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Genetic Diversity And Pathogenic Variability Among *Cercospora beticola* Isolates From Different Egyptian Locations Using Microsatellite DNA Technique

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ABSTRACT

Cercospora leaf spot, caused by *Cercospora beticola*, is one of the most dangerous pathogenic fungi that caused large damage to sugar beet production in Egypt and across the world. The present study is a try to identify some Cercospora isolates collected from six districts in three Egyptian governorates using microsatellite DNA. The results confirmed a wide diversity among the six Cercospora isolates in their disease severity. The highest disease severity was recorded by El-Mansoura isolate, while El-Gemmeiza isolates showed the lowest disease severity among all tested isolates. The differences in the severity of the six isolates may be due to the wide genetic variation among these isolates. According to *RAPD* analysis a complete similarity between all isolations in OP-C3, OP-C15, and OP-O10 genes while the difference was complete between all isolations in both OP-A9 and OP- D1 genes. OP-A3 and OP-A5 genes in this study were associated with the high disease severity of Cercospora isolates while the absence of these two genes correlated with the low disease severity.With respect to DNA Polymorphic analysis, the results revealed that the polymorphic percentage in all tested isolates ranged between 83.33% to zero with a total polymorphic band reached 49%. The polymorphic bands exceeded 50% in the five primers OP-A3, OP-A5, OP-A9, OP-D1, and OP-O11 as clear evidence for the wide genetic diversity among tested isolates in these genes. The highest polymorphic band was showed in the OP-A5 gene (83.33%). As for Cluster analysis for RAPD, the result showed that the isolates were roughly grouped into two major groups according to their geographic origin. The first group consists of the two isolates collected from El-Mansoura, and Dekernes while the second group consists of the four isolates collected from Sidi-Salem, El-Reyad, and SendsesEl-Gemmeiza. The highest similarity between isolates was shownin the sub-sub group of between Sidi-Salem and El-Reyad isolates. In all cases, the similarity indices between all isolates were larger than 80% except for the two isolates collected from El-Mansoura, and Dekernes with Sendses isolates where the similarity index was less than 80%.

Key words: *Cercospora beticola*, Pathogenicity, RAPD fingerprinting.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is one of the most important sugar crops in Egypt, and throughout the world where it is a great source of sugar. In Egypt, sugar beet ranks first followed by sugar cane. Sugar beet is cultivated in the newly reclaimed lands, as the cultivated area in the new lands is 131,308 feddans while the cultivated area in the old lands is about 423633 feddans (Kamel *et al.*,

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2017). In the 2019/2020 seasons total harvested area of sugar beet in Egypt is about 608 thousand feddans producing about 12.25 million tons of sugar beet root and1.53 million tons of sugar in 2018/ 2019 (FAO STAT, 2020).

Cercospora is one of the serious fungi that cause leaf spots on the sugar beet foliage. It often appears in early summer, and then again in the fall when temperatures are closest to the 25 °C optimum (Rosenzweig *et al.,* 2019). The fungal pathogen *Cercospora beticola* Sacc. is the causal agent of the Cercospora leaf spot (Khan *et al.* 2008). *Cercospora beticola* is in the phylum Ascomycota, class Dothideomycetes, order Capnodiales, family Myco-sphaerellaceae, and genus *Cercospora* (Jacobsen and Franc 2009). The pathogen is a filamentous hemi biotroph believed to have originated in the Mediterranean and central Europe region (Skaracis *et al*., 2010). Cercospora leaf spot is one of the most serious diseases that affect sugar beets in temperate regions (Khan *et al*., 2008).Under the critical infection, the total loss in sugar beet roots ranged between 25 to 50% or more (Jacobsen and Franc, 2009).The controlling of *C. beticola* faced several defaults where their population was characterized by wide variabilities in pathogenicity, monocultural and metabolic features such as growth rate, mycelium color, and phytotoxin production.

