{b}	20.00^{g}	Highly pathogenic
MP-4	3.33^{d}	16.67^{a}	20.00^{b}	16.67^{h}	63.33^{b}	Less pathogenic
MP-5	10.00^{b}	13.33^{b}	23.33^{a}	26.67^{e}	50.00^{d}	Moderately pathogenic
MP-6	13.33^{a}	6.67^{d}	20.00^{b}	6.67^{k}	73.33^{a}	Less pathogenic
MP-7	6.67^{c}	16.67^{a}	23.34^{a}	20.00^{g}	56.66^{c}	Moderately pathogenic
MP-8	3.33^{d}	10.00^{c}	13.33^{d}	13.33^{i}	73.34^{a}	Less pathogenic
MP-9	3.33^{d}	10.00^{c}	13.33^{d}	63.33^{a}	23.34^{f}	Highly pathogenic
MP-10	6.67^{c}	16.67^{a}	23.43^{a}	56.67^{c}	19.99^{g}	Highly pathogenic
MP-11	10.00^{b}	13.33^{b}	13.33^{d}	46.67^{d}	30.00^{e}	Highly pathogenic

*Means in each column followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan`s multiple range test. **Each value represents the mean of three replicates.

Confirmation of molecular identification of *M. phaseolina* isolates by specific primers:

The isolates were identified at the molecular level using species specific primers (MpKF/MpKR) as designed by Babu *et al*. (2007). A fragment of 350-bp was amplified by the primer pair in tested isolates (Fig. 2). The result showed that the isolates collected belonged to the species *M. phaseolina*. No fragment was detected in other fungi used as negative control.

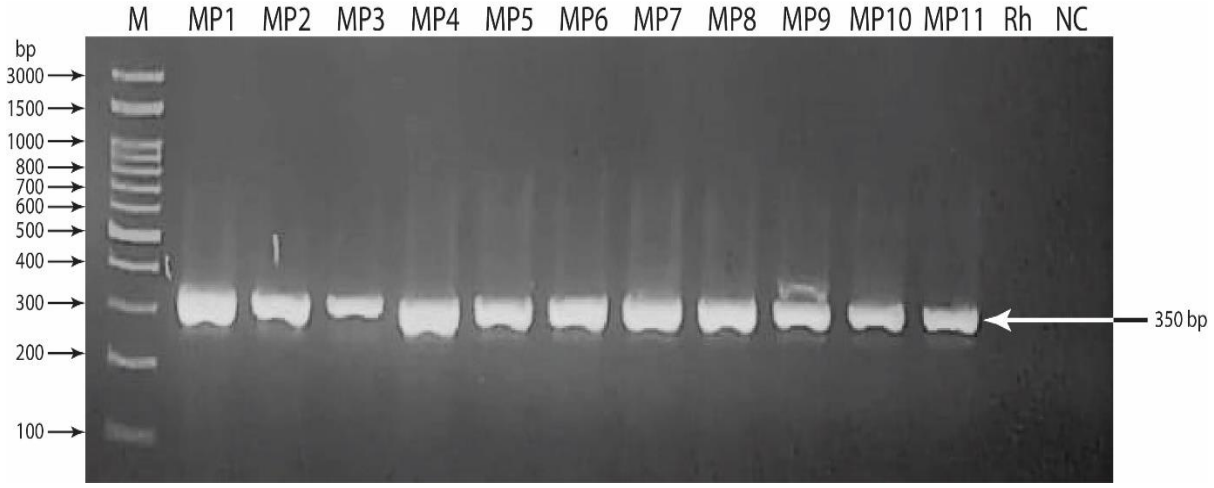

Figure (2): PCR Amplification using specific primer (MpKFI/MpKRI), M: 100bp molecular ladder; 1-11: *M. phaseolina* isolates; Rh: *Rhizoctonia solani* is negative control; NC: negative control.

PCR amplification of ITS region:

Data in Fig. (3) show that the genomic DNA amplification of ITS region of different isolates of *M. phaseolina* using ITS primers produced one fragment of approximately 650 bp.

