Egyptian Journal of Phytopathology, Vol. 49, No. 2, pp 1-9 (2021)
DOI 10.21608/ejp.2021.81678.1038

ORIGNAL PAPER

Pathological and Molecular Characterization of *Magnaporthiopsis maydis* Isolates Causing Late Wilt in Maize

Agag, S.H. [1] ***; Sabry, A.M**[1]**.; EL-Samman, M.G.**[2] **and Mostafa, M.H.**[2]

Received: 21 June 2021 / **Accepted**: 09 July 2021 / **Published online**: 13 July 2021.
© Egyptian Phytopathological Society 2021

ABSTRACT

In this study, sixteen isolates of *Magnaporthiopsis maydis* were isolated from infected maize (*Zea mays* L.) plants collected from different governorates in Egypt. These isolates were identified at the molecular level using a specific primer. All isolates have the same growth pattern form (rhizoid), growth elevation (raise) and growth margin (filiform) on PDA medium but differed in color. The faster isolate in growing on PDA medium was isolate S3 while the slower one was A2. The analysis of variance showed significant differences among isolates. The pathogenicity test was carried out under greenhouse conditions using a Single cross pioneer 3062 hybrid. Isolates F13 and F14 were highly aggressive (33.33%), while isolate Mi5 was the lowest (10%). No correlation was detected between disease incidence and radial growth of *M. maydis* isolates. Genetic diversity among isolates was studied using six RAPD markers and showed a high similarity percentage (95%) between isolate F13 and F14 and between isolate F14 and F15.Whereas, the lowest similarity percentage (26%) was between isolate A2 and KS11. The Cluster analysis using the Dice coefficient divided the studied isolates into five clusters. Cluster 1 contains isolates nos. F14, F15, F13, B12, KS7, and KS16, cluster 2 contains isolates nos. F9, M10, Mi5, and M8, cluster3 contains isolates nos. S3, KS6, and F1-B4, cluster 4 contains isolate number KS11 and cluster 5 contains isolate number A2.

Keywords: Maize, *Zea mays, Magnaporthiopsis maydis*, Late wilt, morphological characterization, RAPD marker.

***Correspondence:** Agag, S.H.
sayedagag82@gmail.com
Sayed H. Agag

https://orcid.org/0000-0002-9394-8866
Ahmed M. Sabry
1- Maize and Sugar Crops Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.
Magdy G. EL-Samman
Mostafa H. Mostafa
2- Plant Pathology Department, Faculty of Agriculture, Ain Shams University P.O. Box: 68, Hadayek Shobra, 11241, Cairo, Egypt.

INTRODUCTION

Late wilt is the most important disease in maize (*Zea mays* L.) grown area in Egypt (Sabet *et al.,*1966 and Fahmy and Mahmoud, 2001). Symptoms first become apparent before tasseling until shortly before maturity (EL-Shafey and Claflin, 1999). The disease symptoms are characterized by the leaves at first become dull green generally showing symptoms of water deficiency and finally turn yellow and look withered and dried up (Abd EL-Rahim *et al.*,1998). Meanwhile, elongated streaks varying in color intensity from yellow through reddish brown to red appeared on the still green stalks and if an infected stalk - cut open lengthwise at this stage, yellow to brown streaks are observed extending into several internodes (EL-Shafey and Claflin, 1999). In advanced cases of infection, the lower internodes become shrunken and hollow. Root tips of the infected plants are stained red at an early stage of the disease development and later on the reddish color spreads along with the fibrous roots several of which become hollow and show decayed. In extreme cases of infection, no cob formation occurs and if any grain is formed it is shrunken and poorly developed (Sabet *et al.,*1961 and Degani and Goldblat, 2014). Late wilt appeared in many countries for example: India in 1969 (Ward and Bateman, 1999), Israel in 2011 (Drori *et al*., 2013), Portugal in 2008 and Spain in 2003 (Molinero-Ruiz and Melero-Vara, 2010). The causal organism is known as *Harpophora maydis* synonym *Cephalosporium maydis* (Ward and Bateman, 1999). They mentioned that methods of spore production in *C. maydis* appear to be typical of *Phialophora* rather than *Cephalosporium*, so *C. maydis* is close to the *Gaeumannomyces-Phialophora* complex. So a distinct group of species formerly included in Phialophora comprises the anamorphs of *Gaeumannomyces* and