This pathogen has not yet been broadly explored by molecular techniques, and not much is known about its population genetic structure. Studies of Random Amplified Polymorphic DNA (RAPD) showed that this fungus had genetic variation, despite the absence of a known teleomorph (Moretti *et al.,* 2004). This diversity caused it so difficult to produce host genotypes with multiple resistance to all pathogen strains or created chemical fungicides against these strains. The first step in the disease management program is defining these strains to determine the suitable cultivar that will be sowing and the effective fungicide that will be used. Recently, molecular detection tools have been developed to facilitate the accurate and rapid detection of *C. beticola* from both pure culture and naturally infected hosts (Groenewald *et al.,* 2005). Cultural variation and the degree of pathogen virulence on cultivars, together with different levels of resistance, are the main criteria used to study the genetic diversity of *C. beticola*. A wide range of phenotypic diversity was found for *C. beticola* isolates (Vaghefi *et al.,* 2016).

Cercospora beticola -specific primer set has been developed based on sequence data from the actin gene, which enabled the successful detection of *C. beticola* from field samples using a PCR assay (Lartey *et al.,* 2003). The present study aims to identify genetic diversity and pathogenic variability among some *C. beticola* isolates from different Egyptian locations using the microsatellite DNA technique. This study was performed in order to investigate the genetic diversity and pathogenic variability among different *C. beticola* Isolates obtained from different Egyptian Locations Using Microsatellite DNA Technique.

MATERIALS AND METHODS

Fungal isolates

Six Cercospora leaf spot isolates with typical disease symptoms were collected from infected sugar beet fields of the six districts i.e. El-Mansoura and Dekernes (El-Dakahlia governorate), Sidi-Salem and El-Ryad (Kafr El-Sheikh governorate) and Sendses and El-Gemmeiza (El-Gharbia governorate) in Egypt. Single-spore isolation technique was used and established on 1.5% potato dextrose agar (PDA) to purify these isolates. Pure cultures were identfied and micrrioscopicaly according to Saccardo (1876) key to verify that all obtained isolates were belonging to *C. bataticola* species.

Artificial infection of the sensitive beet cultivar Pleno was carried out with the six isolates that were previously grown on the agar media under greenhouse conditions, and used to re-isolate the pathogen from the infected leaves and determined the severity of infection of each isolate under controlled conditions. Disease severity was scored 30 days after inoculation using a 1-15 standard scale (Shane and Teng,1992) where scale1 was allocated to the plant leaves with out any symptoms and ascale of 15 to the leaves completely covered with the disease symptoms.

Determination of the relationship among the six isolates using RAPD-PCR and ITS-rDNA analyses

Total genomic DNA was extracted from 6 isolates of C. beticola grown on potato dextrose broth (PDB) and incubated at 25 °C for five days as described by Weiland (2002). The RAPD-PCR was performed as described by Chiusa *et al.*, (1996). The bulked DNA extraction was performed using Qiaprep Spin Miniprep Kit (QIAGEN). Isolation protocol of DNA was as follows:

1- Fungi micilium overnight culture by centrifugation at ›800rpm (6800xg)
2- Resuspend pelleted mysilium cells in 250ul Buffer P1 and transfere to a microcentrefuge tube.
3- Add 250ul Buffer P2 and mix thoroughly by inverting the tube 4-6 times until the solution become clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will run blue.
4- Add 350ul Buffer N3 and mix immedialtly and thoroughly by removing the tube 4-6 times. If using LyseBlue reagent , the solution will turn colorless.
5- Centrifuge for 10 min at 13,000 rpm in a table-top microcentrifuge.
6- Apply 800ul supernatant from step 5 to the QIAprep 2.0 spin column by pipetteing. For centrifuge processing, follow the instructions marked with triangle . For vaccum manifold processing, follow the instructions marked with circle. Centrifuge for 30-60 s and discard the flow-through.
7- Recommended: Wash the QIAprep2.0 spin colmn by adding 0.5 ml Buffer PB. Centrifuge for 30-60s and discard the flow-through.
8- Wash the QIAprep 2.0 spin column by adding 0.75 ml Buffer PE. Centifuge for 30-60s and discard the flow-through.
9- Centrifuge for 1min to remove residual wash buffer.

10- Place the QIAprep 2.0column in a clean 1.5ml microcentifuge tube . To elute DNA , add 50ul Buffer EB (10mM trisCL,ph 8.5) or water to the center of the QIApep 2.0 spin column, let stand for 1min, and centrifuge for 1min.