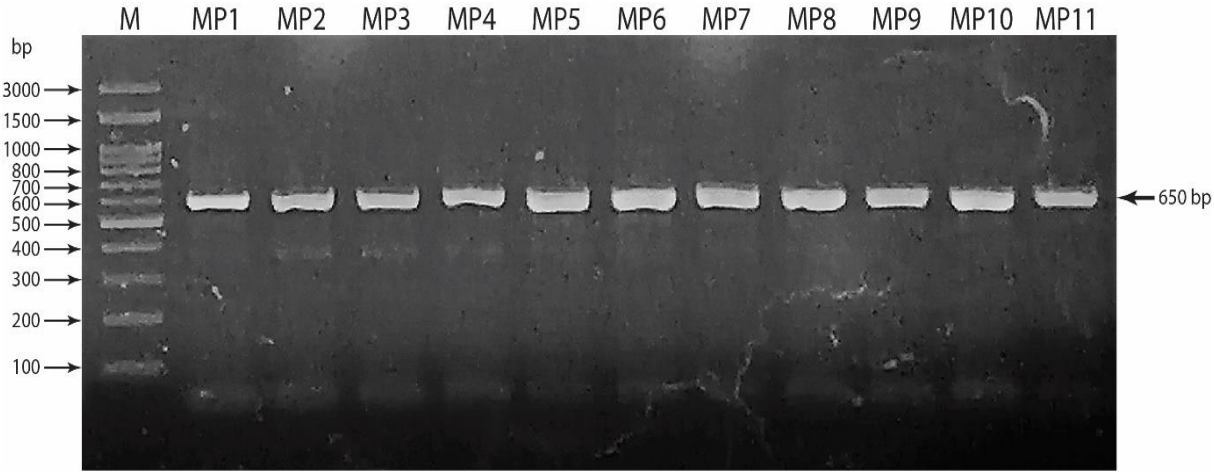

Figure (3): PCR Amplification using Primers ITS-4 and ITS-5 M: 100bp molecular ladder; 1-11: *M. phaseolina* isolates.

RAPD fingerprinting:

RAPD analysis that was carried out on the eleven tested *M. phaseolina* isolates showed genetic variation among the isolates. All 18 random primers yielded amplification products in most of the isolates. Out of the 18 primers used for amplification, primer RAPD-3 showed 80% polymorphism among isolates followed by primers RAPD-5, RAPD-9 and RAPD-12 which showed 70% polymorphism. These primers generated simple banding patterns that revealed DNA polymorphisms among *M. phaseolina* isolates (Fig. 4). The number of scorable bands for corresponding primers ranged from 1 to 8 with an average of six bands with molecular weight ranging approximately from 200 bp to 1500 bp. RAPD profiles were subjected to UPGMA analysis (Fig. 5). Cluster analysis produced by Primers 3, 5, 9 and 12 revealed that all isolates of *M. phaseolina* are divided into two clusters, the first cluster included the highly pathogenic isolates, whereas the second cluster contained the moderately pathogenic isolates and the less pathogenic isolates.