Magnaporthe. The introduction of a new genus for this group was appropriate because the anamorphs are morphologically sufficiently distinct (Gams, 2000). Freeman and Ward (2004) proposed reclassification of *Phialophora* spp. to genus *Harpophora* containing the related fungus *C. maydis*. Based on AFLP, Saleh and Leslie (2004), hypothesized that *C. maydis* is a distinct species in the Gaeumannomyces-Harpophora species complex. In 2013, Luo and Zhang introduced Magnaporthiopsis as a new genus in Magnaporthaceae (Ascomycota). Finally, *C. maydis* is reclassified as *Magnaporthiopsis maydis* (Klaubauf *et al.,* 2014). From the year of identification, the causal organism of late wilt in maize, which was reported in 1963 until 2003, there is no specific primer used to identify the *M. maydis* at the molecular level then, Saleh and Leslie (2004) introduced the first specific primer for *C. maydis* derived from AFLP fragment EAA/MCG-5 amplifies a 300 bp by sequence unique to *H. maydis*. Drori *et al*., (2013) modified the technique for molecular identification by choosing a new set of primers (200 and Am 42/43) using a different reaction mixture. This new sequence is a major part of a large AFLP fragment previously proved to be Species - specific. Random amplified polymorphic DNA (RAPD) is based on the fact that any random sequence of bases is expected to complement to same part of a target genome (Moor and Frazer, 2002). The phylogenetic classification is afield which polymorphism in random molecular marker between isolates has been useful. Phylogenetic tree is valuable both for identifying the relative time and origin of particular taxa and also for analysis of the extent of divergence within groups of organisms (Dickinson,2003). So, RAPD-PCR can detect variation between fungal isolates (Moor and Frazer, 2002). Fewest on the diversity of *M. maydis* using RAPD marker were done (EL-Kazzaz *et al*., 2003; Abd-Rabboh, 2006 and Ashour, 2014). The objectives of the present investigation were to study the pathological and molecular variations of *Magnaporthiopsis maydis* isolates in Egypt.

MATERIALS AND METHODS

Isolation of the maize late wilt pathogen (*Magnaporthiopsis maydis*):

Isolation of fungi associated with diseased maize plants showing typical late wilt symptoms, collected from different maize fields of seven governorates namely Beheira, Kafr-Elsheikh, Menoufia, Fayoum, Minya, Asyut and Sohag in Egypt. The surface of the second internode above the ground of each plant was sterilized by 70% ethanol then flamed as mentioned by Samra *et al*., (1966). Four pieces of plant pith were cultured on Potato dextrose Agar (PDA) medium in Petri plates containing 5gm yeast amended with streptomycin sulfate (100ug/ml) then incubated at 27°C for 6 days. Hyphal tips were taken from the developed colonies and transferred to PDA slants. The pure culture of each isolate was maintained on PDA slants in the refrigerator till further use. Identification of the obtained isolates was preliminary carried out based on taxonomic criteria for *C. maydis* as described by Samra *et al.,* (1971).

Molecular identification of *M. maydis* isolates:

DNA isolation:

Sixteen isolates of *M. maydis* were cultured separately on the PDA medium in Petri plates and incubated at 27°C for 6 days. Then seven mm discs were cut from each plate inoculated into 25 ml conical flask containing liquid PD medium to obtain fungal mass. After incubation for 6 days at 27°C the fungal mass was washed with distilled water and dried through Whatman filter paper No.102 and ground in a mortar using liquid nitrogen to obtain fine powder for each isolate and stored at -20°C until extraction. The extraction was carried out using a Bio-basic DNA extraction kit. DNA was measured using Thermo scientific nanodrop 2000.

PCR reaction and conditions of specific primer:

A200 bp of specific primer, forward (CCGACGCCTAAAATACAGGA) and reverse (GGGCTTTTTAGGGCCTTTTT) was used in the current study. PCR reaction was done in 20 µl total volume of four µl master mix (soles), one µl of each primer, three µl of 85 ng DNA and 11µl double distilled water. Applied biosystems 2720 thermal cycler was used. The cycles of PCR condition were, 94°C for two minutes, 35 cycles of 94°C for 30 second, 55°C for 30 second and 72°C for one minute and finally 72°C for five minutes (Drori *et al.*, 2013).

Gel electrophoresis:

A 15 µl of PCR product were loaded onto 1.7% agarose gel (Bio-basic Inc. Canada) containing 5µl of Ethidium bromide (10 mg /mL^{-1}) in electrophoresis marine (13 × 16 cm) containing TBE buffer. PCR product was run for

two hours at 60 volts. The first lane of each comb was loaded with Thermo Scientific O Gene-Ruler Ready to use 100bp plus DNA ladder containing 14 discrete DNA fragments ranging from 3000bp to 100bp (3000- 2000- 1500-1200- 1000- 900- 800- 700- 600- 500- 400- 300- 200 - 100).

Gel image:

After the DNA fragment runs in the gel for two hours at 60 volts, the gel was exposed to UV light via Hero lab UVT-40 S/L transilluminator, image was captured manually by Sony Cyber-shot camera.

Cultural characterization and mycelial growth of *M. maydis* isolates:

Seven mm disk of six days old of *M. maydis* isolates grown on PDA media were transferred in the center of 12 cm Petri dishes containing PDA medium. The Petri dishes were incubated at 27°C for 6 days. Three replicates were used for each isolate. The radial growth was measured every two days, starting from the second day from incubation to the sixth day of incubation. (Rashid, 2013).