11- If the extracted DNA is to be analyzed on a gel, add 1volum of loading Dye to 5 volume of purified DNA. Mix the solution by pipetting up and down before loading the gel.

Polymerase chain reaction (PCR) condition stock solutions

5X Tris-borate (TBE), pH 8.0

Tris-base	5.40 g
Boric acid	2.75 g
500 mM EDTA, 8.0	0.29 g
H2O (d.w) up to	100.00 ml

Ethidium bromide

1- The stock solution was prepared by dissolving 1 g of ethidium bromide in 100 ml distilled water and mixed well with magnetic stirrer.
2- Transferred to a dark bottle and stored at room temperature.

Sample loading dye (5x)

Na-EDTA, pH 8.0 (500	2.00
Glycerol (100%)	5.00
Bromophenol blue	0.75
H_2O (d.w.)	1.50

PCR was performed in 30-µl volume tubes according to Williams *et al.*, (1990) that contained the following:

dNTPs (2.5 mM)	3.00 µl
$MgCl_2$ (25 mM)	3.00 µl
Buffer (10 x)	3.00 µl
Primer (10 pmol)	2.00 µl
Taq DNA polymerse	0.20 µl
Template DNA (25	2.00 µl
H_2O (d.w.)	16.80

Polymerase chain reaction (PCR) condition for RAPD

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94⁰ C for 4 min followed by amplification 30 cycles of 94⁰ C for 45s, 37⁰ C for 45s and 65⁰ C for 2min 30s. the reaction was finally stored at 72⁰ C for 10 min.

Gel preparation procedure

1- Agarose (1.5 gm) was mixed with (100ml) I x TBE buffer and boiled in microwave.
2- Ethidium bromide (5µl) was added to the melted gel after the temperature became 55°C.
3- The melted gel were poured in the tray of mini-gel apparatus and comb was inserted immediately, then comb was removed when the gel become hardened.
4- The gel was covered by the electrophoretic buffer (1 x TBE).
5- DNA amplified product (15 µl) was loaded in each well
6- DNA ladder (50bp) mix was used as standard DNA with molecular weights of 1500, 1200,1000,900, 800, 700,600, 500, 450, 400, 350, 300, 250,200, 150, 100 and 50 bp. The run was performed for about 30 min at 80 V in mini submarine gel BioRad .

PCR reactions were performed in a DNA thermal cycler (Biometra Co. Germany). All PCR products were analyzed on 1.5% agarose gel (Sambroek *et al*., 1989). Ten primers (University of British Colombia) were used for *C. beticola* isolates amplification, includingOO-A3,OP-A5,OP-A9, OP-C3, OP-C9, OP-C15, OP-D1, OP-K2, OP-O10, and OP-O11. Primer names and sequences are presented in Table 1. PCR reactions were performed for the six isolates collected from the six tested regions. PCR products were digested with EcoR1, Taq1, or Busr1 restriction enzymes under recommended conditions by the manufacturer's protocol. Using agarose gel electrophoresis, the DNA restriction fragments were separated and visualized under UV light **(**Sambroek *et al*., 1989). The reactions were repeated twice to confirm RAPD and ITS-rDNA reproducibility.

Table 1: List of the primer names and their nucleotide sequences used in the study for the RAPD procedure

No	Name	Sequence
1	OP-A3	5´AGT CAG CCA A3`
2	OP-A5	5´ AGG GGT CTT G3`
3	OP-A9	5´GGG TAA CGC C 3`
4	OP-C3	5´ GGG GGT CTT T 3`
5	OP-C9	5´ CTC ACC GTC C 3`
6	OP-C15	5´ GAC GGA TCA G 3`
7	OP-D1	5´ ACC GCG AAG G 3`
8	OP-k2	5´ GTG AGG CGT C3`
9	OP-O10	5´ TCA GAG CGC C3`
10	OP-O11	5´ GAC AGG AGG T3`

Data analysis

The similarity matrices were done using Gel works ID advanced software UVP-England Program. The relationships among genotypes as revealed by dendrograms were done using the SPSS windows (Version 10) program. DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among cultivars (Yang and Quiros, 1993).

