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MOLECULAR AND BIOCHEMICAL EVALUATION OF FUSARIUM OXYSPORUM F. SP. FRAGERIA USING PROTEIN AND RAPD MARKERS ON STRAWBERRY

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ABSTRACT: Gene polymorphism can be used to study the genetic diversity of fungal populations, as well as the relationship between different fungal isolates and strains. Our goal in this study is to use random amplified polymorphic DNA (RAPD) and protein analyses to characterize 10 Fusarium isolates were obtained from five different Egyptian sites. The results revealed that the polymorphism percentage ranged from 62.5 to 91.7, with an average of 81.1 per primer. In terms of polymorphic information content (PIC), RAPD primers had an average of 0.35 and a range of 0.3 to 0.41. Resolving power (RP) values varied from 1.6 and 6.6 with an average of 3.6 per primer. The primer OPB-01 produced the highest effective multiplex ratio (EMR) value (10.1), whilst the primer P-005 produced the lowest value (3.12), with an average value of 6.39 per primer. The range of marker index (MI) values was 0.72 to 4.14, with an average of 1.96 per primer. For primers P-006 and OPB-01, the diversity index (DI) values per polymorphic band ranged from 0.08 to 0.38, respectively. RAPD markers yielded genetic similarity values ranging from 0.62 to 0.92, with an average of 0.75. The ten isolates were divided into two major clusters. Isolates R1, R8, R10, R9, R3, and R2 were placed in the first one, while isolates R5, R6, R4, and R7 were located in the second one. Isolates R8 and R10 had the highest similarity index (0.92). The isolates R4 and R7, on the other hand, had the lowest similarity index score (0.75). The dendrogram of protein banding patterns contains the isolate R7 on one cluster with44% similarity. While the remaining isolates (R1, R2, R4, and R10) are found in the second cluster. The second cluster split into two branches with a commonality of 54%. The isolates R3 and R8 were gathered as a single branch on the first branch. The isolates R1, R4, R2, and R10 were all part of the first subbranch. The isolates R5, R6, and R9 were all part of the second subbranch. Indicators of similarity varied from 44% to 96%. The wide genetic variation among *Fof* isolates is a major challenge for the development of effective disease control strategies. Therefore, our study using protein and RAPD markers for assessing the genetic diversity of *F. oxysporum f. sp. frageria* isolates can be a useful tool for understanding the relationship among different isolates as a base for investigating the relationship between genetic diversity and pathogenicity to develop a suitable program against different *F. oxysporum f. sp. frageria* pathotypes in the future.

Keywords: *Fusarium oxysporumf. sp.frageria*, Pathogenicity, RAPD fingerprinting.

INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch) is an important vegetable crop because of its delicious fruit, distinct flavor, and well-known nutritional benefits. Its fruit is a good source of minerals and vitamin C. Ascorbic acid, polyphenols, anthocyanins, and flavanols are all present in abundance, making it one of the fruits with the highest antioxidant activity (Trejo-Téllez and Gómez-Merino, 2014). Strawberries are typically in high demand by consumers due to their colour, flavour, scent, and nutritional contents (Petran *et al*., 2017; Pradeep and Saravanan, 2018). It is cultivated all over the world using a

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variety of production techniques, harvesting schedules, and genotypes that can respond to shifting environmental conditions. Since its introduction into cultivation in the year 1800, the large strawberry fruit (*Fragaria x ananassa* Duch.) has served as the foundation for the current types that now account for most of the global production (Lopez-Aranda *et al*., 2011). According to FAO data, strawberry production worldwide was 8,885,028 tons. China leads the world in strawberry production in 2019, producing 3,212,814 tons, followed by the United States of America, which produced 1,021,490 tons (Kilic *et al*., 2021). According to the latest data, the production of strawberries has expanded worldwide by 41% over the past ten years. The top exporting nations are China, the United States of America (USA), the European Union (EU), Mexico, Turkey, Egypt, and South Korea. (Simpson, 2018, FAOSTAT, 2021). Egypt was ranked number five in terms of strawberry production worldwide after all of USA, Mexico, Turkey, Spain in 2019 (FAO, 2023). The annual production of strawberries in Egypt reached 544.945 tons, with a cultivable area of 31897 Feddan on both old and new reclaimed soils, which are regarded as the main cash crop for strawberry farmers in several Egyptian governorates (MALR 2019, FAOSTAT, 2020).