Pathogenicity test of *M. maydis* isolates:

The inoculum of *M. maydis* isolates was prepared using a 500ml glucose glass bottle containing 50 g of sorghum grains soaked perversely overnight in a sufficient amount of water and then the bottles were autoclaved. Three bottles for each *M. maydis* isolate were separately inoculated and incubated at room temperature for about 7-14days to obtain sufficient growth. Soil infestation was done by mix 50g of each isolate inoculum with the surface layer of clay soil in 25 cm diameter pots as described by EL-Shafey *et al*., (1985); Sabet *et al*., (1970) and Samra *et al*., (1971). Single cross pioneer 3062 hybrid was used in this greenhouse study, four replicates were used per isolate and five seeds were sown in each pot. Disease incidence was recorded up to 110 days after sowing.

Diversity of *M. maydis* isolates using RAPD Primers:

A set of six RAPD primers (Table1) was used to elucidate diversity for *M. maydis* isolates following method of Singh *et al.,* (2005).

PCR reaction and conditions:

PCR reaction was done in 20 µl total volume of four µl master mix (soles), one µl primer, one µl of 85 ng DNA and 14 µl double distilled water. Applied biosystems 2720 Thermal cycler was used. The cycles of PCR were, 94°C for two minutes, 35 cycles of 94°C for one minute, 37°C for one minute and 72°C for two minutes and finally 72°C for seven minutes.

Table (1): Primer's code and sequence used in RAPD study.

Primer code	(G+C)100%	Sequence
1	70	CACGGCGAGT
2	60	GTCGATGTCG
3	70	AAGCCTCCCC
4	70	CGTCGCCCAT
5	60	GGGTTTGGCA
6	60	AGCGAGCAAG

Gel electrophoresis:

A15 µl of PCR product were loaded onto 1.7% agarose gel (Bio-basic Inc. Canada) containing five µl of Ethidium bromide (10 mg/ mL^{-1}) in electrophoresis marine (13 × 16 cm.) containing TBE buffer. PCR product was run for two hours at 60 volts. The first and the end lanes of the comb were loaded with Thermo Scientific O Gene-Ruler Ready to use 100bp plus DNA ladder containing 14 discrete DNA fragments ranging from 3000bp to 100bp (3000- 2000- 1500- 1200- 1000- 900- 800- 700- 600- 500- 400- 300- 200 - 100).

Gel image:

Image was captured manually by Sony Cyber-shot camera as described before.

Statistical analysis:

Disease incidence was recorded as the percentage of plants showing late wilt infection so- called binomial distribution rather than the normal distribution, so the arcsine transformation was the appropriate for this type of data (Clewer and Scarisbrick, 2001) and this transformation was used to make means and analysis of variance (ANOVA). For molecular studies, every scorable band was considered a single locus in RAPD, zero one data matrix was generated by considering the present band as one and the absent band was zero. Dendrogram of cluster analysis for RAPD molecular markers and similarity matrix was calculated using Dice coefficient. Statistical analysis was done using SPSS program version 15.

RESULTS

Isolates of *M. maydis*:

Isolation trails were carried out from wilted maize plants collected from seven governorates (Table 2). The isolated fungi were purified and identified according to Samra *et al.,* (1971).This preliminary identification was carried out according to their cultural and morphological

characteristics. Out of the isolated fungi, sixteen isolates were identified as *Magnaporthiopsis maydis*, Klaubauf *et al*., (2014).

Table (2): Isolates of *M. maydis* and locations of collection.

Governorate	No. of isolates	Isolate's code
Beheira	2	B4-B12
Kafr-Elsheikh	4	KS6-KS7-KS11-KS16
Menoufia	2	M8-M10
Fayoum	5	F1-F9-F13-F14-F15
Minya	1	Mi5
Asyut	1	A2
Sohag	1	S3

Molecular identification of *M. maydis* isolates:

All the 16 isolates of *M. maydis* isolated from diseased plants collected from seven different governorates were identified at the molecular level using a specific primer. All isolates had the same molecular weight (200bp) which is specific for *M. maydis*. (Figure1)

Cultural characterization of *M. maydis* on PDA medium:

All isolates of *M. maydis* showed the same growth pattern form (rhizoid), growth elevation (raise) and growth margin (filiform) but differed in cultural color. The majority of isolates under study have shown whitish color. Whereas only one isolate was white grayish color (F15) and five isolates exhibited dark olive color (A2, B4, KS6, F9 & M10).