RESULT

Severity of the six *Cercospora beticola* isolates:

The results in Table 2 confirmed a wide diversity among the six Cercospora isolates in their disease severity. The highest disease severity was recorded from El-Mansoura isolates (68.70%), followed by Dekernes isolate (63.70%), then El-Reyad isolates (45.00%).On the other hand,El-Gemmeiza isolate showed the lowest disease severity among all tested isolates. The differences inthe severity of the six isolates may be due to the wide genetic variation among these isolates.

Table 2: disease severity of the six *Cercospora beticola* isolates.

Isolate No.	Location	District	Disease severity
1	El-Mansoura	El-Dakahlia	68.70
2	Dekernes	El-Dakahlia	63.70
3	Sidi-Salem	Kafr El-Sheikh	40.00
4	El-Reyad	Kafr El-Sheikh	45.00
5	Sendses	El-Gaharbia	28.77
6	El-Gemmeiza	El-Gaharbia	21.30

1. ***Random Amplified Polymorphic DNA (RAPD)*analysis:**

1.1. Restriction analysis of the *RAPD* region

In this part 10 gene primers were used to determine the relationship between the six isolates. Data presented in Fig. 1 and Table 3 cleared that there is a complete similarity between all isolations in OP-C3, OP-C15, and OP-O10 genes, where the gene band clearly appeared in all isolations under all molecular weights, while the difference was complete between all isolations in both OP-A9 and OP- D1 genes, where the gene, appeared at the lowest molecular weight of 340 and 230 for the two genes respectively while the two genes varied in their appearance in isolations, with the amplification of the genes in the high molecular weights. There was a great similarity between El-Mansoura, and Dekernes isolates in both OP-A3 and OP-A5 genes, where the OP-A3 gene appeared in the two isolates at the same molecular weights 265, 400, and 510 pb, while the OP-A5 gene appeared in the two isolations at 285 and 420 pb. Both El-Reyad and Sendses isolates were similar in OP-A3, OP-K2, and OP-O11 genes confirminga great similarity between the two isolates in many genes. On the other hand, there was a clear similarity between the El-Mansoura and Gemmeiza isolates in OP-K2 and OP-C9 genes. The similarity was clear between the isolation of Sidi Salem and Gemmeiza in OP-A3 and OP-A5 genes. OP-A3 and OP-A5 genes in this study were associated with the high disease severity of *Cercospora* isolates while the absence of these two genes correlated with the low disease severity.