However, because its high susceptibility to fungal diseases including fusarium wilt and fusarium root rot, strawberry production is extremely susceptible to field and post-harvest losses. (Hutton *et al*, 2013, Koike, and Gordon, 2015, Nam *et al*., 2009, Zveibil *et al*., 2012). The pathogen was initially identified in 1962 in Australia and Japan (Winks and Williams, 1965). The *Fusarium oxysporum* species complex is a large group of soil-borne filamentous, ascomycetous fungi that can infect numerous commercially significant crop species and cause disease (Gordon 2017). Noteworthy, *Fusarium oxysporum f. sp. Fragariae* is long-lived and has strong saprophytic abilities (Koike, and Gordon, 2013). Common indications of fusarium wilt included wilted foliage, distorted and very chlorotic leaflets, and discoloured crowns (Dilla-Ermita, *et al*., 2023). Through the root system, the fungus infects strawberry plants, causing root rot and vascular darkening of the crown tissues (Fang *et al*., 2012, Koike, and Gordon, 2013). Reduced yields and catastrophic losses can result from the fugus' rapid withering of strawberry plants, particularly when it is worsened by heat and water stress (Juber *et al*., 2014, Koike and Gordon, 2015 Henry *et al*., 2017). It frequently manifests in the early summer and is most severe when temperatures exceed 25°C (Bosland, *et al*. 1997). Recently, molecular detection tools have been developed to facilitate accurate and rapid detection of *F. oxysporum* from both pure culture and naturally infected hosts (Dilla-Ermita, *et al*., 2023). Cultural variation and the degree of pathogen virulence on cultivars, together with diverse levels of resistance, are the main criteria used to study the genetic diversity of *Fusarium wilt disease*. A wide range of phenotypic diversity was found for *Fusarium* isolates (Williams *et al* 1990, Burkhardt, 2019). Controlling of Fusarium faced several difficulties where their population have a wide variability in pathogenicity, morpho-cultural and metabolic features such as growth rate, mycelium color and phytotoxin production.

Genetic diversity of *Fusarium oxysporum f. sp. frageria* isolates is important for understanding the epidemiology of the disease and for developing effective management strategies. Previous studies have used a variety of molecular markers to assess genetic diversity among *Fusarium oxysporum f. sp. lycopersici* isolates (Anand, and Kapoor, 2018), *Fusarium oxysporum f. sp. carthami* isolates (Bogale, *et al*, 2005), *Fusarium oxysporum f. sp. pisi* isolates (Dubey and Singh, 2008, Sharma et al 2014), *Fusarium oxysporum f. sp. vasinfectum* isolates (Greenewald *et al*, 2006), *Fusarium oxysporum f. sp. melonis* isolates (Mahfooz *et al*, 2012, Yuan *et al*, 2013), including protein markers, RAPD, ISSR markers, and AFLP markers. They showed that there was significant genetic diversity among the isolates, with most of the variation being explained by the protein markers. They also found that there was a correlation between genetic diversity and pathogenicity, with the more virulent isolates being more genetically diverse. To study the genetic diversity of *F.*

oxysporum f. sp. fragariae in strawberry, AFLP was conducted on population of 91 strains of Fusarium wilt pathogen. Additionally, to assess the connections between genetic diversity and each strain's characteristic and sampling site (Choaand and Kwak, 2022). Studies of Random Amplified Polymorphic DNA showed that this fungus had genetic variation, despite the absence of a known teleomorph (Irom Manoj Singha *et al*. 2016). Due to this diversity, it is extremely challenging to develop host genotypes that are resistant to all disease strains or to develop chemical fungicides to combat these strains. To select the appropriate cultivar to sow and the efficient fungicide to employ, the controlling program's initial step is to define these strains. The present study aims to identify genetic diversity among ten *Fusarium oxysporum* isolates using SDS-PAGE and RAPD-PCR techniques.