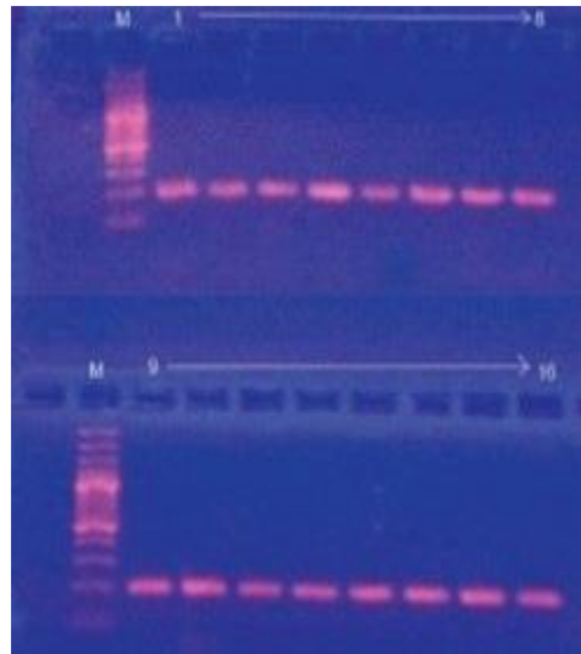

Figure (1): Gel electrophoresis for 16 isolates of *M. maydis*, upper comb exhibits isolate from 1 to 8 while lower comb exhibits isolate from 9 to 16. The first lane of each comb was loaded with 100 bp of DNA ladder.

Mycelial growth of *M. maydis* isolates:

Data in Figure (2) indicate that there is a variation in mycelial growth of the 16 isolates of *M. maydis* on PDA medium. The fastest growing isolate was S3 while the slowest one was A2. The analysis of variance showed significance among isolates, the calculated F value was 9.613 ($p< 0.05$).

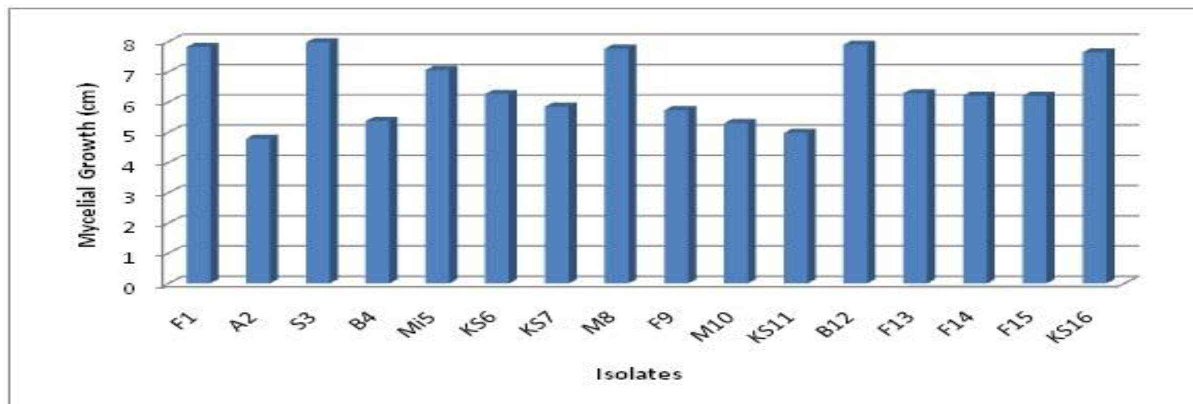

Figure (2): Mycelial growth (cm) of *M. maydis* isolates after six days incubation on PDA in petri plates at 27°C.

Pathogenicity test of *M. maydis* isolates:

A single cross 3062 maize hybrid was used in the greenhouse experiment to elucidate the pathogenic variations among 16 isolates of *M. maydis*. Isolates F13 and F14 were highly aggressive isolates 33.33%, while the Mi5 isolate was the lowest 10%. The analysis of variance showed no significant differences among isolates and the calculated F value was 1.711 at ($p =0.081$). (Figure 3) There was no correlation between disease incidence and radial growth of *M. maydis* isolates.