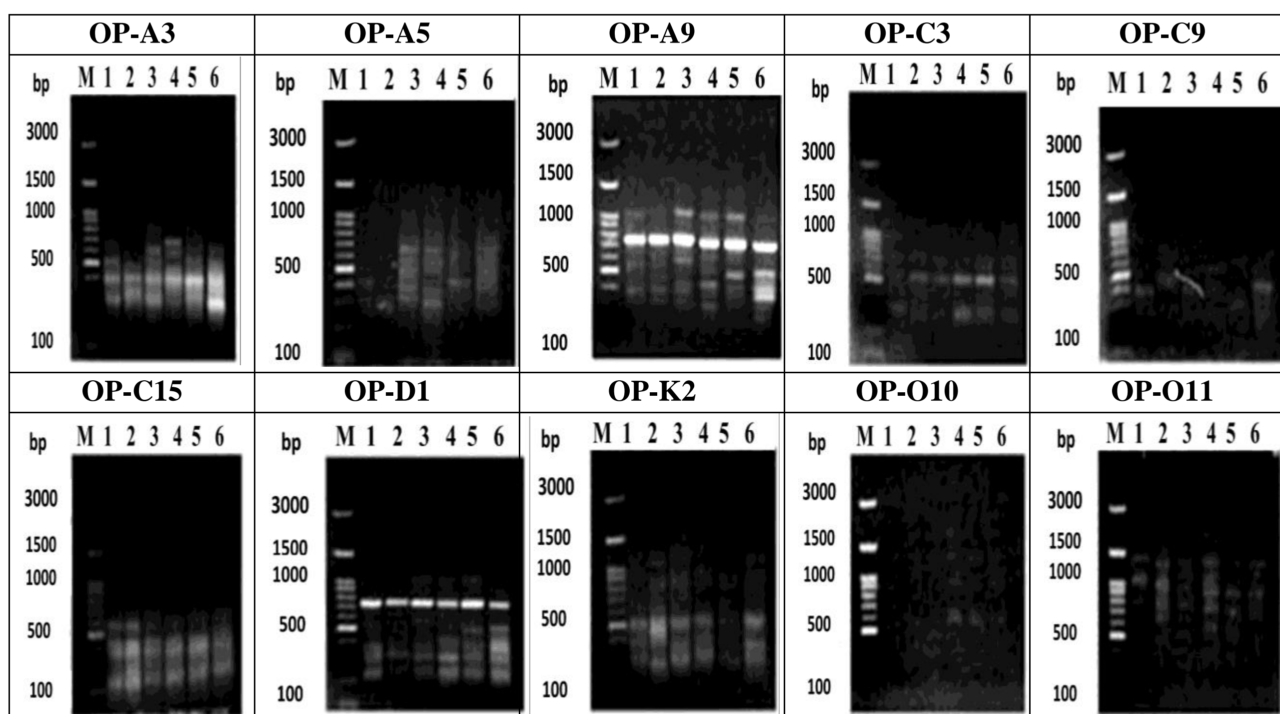

Fig.1: Restriction analysis of RAPD region.

Table 3: Restriction analysis of the RAPD region

BandNo	M.W bp	Strains					
		1	2	3	4	5	6
		OP-A3					
1	670	0	0	0	1	1	0
2	580	0	0	1	0	0	1
3	510	1	1	0	0	0	0
4	400	1	1	1	1	1	1
5	265	1	1	1	1	1	1
Total		3	3	3	3	3	3
		OP-A5					
1	660	0	0	1	1	0	1
2	560	0	0	1	1	0	1
3	480	0	0	1	1	0	1
4	420	1	1	1	0	1	1
5	345	0	0	1	1	1	1
6	285	1	1	1	1	1	1
Total		2	2	6	5	3	6
		OP-A9					
1	1165	0	1	1	1	1	0
2	740	1	1	1	1	1	1
3	530	1	1	1	0	0	0
4	445	0	1	0	0	1	1
5	430	0	0	1	1	1	1
6	340	1	1	1	1	1	1
Total		4	5	5	4	5	4
		OP-C3					
1	760	1	1	1	1	1	1
2	540	1	1	1	1	1	1
3	310	1	1	1	1	1	1
Total		3	3	3	3	3	3
		OP-C9					
1	615	0	1	0	0	1	0
2	535	0	0	0	1	0	0
3	460	1	1	1	1	1	1
4	400	1	1	1	1	1	1
Total		2	3	2	3	3	2

Band No	M.W bp	Strains					
		1	2	3	4	5	6
		OP-C15					
1	600	1	1	1	1	1	1
2	470	1	1	1	1	1	1
3	385	1	1	1	1	1	1
4	225	1	1	1	1	1	1
Total		4	4	4	4	4	4
		OP-D1					
1	1065	0	0	1	1	1	0
2	740	1	1	1	1	1	1
3	560	1	0	1	0	0	0
4	490	0	0	0	0	1	1
5	375	1	0	1	1	1	1
6	315	1	1	1	1	1	1
7	230	1	1	1	1	1	1
Total		5	3	6	5	6	5
		OP-K2					
1	1235	1	0	1	1	1	1
2	640	1	1	1	1	1	1
3	520	1	1	1	1	1	1
4	430	0	1	0	0	0	0
5	370	1	1	1	1	0	1
6	240	1	1	1	1	1	1
Total		5	5	5	5	4	5
		OP-O10					
1	1340	1	1	1	1	1	1
2	830	0	1	1	1	1	1
3	420	1	1	1	1	1	1
Total		2	3	3	3	3	3
		OP-O11					
1	1375	1	1	1	1	0	1
2	1230	0	0	1	1	0	0
3	1080	1	1	1	1	0	0
4	840	0	1	1	1	1	1
5	750	1	1	1	1	1	1
6	640	1	1	1	1	1	1
7	500	1	1	1	1	1	1
Total		5	6	7	7	4	5

1.1. DNA Polymorphic analysis:

Results illustrated in Table 4 revealed that the polymorphic percentage in all tested isolates ranged between 83.33% to zero with a total polymorphic band reached 49%. The polymorphic bands exceeded 50% in the five primers OP-A3, OP-A5, OP-A9, OP-D1, and OP-O11 as clear evidence for the wide genetic diversity among tested isolates in these genes. The highest polymorphic band was showed in the OP-A5 gene (83.33%). On the other side, polymorphic bands were less than 50% under OP-C3, OP-C9, OP-C15, OP-K2, and OP-O10 primers. The polymorphic band wasabsentin OP-C3 and OP-C15 genes where the monomorphic band was100% indicatingthe complete similarity between all tested isolates in both genes.