MATERIALS AND METHODS

Fungal isolates and genomic DNA isolation and RAPD-PCR amplification

Ten Fusarium isolates with typical disease symptoms of wilting disease were collected from infected strawberry fields of five distinct locations of Buhayra governorate in Egypt. Single spore technique for each isolate was used and established for a week at 25 Co on potato dextrose agar on 1.5% potato dextrose agar (PDA), (0.8 g of potato starch, 4.0 g of dextrose, and 20 g of agar in L). After the cultivation, the mycelium was raked and put into 1.5 mL tube. incubated in liquid culture in 50-ml flasks at 25 °C for five days collected using double-layer cheesecloth, followed by rinsing with distilled water and removing the extra water using filter paper as described by Burkhardt *et al*, (2019). The mycelial pads were ground in an atmosphere of liquid nitrogen for DNA extraction using the CTAB protocol. RNA was removed from the DNA sample with RNase (100 µg/µL). The quality and quantity of DNA was checked by agarose gel electrophoresis. The final DNA concentration of each sample was adjusted to 100 ng/µL using an ND-1000 spectrophotometer (Thermo Scientific Inc, Grand Island, NY) and stored at -20 °C. PCR reactions were performed in a DNA thermal cycler (Biometra Co. Germany). The RAPD-PCR was performed using six primers obtained from Agricultural Research Center (ARC); Egypt was used for *F. oxysporum* isolates amplification. Primer's name and sequences are presented in Table (1). A reaction mixture of 25 µL contained 2.5 µL 10x buffer (Fermentas), 1.0 µL Taq polymerase, 10 pmol of each primer, 5 µL dNTPs (0.2 mM), and 100 ng template DNA. The amplification cycles were 1 min denaturation at 94◦C, 1 min annealing at 35◦C, and 1.5 min extension at 72◦C for 35 cycles. The reaction started by denaturation step for 4 min at 94◦C and ended with a final extension for 10 min at 72◦C. The amplified DNA fragments were electrophoresed in 1.5% agarose gel and stained by ethidium bromide (Sambroek *et al*., 1989) and photographed under UV illumination.

Table (1): The primers sequences employed in the RAPD method.

S/No.	Primer name	sequence ('5--3')
1	OPE-02	GGTGCGGGAA
2	OPB-01	GTTTCGCTCC
3	OPB-11	GTAGACCCGT
4	P-004	AAGAGCCCGT
5	P-005	AACGCGCAAC
6	P-006	CCCGTCAGCA

Total protein profiling

Protein was isolated and detected according to a method of Laemmli (1970) with slight modifications was adopted to use in the present study. The modification was reduced TEMED from 30µl to 25 µl and APS was reduced from 1.5 ml to 1.3 ml. Approximately 1 g freeze dry mycelial growth from each tested isolate of fungi was ground in a mortar and pestle in liquid nitrogen. The sample was crushed repeatedly until it became entirely homogeneous. The crushed samples were then added to a 1 ml Eppendorf tube that had been filled to 200 l with extraction buffer (50 m Mtris-HCl buffer, pH 6.8, glycerol 10% by weight, ascorbic acid 0.1%, and cysteine hydrochloride 0.1 by weight). Debris was removed using centrifugation at 18,000 rpm for around 30 minutes. The protein content in supernatant was estimated according to the method of Bradford (1976) by using bovine serum albumin as a standard protein. Protein content was adjusted to 2 mg / ml per sample.