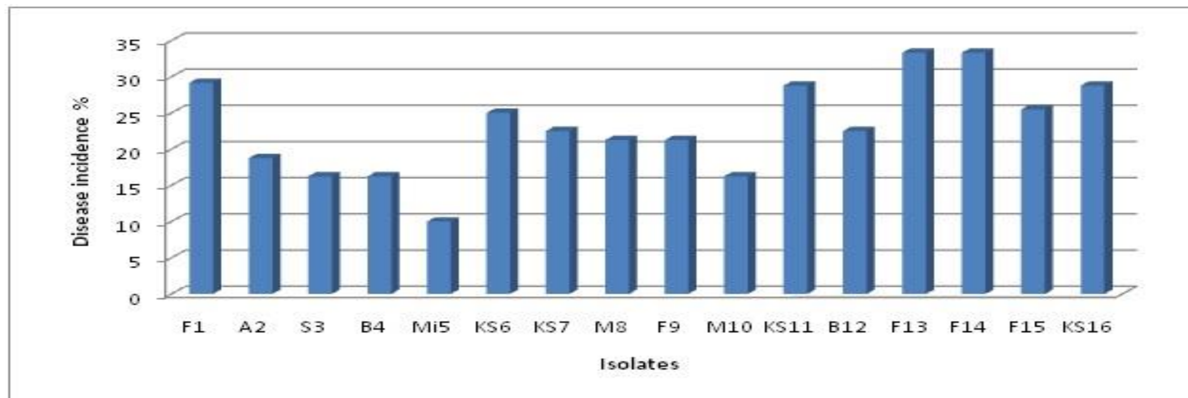

Figure (3): Means of disease incidence of *M. maydis* isolates.

Molecular characterization of *M. maydis* isolates:

Six primers of RAPD produced 32 DNA fragments (Figure 4). with an average of 5.33 bands per primer (Table 3). Among 32 amplified fragments 93.88% were polymorphic with an average of 5 polymorphic bands per primer, the most polymorphic primers were numbers 1,2,3 and 6. The polymorphic information content (PIC) values ranged from 0.20 to 0.39 with an average of 0.29. The similarity matrix (Table 4) for 16 isolates of *M. maydis* using six RAPD primers revealed that the highest similarity percentage (95%) was between isolates numbers F13 and F14, also between isolates numbers F14 and F15 while the lowest similarity percentage (26 %) was between isolates number A2 and KS11. The cluster analysis was done using Dice coefficient and divided the isolates into five clusters. Cluster 1 contains isolates numbers F14, F15, F13, B12, KS7, and KS16, cluster 2 contains isolates numbers F9, M10, Mi5, and M8, cluster 3 contains isolates numbers S3, KS6, F1, and B4, cluster 4 contains isolate number KS11 and the final cluster contains isolate number A2. (Figure 5).

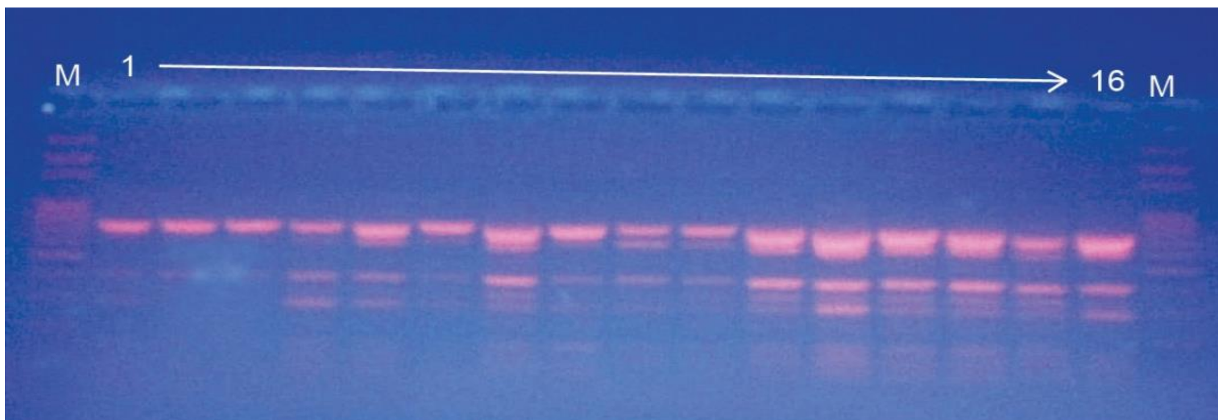

Figure (4): RAPD gel electrophoresis of 16 isolates of *M. maydis* using primer No. 5, the first and end lane were loaded with 100 bp DNA ladder.

Table (3): Total number of bands (TNB), polymorphic bands (PB), percentage of polymorphic bands (PPB)and the polymorphic information content (PIC)in the six RAPD primers.

RAPD Primers	TNB	PB	PPB	PIC
1	5	5	100	0.39
2	7	7	100	0.31
3	2	2	100	0.28
4	6	5	83.33	0.20
5	5	4	80	0.37
6	7	7	100	0.20
Total	32	30	--	--
Average	5.33	5	93.88	0.29

Table (4): The similarity matrix based on RAPD data among 16 isolates of *M. maydis*.