1.1. Cluster analysis for RAPD.

The cluster analysis for RAPD is shown in Fig.2. The isolates were clustered in distinct groups, with different rates of similarities among each other.The isolates were roughly grouped into two major groups according to their geographic origin.The first group consists of the two isolates collected from El-Mansoura, and Dekernes while the second group consists of the four isolates collected from Sidi- Salem, El-Reyad, Sendses, and El-Gemmeiza. The second group was also, divided into a sub and sub-sub groups according to the similarity between the isolates. The highest similarity between isolates was shown in the sub-sub group of between Sidi- Salem and El-Reyad isolates. In all cases, the similarity indices between all isolates were larger than 80% except for the two isolates collected from El-Mansoura and Dekernes with Sendses isolates where the similarity index wasless than80% (Table 5).

Table 4: DNA Polymorphic analysis for all tested primers.

Primer Name	Total band	Monomorphic Band	Polymorphic Band	Unique Band	Polymorphic %
OP-A3	5	2	3	-	60%
OP-A5	6	1	5	1	83.33%
OP-A9	6	2	4	1	66.66%
OP-C3	3	3	-	-	-
OP-C9	4	2	2	1	50%
OP-C15	4	4	-	-	-
OP-D1	7	3	4	1	57.14
OP-K2	6	4	2	2	33.33%
OP-O10	3	2	1	1	33.33%
OP-O11	7	3	4	2	57.14%
Total	51	26	25	10	49%