Scored data and statistical analysis

For each of the ten F. oxysporum isolates, RAPD bands were counted as either present (1) or absent (0) for each primer used. The total number of bands, the number of polymorphic bands and the percentage of polymorphism were determined. The Simqual module of the NTSYS-pc software 2.01e was used to compare all genotypes pairwise (Rohlf, 1998), the Jaccard coefficient was used to calculate genetic distances (Jaccard, 1908). The distance coefficients obtained were used to construct dendrograms using UPGMA (the unweighted pair group method with arithmetic averages) employing the SAHN (sequential, agglomerative, hierarchical, and nested clustering) algorithm in same software package. Effective multiplex ratio (EMR) and marker index (MI) for both marker systems were calculated to measure the usefulness of the marker system according to Powell *et al*., (1996). To calculate the multiplex ratio (MR), the total number of bands amplified was divided by the total number of assays. The effective multiplex ratio (EMR) is the number of polymorphic fragments detected per assay. Using the formula of Roldán-Ruiz *et al*., (2000), polymorphic information content (PIC) or heterozygosity (H) was calculated: PIC = 2fi (1-fi), where fi is the frequency of the amplified allele. Average heterozygosity (Hav) is estimated by taking the average of PIC values obtained for all the markers. According to Powell *et al.* (1996), the marker index (MI) was derived by multiplying the average heterozygosity (Hav) by the EMR. According to Prevost and Wilkinson (1999), resolving power (Rp), which measures the most informative primers' capacity to distinguish between genotypes, was calculated as follows: $Rp = \sum Ib$, where Ib is the band informativeness, with $Ib = 1 - [2 \times (0.5-p)]$ where p is the percentage of clones that contain the band. The distribution of bands within the sampled genotypes determines the resolving power.

RESULTS AND DISCUSSION

Many methods used to determine the degree of variation among the isolated fungus taken from various locations. Some of these methods depends on the appearance of the fungus or the severity of the injury. 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 the modern techniques such as molecular markers and protein analysis and determining the degree of similarity among these strains.

Random Amplified Polymorphic DNA (RAPD) Analysis

a. Characterization of polymorphism and genetic diversity

In this study, the genetic diversity was estimated among 10 *Fusarium* isolates using RAPD primers (Figure 1 and Table 2). The percentage of polymorphism ranged from 62.5 to 91.7 with primers P-005 and OPB-01, respectively, with an average of 81.1 per primer. The six primers amplified a total of 56 bands, of which 46 were polymorphic with an average of 7.7 polymorphic bands per primer. The maximum number of 12 bands was amplified with OPB-01, followed by P-004 and P-006 which amplified 10 bands each. The minimum

amplified bands were shown by RAPD primers OPE-02, OPB-11 and P-005 with 8 bands each. The percentage of polymorphism as a measure of genetic diversity in *Fusarium oxysporum f. sp. frageria* varies depending on the population studied and the markers used. However, studies have shown that this percentage can be extremely high, with some populations showing over 99% polymorphism. For example, a study by Yang *et al.,* (2009) used microsatellite markers to analyze the genetic diversity of *F. oxysporum f. sp. frageria* isolates from six different countries. They found that the overall percentage of polymorphism was 99%, with the number of polymorphic loci (r) ranging from 13 to 15. Another study by El-Mounadi *et al.,* (2014) used random amplified polymorphic DNA (RAPD) markers to analyze the genetic diversity of *F. oxysporum f. sp. frageria* isolates from Morocco. They found that the overall percentage of polymorphism was 98.7%, with the number of polymorphic loci (r) ranging from 15 to 17. These studies suggest that the percentage of polymorphism can be a very useful measure of genetic diversity in *F. oxysporum f. sp. frageria*. However, it is important to note that the percentage of polymorphism can be affected by several factors, including the population size, the number of markers used, and the type of markers used.