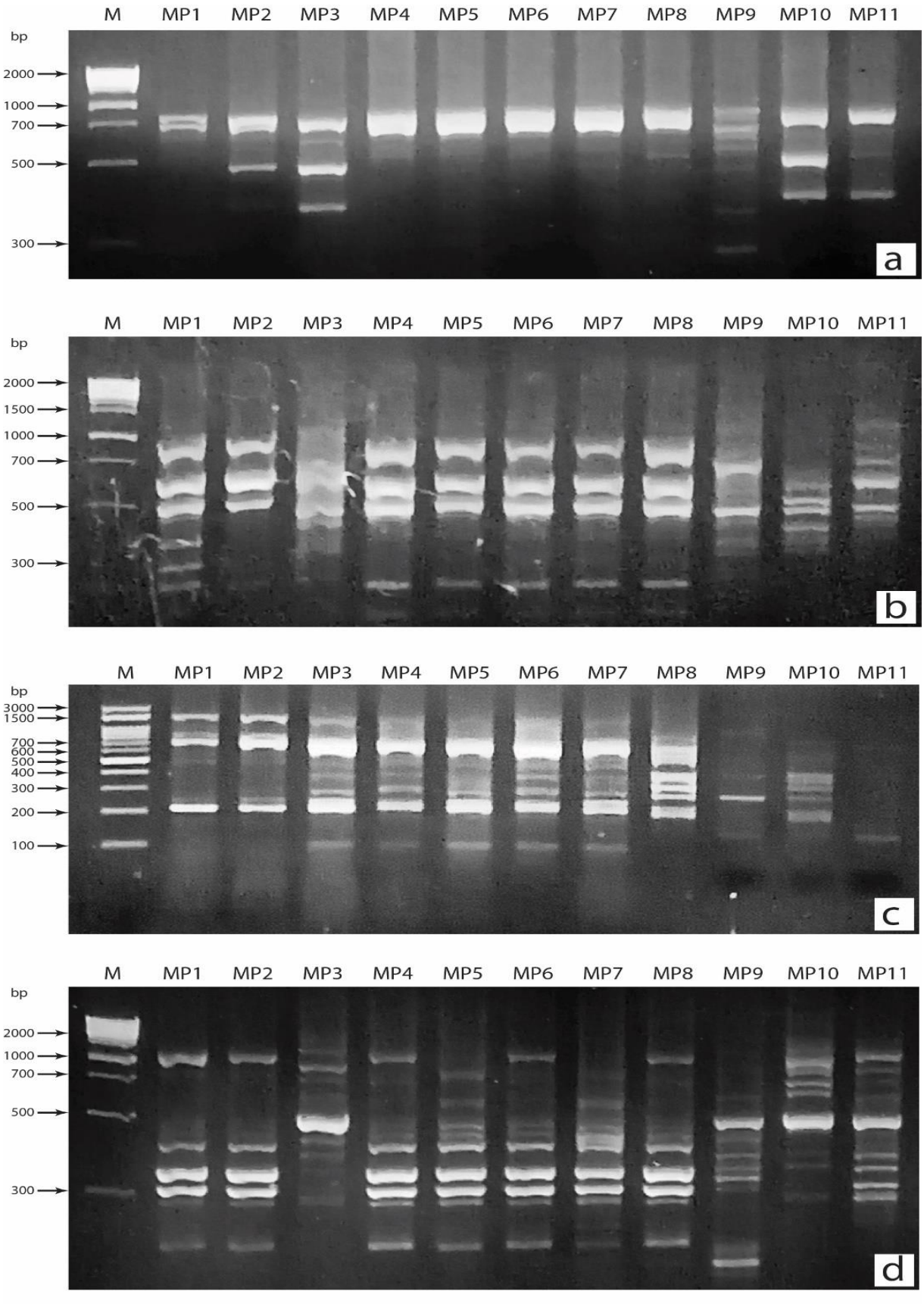

Figure (4): RAPD profiles of eleven different isolates of *M. phaseolina* obtained with random primers (a) RAPD-3, (b) RAPD-5, (c) RAPD-9 and (d) RAPD-12. lanes 1–11 represent the amplicon profile of isolates and M: molecular weight marker.