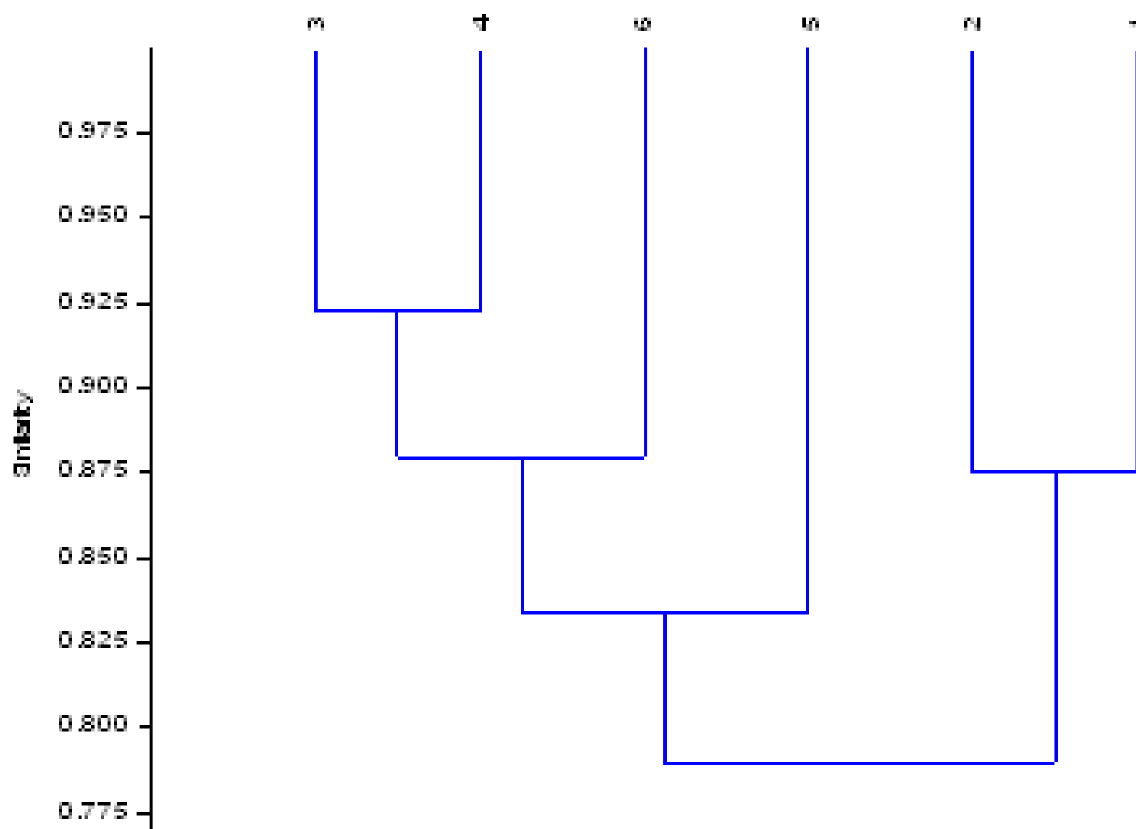

Fig. 2: Dendrogram of cluster analysis for RAPD data conducted within six different Cercospora isolates.

Taple5: Similarity Index Using RAPD analysis for six Fungi isolates.

Isolates	Locations	El-Mansoura	Dekernes	Sidi-Salem	El-Reyad	Sendses	El-Gemmeiza
1	El-	1.0					
2	Dekernes	0.82	1.0				
3	Sidi- Salem	0.87	0.88	1.0			
4	El-Reyad	0.87	0.88	1	1.0		
5	Sendses	0.74	0.76	0.82	0.82	1.0	
6	El-	0.86	0.87	0.92	0.927	0.90	1.0

DISCUSSION

Fungi in general, and Cercospora leaf spots especially, have a wide genetic diversity. This diversity considers a great tool that helps fungi to stay alive indiverse environmental conditions, as well as help them in preserving their species from extinction. The development of host resistance to a fungus or the emergence of an effective fungicide in controlling the fungus is usually for

a limited period, as genetic diversity allows the fungus to develop itself quickly and produce new strains that are more virulent on the host or resistant to the effective pesticide. Several studies conducted on Cercospora fungus concluded that there is significant genetic variation for strains isolated from different regions, whether these regions have a limited or wide geographical range. Despite this wide diversity among these isolates, they are similar in many morphological and genetic traits, which establish the rule of one origin for those isolates.

Many methods are used to determine the degree of similarity between the isolated fungus taken from different locations. Some of these methods depend on the appearance of the fungus or the severity of the injury. These methods are inaccurate, as they are often affected by environmental conditions and the resistance degree of the host plant. The progress in the process of isolation and definition of fungi has given a wide field in the process of describing fungal isolation more accurately through modern techniques such as DNA isolation and the use of microscopes in determining the degree of similarity between these strains or isolates. It has become easy with the amplification of the DNA under different gene primers, determining the polymorphism of the gene, and thus determining the degree of similarity between the same fungi isolations or the different fungi strains. In the currentstudy, we defined six *Cercospora* isolates from the Egyptian population collected from different locations using *Random Amplified Polymorphic DNA* (*RAPD*) analysis.

Our results confirmed a wide diversity among the six Cercospora isolates in their disease severity where the highest disease severity was recorded from El-Mansoura followed by Dekernes isolates while El-Gemmeiza isolate showed the lowest disease severity among all tested isolates. The differences inthe severity of the six isolates may be due to the wide genetic variation among these isolates. Similar results havebeen reported by Mahmoudi *et al.,* (2018) who studied the pathogenic and genotypic variation of 24 *C. beticola* isolates collected from different regions of Iran and found that all of the 24 isolates tested were found to be pathogenic on the cultivars with significant variation in disease severity. Moreover, Vaghefi *et al.,* (2016) characterize the genetic structure of *C. beticola* populations in sugar beet using 12 microsatellite markers in New York and they found high genotypic diversity, detection of admixed genotypes by Bayesian clustering and DAPC analyses which were suggestive of recombination in the *C. beticola* population. This variation in the severity of infection between different locations may be due to the different environmental conditions between those locations. In this regard, EL-Sayed (2000) surveyed sugar beet leaf spot disease in four major productive