The Polymorphic information content (PIC) of RAPD primers ranged from 0.3 (OPE-02) to 0.41 (OPB-01), with an average of 0.35. A higher PIC value indicates a more informative marker. The PIC values were in harmony with that found by De Riek *et al.,* (2001) that the maximum value of PIC for dominant markers such as RAPD is 0.5. The polymorphic information content (PIC) value of a dominant marker like RAPD among *Fusarium oxysporum* isolates can vary depending on the population of isolates being studied and the specific RAPD primers used. However, studies have shown that RAPD markers can be highly informative for differentiating between *Fusarium oxysporum* isolates. For example, one study found that the average PIC value of RAPD markers among 25 *F. oxysporum* isolates was 0.48, with a range of 0.29 to 0.68 (Sharma *et al*., 2014). Another study found that the average PIC value of RAPD markers among 30 *F. oxysporum* isolates was 0.56, with a range of 0.38 to 0.73 (Mahfooz *et al*., 2012). These studies suggest that RAPD markers can be a valuable tool for studying the genetic diversity of *Fusarium oxysporum* populations. However, it is important to note that RAPD markers are dominant markers, which means that they cannot distinguish between homozygous and heterozygous individuals. This can limit their usefulness for certain applications, such as genetic mapping.

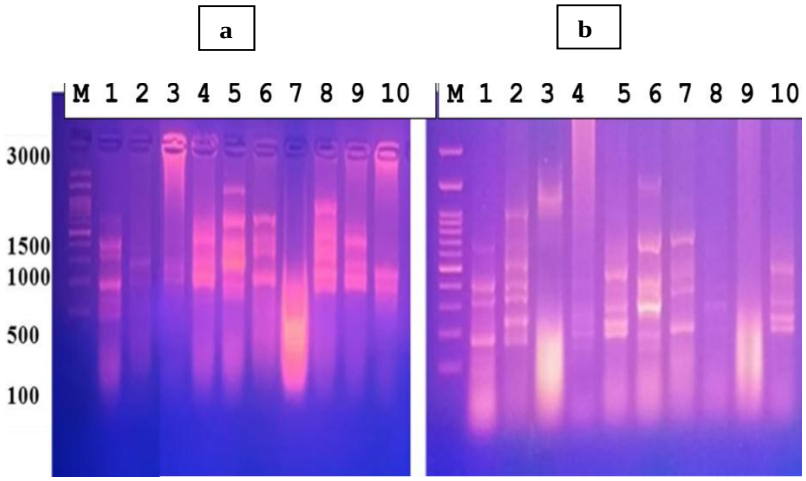

Figure 1: RAPD banding patterns on 1.5% agarose gel of amplified fragments generated from ten isolates of *Fusarium oxysporum f. sp. frageria* with six random primers. (a) OPE-02; and (b) OPB-11 chosen as examples; M., DNA marker 1 kb), lanes 1 - 10 are the number of isolates.

Table 2: primer code, total number of bands, number of common bands, number of polymorphic bands, percentage of polymorphism, polymorphic information content, resolving power, effective multiplex ratio, marker index and gene diversity index (diversity index per polymorphic band).

Primer code	Total no. of alleles	No. of polymorphic alleles	Polymorphism %	No. of Common alleles	PIC	RP	EMR	MI	DI
OPE-02	8	7	87.5	1	0.30	1.6	6.125	1.84	0.26
OPB-01	12	11	91.7	1	0.41	6.6	10.087	4.14	0.38
OPB-11	8	6	75	2	0.35	3.2	4.5	1.58	0.26
P-004	10	8	80	2	0.37	3.6	6.4	2.37	0.30
P-005	8	5	62.5	3	0.35	2.8	3.125	1.1	0.22
P-006	10	9	90	1	0.34	4	8.1	0.72	0.08
Total	56	46	486.7	10	2.12	21.8	38.34	11.75	0.25
Average	9.3	7.7	81.1	1.67	0.35	3.6	6.39	1.96	0.25