Isolate	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10	Isolate 11	Isolate 12	Isolate 13	Isolate 14	Isolate 15	Isolate 16
Isolate1	1															
Isolate 2	0.692	1														
Isolate 3	0.828	0.609	1													
Isolate 4	0.833	0.600	0.727	1												
Isolate 5	0.683	0.457	0.632	0.800	1											
Isolate 6	0.706	0.500	0.839	0.737	0.791	1										
Isolate 7	0.647	0.286	0.645	0.737	0.791	0.722	1									
Isolate 8	0.703	0.452	0.706	0.78	0.783	0.769	0.667	1								
Isolate 9	0.789	0.563	0.743	0.762	0.894	0.800	0.700	0.837	1							
Isolate10	0.722	0.533	0.667	0.750	0.800	0.737	0.632	0.829	0.905	1						
Isolate11	0.552	0.261	0.615	0.545	0.632	0.645	0.839	0.529	0.629	0.606	1					
Isolate12	0.75	0.529	0.649	0.818	0.857	0.714	0.810	0.756	0.826	0.773	0.703	1				
Isolate13	0.737	0.438	0.571	0.810	0.851	0.700	0.900	0.698	0.773	0.714	0.743	0.913	1			
Isolate14	0.789	0.500	0.629	0.810	0.851	0.75	0.850	0.698	0.818	0.762	0.743	0.913	0.955	1		
Isolate15	0.737	0.438	0.571	0.810	0.809	0.700	0.80	0.744	0.773	0.762	0.686	0.870	0.909	0.955	1	
Isolate16	0.647	0.357	0.645	0.684	0.837	0.778	0.833	0.667	0.750	0.737	0.774	0.762	0.800	0.850	0.800	1

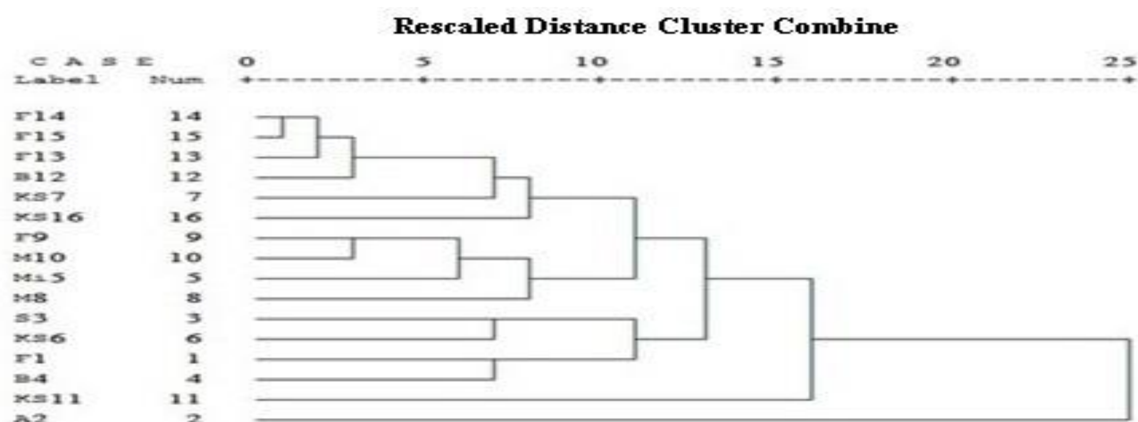

Figure (5): Dendrogram of cluster analysis based on RAPD data using Dice coefficient for 16 isolates of *M. maydis*.

DISCUSSION

In the current study, sixteen isolates of *M. maydis* the causal organism of maize's late wilt were isolated from diseased plants collected from seven different governorates in Egypt. Since Saleh and Leslie (2004) introduced the first specific primer for *C. maydis* and subsequently Drori *et al.* (2013) modified technique for molecular identification by choosing a new set of primers (200 and Am 42/43), it is easy to identify *M. maydis* isolates even in infected stalks or seeds. In the present study, sixteen isolates of *M. maydis* were confirmed using PCR.

Three different colors of isolates were detected *i.e*., white, white gray and dark olive however the cultural color of *M. maydis* varying between white to black-gray as reported by Khalil (1964) who studied the morphological characteristics of 15 isolates of *C. maydis* on PDA medium containing yeast extract and found that the color of isolates was white gray and pale white. Also, Mansour (1969) found that the

cultural color was white and gray. Three different colors white, gray and dark gray were found by Awad (2002). Likewise, El-Bakery (2010) found three different colors but white, dirty white and grayish white. Finally, two different colors, white-gray and black-gray were found by Rashid (2013). Isolates also showed variance in mycelial growth rate on PDA medium as the fastest growing isolate was isolate S3 while the slowest was A2.However,there is no correlation between isolates color, radial growth and origin of different governorates. In the Pathogenicity test using Hybrid pioneer 3062, the results revealed that isolates F13 and F14 were highly aggressive whereas isolate Mi5 was the lowest. Disease incidence values for isolates (F13 and F14) and (KS7 and B12) were similar 33.33%, and 22.50%, respectively, which laying together in cluster number one by RAPD marker. Also isolates number M8 and F9 caused similar disease incidence 21.25%, which laying together in cluster number two by RAPD marker. Finally, isolates S3, B4 and M10 were similar in disease incidence 16.25% which lies in cluster number three for isolates S3 and B4 and cluster number two for isolate number M10 by RAPD marker. These data revealed that there is no correlation between the pathogenicity of isolates and geographical origin of *M. maydis* isolates in governorates of Egypt and also, there is no correlation between cluster analysis and geographical distribution of *M. maydis* isolates. These data are contrary to that obtained by Abd-Rabboh (2006) who found genetic differences among the pathogen isolates in relation to concerning their pathogenicity as he used only one primer of RAPD to differentiate among ten isolates of *C. maydis.*