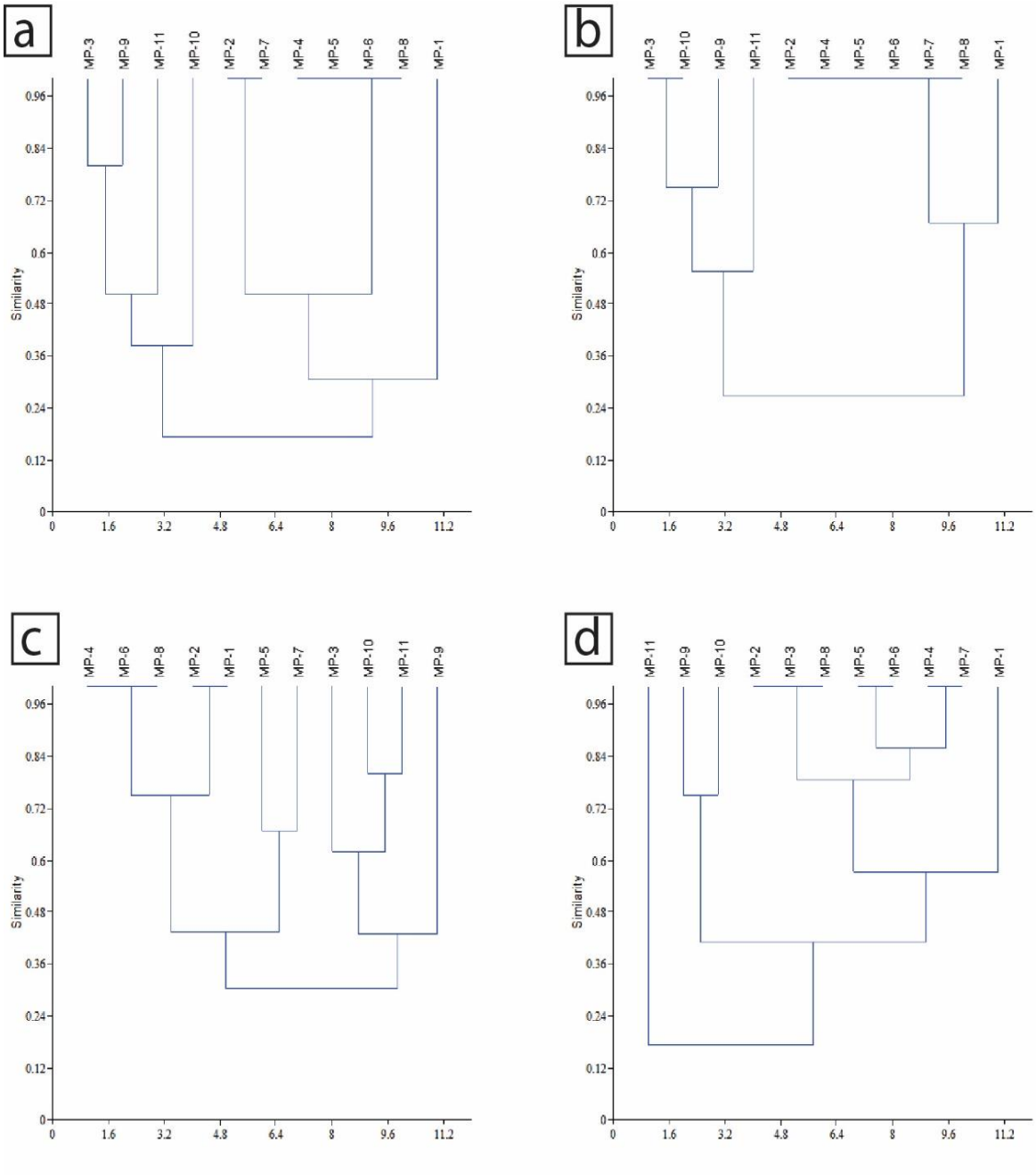

Figure (5): Dendrogram obtained from 11isolates of *M. phaseolina* with Unweighted pair-group method with arithmetic mean (UPGMA) clustering method based on Jaccards coefficient. Distance is based on genetic similarity coefficient.

ISSR Polymorphism:

All the used 15 ISSR primers successfully generated amplifications with isolates. Four representative primers (ISSR-3, ISSR-6, ISSR-10 and ISSR-14) yielded reproducible and polymorphic amplification patterns on agarose gel. Each reproducible primer amplified 1-8 bands and revealed an average number of 5 bands per ISSR primer. The band size ranged between 200 to 2,000 bp, and 85% of the bands were revealed as polymorphic in these isolates (Fig. 6).

UPGMA clustering of ISSR data produced a dendrogram that could separate the eleven isolates into two groups at an arbitrary level of 96% similarity (Fig. 7). Dendrogram results showed that the M. *phaseolina* isolates were clearly differentiated to the specific group according to the geographical origins, however, usually the isolates from nearly geographic origin tend to group closely.