The resolving power (RP) values ranged from 1.6 for primer OPE-02 to 6.6 for primer OPB-01 with an average of 3.6 per primer. In a study by Sharma *et al*. (2013), RAPD markers were used to analyze 15 isolates of *Fusarium oxysporum f. sp. ciceri*, the causal agent of chickpea wilt. The study found that RAPD markers were able to distinguish all 15 isolates, with an average RP of 93%. This suggests that RAPD markers are a powerful tool for differentiating *Fusarium oxysporum* isolates. In another work, 20 isolates of Fusarium oxysporum f. sp. lycopersici, the cause of tomato wilt, were examined by El-Kassas *et al*. (2008) using RAPD markers. The research discovered that RAPD markers, with an average RP of 95%, were capable of differentiating all 20 isolates. This provides more evidence in favour of using RAPD markers to distinguish Fusarium oxysporum isolates. However, it is important to note that the RP of RAPD markers can vary depending on the number and type of markers used, as well as the genetic diversity of the isolates being analyzed. In addition, RAPD markers are dominant markers, meaning that they cannot distinguish between heterozygous and homozygous individuals. This can be a limitation in some studies, such as those that are investigating the inheritance of traits. Overall, RAPD markers are a powerful tool for differentiating *Fusarium oxysporum* isolates. They have a high RP and are relatively easy to use. However, it is important to note that the RP of RAPD markers can vary depending on the experimental conditions, and that RAPD markers are dominant markers.

In the current study, the highest effective multiplex ratio (EMR) value (10.1) was recorded with primer OPB-01 while the lowest value (3.12) was observed with primer P-005 with an average value of 6.39 per primer. The markers that showed higher polymorphism exhibited higher EMR. One way to measure genetic diversity is to use the effective multiplex ratio (EMR). The EMR is a measure of the number of different alleles at a given locus in a population. A study of *Fof* populations in California found that the EMR ranged from 1.5 to 3.0, with an average of 2.2. This suggests that there is a moderate level of genetic diversity in *Fof* populations in California (Elmer *et al.,* 2010). Additional study of *Fof* populations in Europe found that the EMR ranged from 1.2 to 3.0, with an average of 2.0. According to Elmer *et al.* (2014), this indicates that the genetic diversity of *Fof* populations in Europe and California is comparable.

The results of our study revealed that the marker index (MI) values ranged from 0.72 to 4.14 for primers P-006 and OPB-01, respectively, with an average of 1.96 per primer. The diversity index (DI) values per polymorphic band ranged from 0.08 to 0.38 for primers P-006 and OPB-01, respectively. MI has been used to assess genetic diversity in *Fusarium oxysporum f. sp. frageria*, the causal agent of Fusarium wilt of strawberry. In a study by Nagarajan *et al.* (2004), RAPD markers were used to assess genetic diversity in 50 isolates of *F. oxysporum f. sp. frageria* from different geographical locations in India. The MI values for the different isolates ranged from 0.83 to 0.97, with an average value of 0.91. This shows that F. oxysporum f. sp. frageria isolates in India exhibit a significant degree of genetic diversity%. In another study, by Smujo *et al.* (2017), SRAP markers were used to assess genetic diversity in 30 isolates of *F. oxysporum f. sp. frageria* from different geographical locations in Indonesia. The MI values for the different isolates ranged from 0.78 to 0.92, with an average value of 0.85. This indicates that F. oxysporum f. sp. frageria isolates in Indonesia also exhibit a significant degree of genetic diversity. The MI has been used to assess the genetic diversity of *Fof* populations from a variety of geographical regions. For example, a study of *Fof* isolates from Spain found that the MI ranged from 0.47 to 0.93, with an average of 0.76. This suggests that *Fof* populations in Spain have a high level of genetic diversity (López-Herrera *et al* 2005). Another study of *Fof* isolates from Iran found that the MI ranged from 0.228 to 0.442, with an average of 0.330. This suggests that *Fof* populations in Iran have a lower level of genetic diversity than *Fof* populations in Spain **(**Pourghasemi *et al*, 2014). The high MI values observed in these studies suggest that MI is a useful measure of genetic diversity in *Fof*. because it considers both the number of alleles per marker and the informativeness of the marker. It is also a relatively easy measure to calculate, and it can be used with a variety of different marker systems. Overall, the MI is a useful tool for assessing the genetic diversity of *Fof* populations. It can be used to compare the genetic diversity of *Fof* populations from various sources, such as different cultivars of strawberry or different geographical regions.