RAPD markers are well suited for genetic mapping, plant and animal breeding applications and for DNA fingerprinting with particular utility for studies of population genetics. RAPD markers can also provide an efficient assay for polymorphisms which should allow rapid identification and isolation of chromosome - specific DNA fragment (Williams *et al.,* 1990 and Kumari and Thakur, 2014). In the present study, five clusters analysis were detected by using six RAPD primers. The highest similarity (95%) was detected between isolates F14 and F15, between isolates F13 and F14 which were isolated from Fayoum governorate, isolates F9 from Fayoum and M10 from Menoufia are very closed to each other. Also, isolate S3 from Sohag and KS6 from Kafr-Elsheikh are very closed to each other. Also, isolate F1 from Fayoum and B4 from Beheira are very closed to each other. These obtained data do not contrast with EL-Kazzaz *et al*. (2003) who used five RAPD primers to differentiate among 14 isolates of *C. maydis* according to geographical distribution. On the other hand, our results agree with Zeller *et al.* (2000) that, there is no detectable correlation between geographical location and pathogen subgroup. Our results support an earlier study in Egypt by Saleh *et al.* (2003) who characterized 866 isolates of *C. maydis* collected from 14 governorates with AFLP and found three lineages in both the Nile River Delta and southern.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

REFERENCES

Abd EL-Rahim, M.F.; Fahmy, G.M. and Fahmy, Z.M.1998.Alternations in transpiration and stem vascular tissues of two maize cultivars under conditions of water stress and late wilt disease. Plant Pathology,47: 216-223.

Abd-Rabboh, M.S. 2006. Studies on late wilt disease of maize. Ph.D. Thesis, Dept. of Agric. Botany, Fac. of Agric., Menoufia Univ., Egypt, 139pp.

Ashour, A.M.A.; Sabet, K.K.A.; EL-Shabrawy, E.M. and Alhanshoul, A.M. 2014. Morphological, pathological, and genetic diversity among *Cephalosporium maydis* isolates. Egypt. J. Phytopathol.42(1): 25-42.

Awad, H.E.M. 2002. Studies on late wilt disease of maize. Ph.D. Thesis, Dept. of Agricu. Botany, Faculty of Agricu., Tanta Univ., Egypt, 113pp.

Clewer, A.G. and Scarisbrick, D.H. 2001. Practical statistics and experimental design for plant and crop science. John Wiley & Sons, LTD. 332pp.

Degani, O. and Goldblat, Y. 2014. Ambient stresses regulate the development of the maize late wilt causing agent, *Harpophora maydis*. Agricultural Sciences, 5: 571-582.

Dickinson, M.2003. Molecular plant pathology. Bios Scientific Publishers, London and New York.244 pp.

Drori, R.; Sharon, A.; Goldberg, D.; Rabinovitz, O.; Levy, M. and Degani, O. 2013. Molecular diagnosis for *Harpophora maydis*, the cause of maize late wilt in Israel. Phytopathologia Mediterranea, 52(1): 16-29.

EL-Bakery, A.M. 2010. Biological control of *Cephalosporium maydis* the causal organism of the late wilt disease on maize. M.Sc. Thesis, Dept. of Botany, Fac. of Science, Zagazig Univ., Egypt, 125pp.

EL-Kazzaz, M.K.; Hassan, M.A.; EL-Mersawy, E.M. and Awad, H.M. 2003. Differentiate between certain isolates of *Cephalosporium maydis*, the causal fungus of late wilt disease of corn by Random Amplified Polymorphic DNA (RAPD) analysis. The Egyptian Phytopathological Society, 10th Congress of Phytopathology, December 9-10, 2003, Giza-Egypt., 381-392.

EL-Shafey, H.A.; Mahmoud, S.A.Z.; Dawood, N.A.; EL-Borollasy, M.A. and Ibrahim, T.F.1985. Antagonism between soil microflora and late wilt of maize fungus *Cephalosporium maydis*. Proc. Egypt. Bot. Soc. 4: 1194-1207.

EL-Shafey, H.A. and Claflin, L.E. 1999. Late wilt. pp. 43-44. in: White, D.G. (ed) Compendium of corn diseases. Third Edition, APS Press, St. Paul. 78pp.

Fahmy, Z.M. and Mahmoud, A.H. 2001. Allelopathy of *Eucalyptus rostrata* leaves in controlling late wilt disease of maize. Egypt. J. Appl. Sci, 16(4).