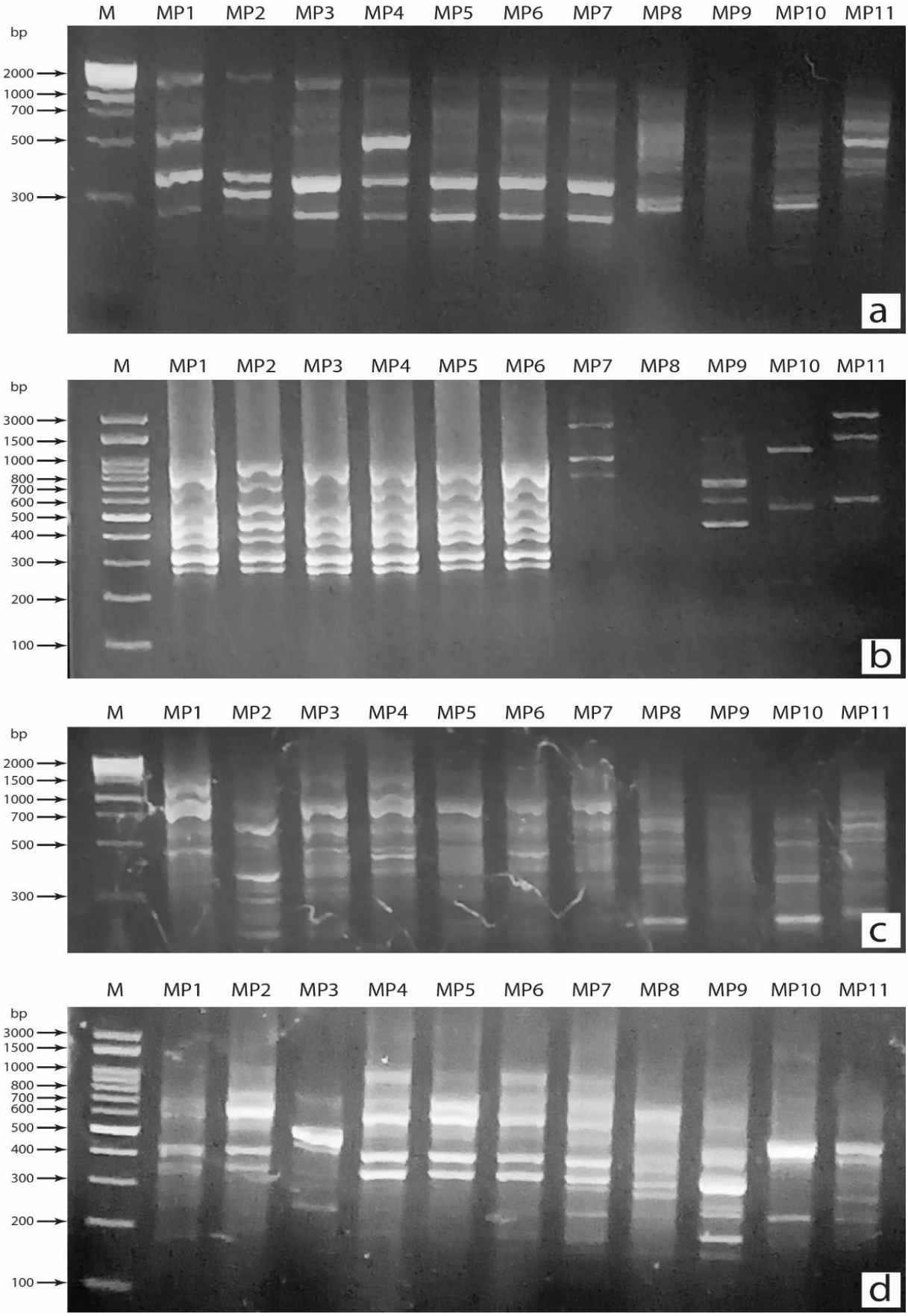

Figure (6): DNA banding patterns of eleven *M. phaseolina* isolates obtained with the ISSR primer (a) ISSR -3, (b) ISSR -6, (c) ISSR -10 and (d) ISSR -14. lanes 1–11 represent the amplicon profile of isolates and M: molecular weight marker.