In respect of the diversity index, different studies found that *Fof* populations are highly diverse, with a large number of different alleles present. The diversity indices varied depending on the population studied, but they were generally high, indicating that *Fof* populations are well-adapted to a variety of different environmental conditions **(**Al-Mughrabi *et al.*, 2021, Dubey *et al*, 2020, Fouly *et al*, 2019).

b. Genetic relationship and cluster analysis

The genetic relationship among the *Fusarium* isolates was estimated based on Jaccard's pairwise similarity matrix coefficient as described by the dendrogram shown in (Figure 2). The minimum and maximum genetic similarity values obtained by RAPD markers for *Fof* isolates ranged from 0.62 to 0.92 with an average of 0.75. The dendrogram generated from RAPD data grouped the ten isolates in two main clusters. The first one contains isolates R1, R8, R10, R9, R3 and R2 while the second includes isolates R5, R6, R4 and R7. The highest similarity index (0.92) was shown between isolates R8 and R10 followed by isolates R8 and R10 with R9 with similarity index value 0.90. On the other hand, the isolates R4 and R7 showed the lowest similarity index value (0.75) followed by R5 and R6 with (0.76). Some studies have shown that the genetic relationship and similarity index of *F. oxysporum f. sp. frageria* isolates can vary depending on the geographical origin of the isolates. For instance, Alabouvette *et al* (1995) discovered that French isolates were more genetically similar to one another than isolates from other nations. On the other hand, a study using RAPD markers found that *F. oxysporum f. sp. frageria* isolates from different geographic regions were clustered together, suggesting that there is some degree of isolation between

populations. However, the study also found that there was a high degree of genetic diversity within each region, suggesting that the fungus can adapt to local conditions (Al-Aghbari and Abdel-Kawi, 2018, Chen *et al*., 2016). Using ISSR and SSR markers, another study discovered that isolates of F. oxysporum f. sp. frageria could be divided into two main clusters based on their genetic similarity. But the study also discovered that each cluster has a high level of genetic variety, indicating that the fungus is continuously developing (Zhang *et al.,* 2018). A study of *Fof* isolates from Brazil by Alves *et al*., (2018) using seven SSR markers revealed a similarity index ranging from 0.29 to 0.93. A study of *Fof* isolates from India by Singh and Srivastava (2017) using RAPD and ISSR markers revealed a similarity index ranging from 0.38 to 0.96. These results suggest that there is a wide range of genetic diversity among *Fof* isolates. The information on the genetic relationship and similarity index of *F. oxysporum f. sp. frageria* isolates can be used to develop strategies for managing this pathogen. For example, if it is known that certain isolates are more closely related to each other, these isolates may be more likely to share virulence factors. This information could be used to develop targeted control measures for these isolates. The higher the similarity index between two isolates, the more closely related they are. Similarity indices are often used to group isolates into clusters, which can help to identify populations of *F. oxysporum f. sp. frageria* that are more likely to cause disease.