Governorates. The survey was carried out in 1996-1997 and 1997-1998 growing seasons in different districts in Kafr El-Sheikh, El-Behaira, El-Gharbia, El-Sharkia, and El-Dakahlia Governorates and revealed that Cercospora leaf spot showed the highest disease severity in all districts ranged from 50% in Sakha to 72% in Sidi Salem (Kafr El-Sheikh) on Pleno cultivar. The disease severity was 30% on Ras Poly in the Damanhour district (El-Behaira), 28% on the Kawamira cultivar in the Etai El-Baroud district, 56% on the Maribo marina cultivar in El-Reyad district (Kafr El-Sheikh) and 25% on Top cultivar in Mehalla district (El-Gharbia). In another study, Asif Khan *et al.* (2007) found a wide diversity in disease severity in different locations. The highest mean disease of Cercospora leaf spot was recorded in Dera Ismail Khan District which was 27.41% followed by Bannu district (26.98%). In the districts of Mardan, Charsda, and Peshawar the mean disease severity of Cercospora leaf spot was recorded as 23.74%, 26.44%, and 26.28% respectively.

According to *RAPD* analysis, this study confirmed a complete similarity between all isolations in OP-C3, OP-C15, and OP-O10 genes while the difference was complete between all isolations in both OP-A9 and OP- D1 genes. OP-A3 and OP-A5 genes in this study were associated with the high disease severity ofCercospora isolates while the absence of these two genes correlated with the low disease severity.In this respect, Mahmoudi *et al.,* (2018) studied the pathogenic and genotypic variation of 24 *C. beticola* isolates collected from different regions of Iran using RFLP of the Internal Transcribed Spacer (ITS-RFLP), and Random Amplified Polymorphic DNA (RAPD-PCR).Results of RAPD analysis showed wide DNA polymorphism among the Iranian C. beticola isolates. RAPD and ITS-RFLP markers showed the highest level of genetic diversity which confirms the variation in *C.beticola* detection. Moretti *et al*., (2006) surveyed genetic variability and population structure of *C. beticola*, the causal agent of Cercospora leaf spot in sugar beet, from four sugar beet growing regions of Greece were investigated using DNA finger-printing. High diversity was found with an average gene diversity of 0.21, and no significant differences among populations. Among the 46 isolates, 45 different genotypes were identified, showing a high degree of genotype diversity.

With respect to DNA Polymorphic analysis, the results revealed that the polymorphic percentage in all tested isolates ranged between 83.33% to zero with a total polymorphic band reached 49%. The polymorphic bands exceeded 50% in the five primers OP-A3, OP-A5, OP-A9, OP-D1, and OP-O11 as clear evidence for the wide genetic diversity among tested isolates in these genes. The highest polymorphic band was showed in the OP-A5 gene (83.33%). As for Cluster analysis for RAPD, the result showed that the

isolates were roughly grouped into two major groups according to their geographic origin. The first group consists of the two isolates collected from El-Mansoura and Dekernes while the second group consists of the four isolates collected from Sidi-Salem, El-Reyad, Sendses El-Gemmeiza. The highest similarity between isolates wasshownin the sub-sub group of between Sidi-Salem and El-Reyad isolates. In all cases, the similarity indices between all isolates were larger than 0.80 except for the two isolates collected from El-Mansoura and Dekernes with Sendses isolates where the similarity index wasless than 80%. These results are in agreement with those of Turgay *et al.,* (2010) who identified the pathotypes of *C. beticola,* the causal agent of sugar beet leaf spot disease, by application of a pathogenicity test using 100 isolates obtained from the provinces with intensive sugar beet cultivation. 9874 polymorphic fragments of sizes between 100 and 500 bp were analyzed which were generated by nine primers. The dendrogram derived from AFLP analysis depicted the existence of five different subgroups. The polymorphism rate among isolates was 91.13% and the dendrogram distribution of the pathotypes obtained by pathogenicity indicated that pathotypes were not discriminated and did not compose any groups. Mahmoudi *et al.,* (2018) studied the pathogenic and genotypic variation of 24 *C. beticola* isolates collected from different regions of Iran using RFLP of the Internal Transcribed Spacer (ITS-RFLP), and Random Amplified Polymorphic DNA (RAPD-PCR). Results of RAPD analysis showed wide DNA polymorphism among the Iranian *C. beticola* isolates.

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