Freeman, J. and Ward, E. 2004. *Gaeumannomyces graminis*, the take-all fungus and its relatives. Molecular Plant Pathology, 5(4): 235-252.

Gams, W. 2000. Phialophora and some similar morphological little-differentiated anamorphs of divergent ascomycetes. Studies in Mycology, 45:187-199.

Khalil, I.H.I. 1964. Studies in stalk rots of maize in U.A.R. variation in the late wilt fungus *Cephalosporium maydis*, M.Sc. Thesis, Dept. of Agric. Botany, Fac. of Agric., Cairo Univ., Egypt, 116pp.

Klaubauf, S.; Tharreau, D.; Foumier, E.; Groenewald, J.Z.; Crous, P.W.; deVries, R.P. and Lebrun, M.H. 2014. Resolving the polyphyletic nature of Pyricularia (Pyriculariaceae). Studies in Mycology, 79: 85-120.

Kumari, N. and Thakur, S.K. 2014. Randomly amplified polymorphic DNA-A brief review. Am. J. Anim. Vet. Sci., 9(1):6-13.

Luo, J. and Zhang, N. 2013. Magnapothiopsis, a new genus in Magnaporthaceae (Ascomycota). Mycologia, 105(4): 1019-1029.

Mansour, I.M.1969. Studies on stalk rots of maize in U.A.R. Further investigations on the late wilt and black bundle diseases. Ph.D. Thesis, Dept. of Agric. Botany, Fac. of Agric., Cairo univ., Egypt, 190 pp.

Molinero-Ruiz, M.L. and Melero-Vara, J.M. 2010.*Cephalosporium maydis*, the cause of late wilt disease, a pathogen new to Portugal and Spain. Plant Disease, 94(3): 379.

Moor, D. and Frazer, L.A.N. 2002. Essential fungal genetics, Springer, 356 pp.

Rashid, D.M. 2013. Pathological and Biochemical Studies on *Cephalosporium maydis* of Maize. M.Sc. Thesis, Dept. of Botany, Fac. of Science, Benha Univ., Egypt, 74 pp.

Sabet, K.A.; Samara, A.S.; Hingorani, M.K.; Mansour, I.M.; Abdel Rahim, M.F. and Khalil, I.I. 1961. Investigation on root and stalk rots of maize in Egypt (Etiological studies). United Arab Republic, Ministry of Agriculture, Agricultural Extension Department, Editing and Publication Administration, Technical Bulletin,1-29.

Sabet, K.A.; Samra, A.S. and Mansour, I.S. 1966. Interaction between *Fusarium oxysporum* f. sp. *vasinfectum* and *Cephalosporium maydis* on cotton and maize. Ann. Appl. Biol., 58: 93-101.

Sabet, K.A.; Zaher, A.M.; Samra, A.S. and Mansour, I.M. 1970. Pathogenic behavior of *Cephalosporium maydis* and *C.acremonium*. Ann. Appl. Biol., 66: 257-263.

Saleh, A.A.; Zeller, K.A.; Abou-Serie M.I.; Fahmy, Z.M.; El-Assiuty, E.M. and Leslie, J.F. 2003. Amplified fragment length polymorphism diversity in *Cephalosproium maydis* from Egypt. Phytopathology, 93: 7.

Saleh, A.A. and Leslie, J.F. 2004. *Cephalosporium maydis* is a distinct species in the Gaeymanomyces-Harpophora species complex. Mycologia, 96(6):1294-1305.

Samra, A.S.; Sabet, K.A.; Abdel Rahim, M.F.; Elshafey, H.A.; Mansour, I.M.; Fadl, F.A.; Dawood, N.A. and Khalil, I.H. 1966. Investigations on stalk-rot disease of maize in U.A.R. Ministry of Agriculture, Government Printing Offices, Cairo, Egypt, 1-204.

Samra, A.S.; Mansour, I.M. and Sabet, K.A. 1971. Cultural and pathogenic variation in *Cephalosporium maydis*. Agricultural Research Review, 49: 47-65.

Singh, N.; Somai, B.M. and Pillay, D. 2005. Molecular profiling demonstrates limited diversity amongst geographically separate strains of *Usitlago scitamineae*. FEMS Microbiology Letters.247: 7-15.

Ward, E. and Bateman, D.L. 1999. Comparison of Gaeumannomyces- and Phialophora-like fungal pathogens from maize and other plants using DNA methods. New Phytol.,141: 323-331.

Williams, J.G.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18(22): 6531-6535.

Zeller, K.A.; Jurgenson, J.E.; EL-Assiuty, E.M. and Leslie, J.F. 2000. Isozyme and amplified length polymorphisms from *Cephalosporium maydis* in Egypt. Phytoparasitica, 28(2): 121-130.