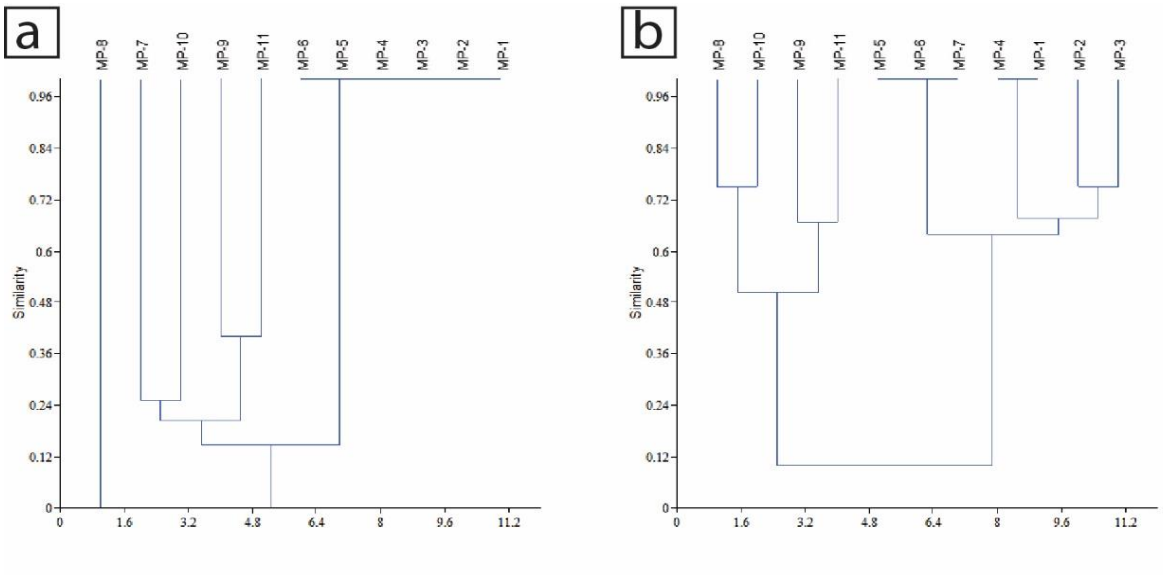

Figure (7): Dendrogram obtained with UPGMA from the Jaccard dissimilarity matrix of eleven isolates of *M. phaseolina* derived from ISSR analysis. Distance is based on genetic similarity coefficient.

DISCUSSION

In this study, *M. phaseolina* sesame isolates obtained from eleven different geographical regions in Egypt have been characterized through morphological characteristics, pathogenic variations, and molecular characterizations. In this respect, the obtained results showed some cultural variations among the isolates; however, morphological characteristics of the *M. phaseolina* isolates showed a wide range of diversity in colony color and colony sizes. The variation in mycelial growth and sclerotia diameter were statistically significant. This diversity indicated that this pathogen has improved its ability to adapt to a variety of environments and overcome grower-implemented host resistance (Trigiano *et al*., 2008), since environmental factors such as temperature, moisture, and soil types, as well as interactions with products of unknown genes in the genetic context, may alter these phenotypes (Marquez *et al.,* 2021). Iqbal and Mukhtar (2014) found morphological variability across various isolates of *M. phaseolina* on multiple hosts in terms of growth, color, and pycnidium production. Similarly, Beas-Fern´andez *et al*. (2006) observed differences in morphology and pathogenicity among *M. phaseolina* isolates from various hosts as well as different parts of the same host.

The experiment of the pathogenicity demonstrated that all the isolates showed statistically significant variation in pathogenicity on sesame. *M. phaseolina,* has a wide variety of hosts and exits in two asexual forms which maintain its survival better (Almeida *et al*., 2003). *M. phaseolina* can survive in soil for two or more years in the absence of a host, depending on soil conditions (Gupta *et al*., 2012), which makes charcoal rot a difficult disease to control. Primary inoculum comes from seed, soil, and plant debris, and the severity of the disease is proportional to the amount of survived sclerotia in the soil (Ijaz *et al*., 2013). The present study proved that there is a relationship between the morphological characteristics and pathogenicity of the isolates. The highly pathogenic isolates of *M. phaseolina* MP-3, MP-9, MP-10 and MP-11 against sesame, produced large sized sclerotia. Likewise, isolates MP-1, MP-4, MP-6, and MP-8 which produced low sized sclerotia showed less pathogenicity against sesame. A similar pattern was observed in moderately pathogenic isolates.

The morphological heterogeneity might well be due to the fungi responses to environmental stimuli or variation in their hosts (Tok, 2019 and Pandey *et al*., 2020). Similarly, a high correlation between virulence and morphological variations has been reported by Tok (2019).

This finding is also consistent with the work of Edraki and Banihashemi (2010) and Purkayastha *et al*. (2004) who reported a relationship between morphological variations and pathogenicity. On the contrary, Beas-Fern´andez *et al*. (2006) and Iqbal and Mukhtar (2014) reported that pathogenicity has no relation with the size and weight of sclerotia.

(ITS) regions of the ribosomal RNA genes possess characteristics that allow pathogen

identification (Mahmoud and Zaher, 2015). ITS sequencing of the 18S rRNA region has previously been used to identify *M. phaseolina* from a variety of hosts (Babu *et al*., 2007; Romanelli *et al*., 2014 and Khan *et al*., 2017). The ITS region was amplified using ITS1 and ITS4 primers for the eleven isolates, and the amplified PCR products produced one fragment of approximately 650 bp. Furthermore, the primers, MpKF1 and MpKR1 designed from the conserved sequences of the ITS region were highly specific and yielded a specific 350 bp products. In the present study, all isolates belonged to the species *M. phaseolina*. Since this 350 bp amplicon was absent from other soil-borne pathogens, it can be used for the species-specific identification of *M. phaseolina* (Babu *et al*., 2007).

To develop disease control systems and sesame breeding programs against charcoal rot, genetic variation within and between species must be understood (Salahlou *et al*., 2019). Molecular markers are ideal tools for assessing variation quickly within and among species (Sharma *et al*., 2013). In the present study, RAPD analysis clearly indicated high polymorphism among the *M. phaseolina* isolates from different geographical origins possessing different pathogenicity on sesame. The obtained molecular polymorphism was largely independent of geographical origin. Polymorphism generated by cluster analysis using the UPGMA method was correlated with the variation observed in the pathogenicity of isolates. The highly pathogenic isolates were separated in a different cluster from the less pathogenic. Isolates in a cluster which were collected from different geographical regions might share the same pathogenicity. In other molecular studies, high genetic diversity among isolates of *Macrophomina* was found considering different hosts and geographical origins. This is in agreement with Purkayastha et al. (2006) who found highly aggressive isolates for beans grouped separately from less aggressive ones, using PCR-RFLP. Živanov *et al*. (2019), when using RAPD markers, observed high variability among *M. phaseolina* isolates collected from different hosts in different countries.

Furthermore, the high genetic diversity among *M. phaseolina* isolates may be due to its pathogenic variability in plants as well as its ability to adapt to different agroecological conditions (Babu *et al*., 2010). Csondes *et al*. (2012) found that the pathogen's ability to grow and evolve under varying temperatures and pH conditions, cropping patterns, and transportation of the pathogen, these characteristics make it able to adapt to different environments and hosts, which are correlated with the genetic diversity of *M. phaseolina* isolates, which were obtained from different regions (Aghakhani and Dubey, 2009; Baird *et al*. 2010 and Iqbal and Mukhtar, 2014).

Similar results were reported by Khan *et al*. (2017), and Mahdizadeh *et al*. (2011) who indicated that different *M. phaseolina* isolates could not clearly be grouped according to geographical origins. This is in disagreement with the findings of Mayék-Pérez *et al*., (2001) and Jana *et al*., (2005) who reported that molecular markers could helpfully be used to group *M. phaseolina* isolates based on their geographical regions.

(ISSR) or microsatellites have been shown to be one of the most effective genetic markers in mycology (Bahkali *et al*., 2012). Microsatellite markers have been generated for several plant pathogenic fungi (Arias *et al*., 2011 and Mahdizadeh *et al*., 2012). In this study, ISSR genomic fingerprinting revealed a high level of heterogeneity among *M. phaseoina* populations. It is clear from the cluster analysis that isolates from closely geographical origins tend to group together, whereas in some cases they were placed in different clusters. The results of this study correspond with Mahdizadeh *et al*. (2011) who reported that isolates from the same host or the same geographic origin tend to group nearer.

Sánchez *et al*. (2017) and Tarakanta, *et al*. (2005), using SSR markers, reported that *M. phaseolina* isolates strongly tended to group according to their geographical origin. However, by using RAPD markers, clusters of M. pseudophaseolina isolates were not linked to the host or the location where the pathogen was detected (Živanov *et al*., 2019).

RAPD and ISSR profiling techniques may provide useful information on the level of polymorphism and diversity in sesame isolates of *M. phaseolina*. The higher level of polymorphism detected by RAPD markers than with ISSR reveals the discriminating ability of both markers. The presence of genetic variants represented by the number of alleles at a locus and their frequency of distribution in a population is often the cause of polymorphism in each population (Powell *et al*., 1996). The disparity in resolution between RAPDs and ISSRs is most likely due to the fact that the two marker approaches target different parts of the genome (Bhattacharya *et al*., 2010).

CONCLUSION

The increase in charcoal rot disease incidence still poses a major challenge and a great economic threat to important crops, even though chemicals and fertilizers are being widely used. Therefore, molecular characterization of *M. phaseolina* can help the understanding of the population genetics and biological parameters. The understanding of existing variability can be efficiently utilized to manage this disease, improve sesame yield, and help sesame breeding programs.

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