Protein Analysis

Protein analysis is a valuable tool for identifying different races of *Fusarium oxysporum f. sp. frageria* and tracking the spread of the disease. It can also be used to compare *Fof* isolates from different geographical regions to better understand the population dynamics of this fungus. The protein analysis of *Fusarium oxysporum f. sp. frageria* isolates is important for several reasons. First, it can help to identify new strains of the fungus and to track the spread of existing strains. Second, it can help to understand the genetic diversity of the fungus and to identify potential targets for disease control. Third, it can help to develop new diagnostic tools and vaccines. In this study, isolated protein of the ten *Fusarium oxysporum f. sp. frageria* isolates were electrophoretic analyzed on SDS-PAGE (Figure 3 and 4). The protein pattern data were subjected to the Jaccard's similarity to assess genetic relationship among the fungal isolates. The resulted dendrogram from protein banding patterns divided into two main clusters. The first one with about 44% similarity which includes only the isolate R7. While the second cluster contains the rest of the isolates (R1, R2, R4, and R10). The second cluster subdivided into two branches with about 54% similarity. The first branch collected the isolates R3 and R8 together and the second branch contains two subbranches. The first subbranch includes the isolates R1, R4, R2, and R10 and other one collected the isolates R5, R6 and R9. The similarity index values ranged from 44% to 96. The Maximum similarity index was between R1 and R4 isolates with 96% similarity. Protein analysis can also be used to track the spread of Fof. For example, researchers in the Netherlands used protein analysis to track the spread of Fof race 2 in strawberry fields. They found that the race 2 isolates were very similar to each other, suggesting that they had been introduced into the Netherlands from a single source (Scherm *et al.* 2008). A study by Al-Sadi *et al.* (2018) used gel electrophoresis to compare the protein profiles of 10 *Fof* isolates from different locations in Jordan. They found that the isolates had a high degree of similarity, with a mean similarity percentage of 85%. However, there was some variation between isolates, suggesting that there may be different strains of *Fof* present in Jordan. Another study by Chen *et al.* (2019) used mass spectrometry to compare the protein profiles of 20 *Fof* isolates from different locations in China. They found that the isolates had a mean similarity percentage of 90%. However, they also identified some unique proteins in some isolates, suggesting that there may be different strains of *Fof* present in China.

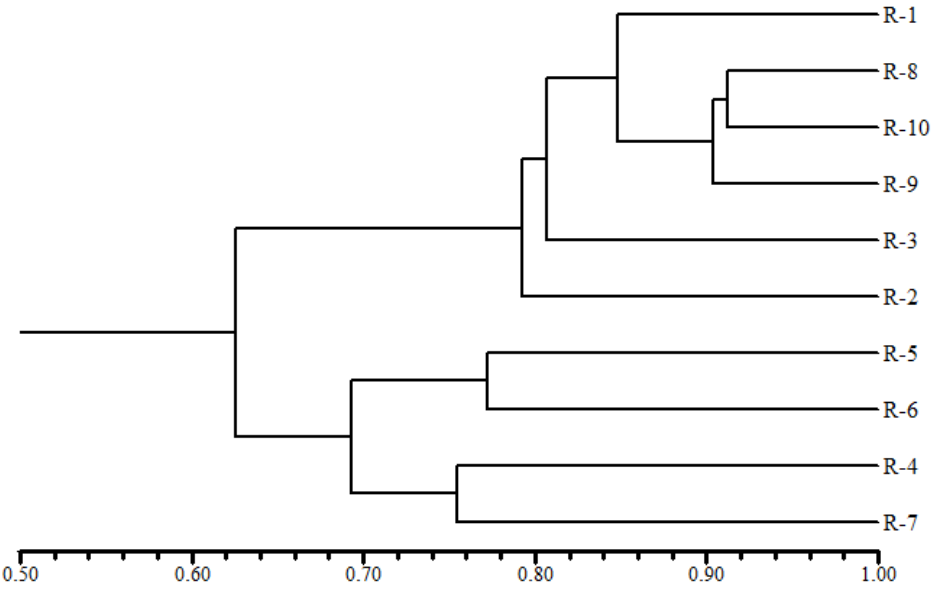

Figure 2: Dendrogram derived from cluster analysis (UPGMA) showing genetic relationship among the ten *Fusarium oxysporum f. sp. frageria* isolates. Genetic similarity was obtained based on RAPD markers and simple matching coefficient. (R: represents the fungal isolate numbered from 1-10).

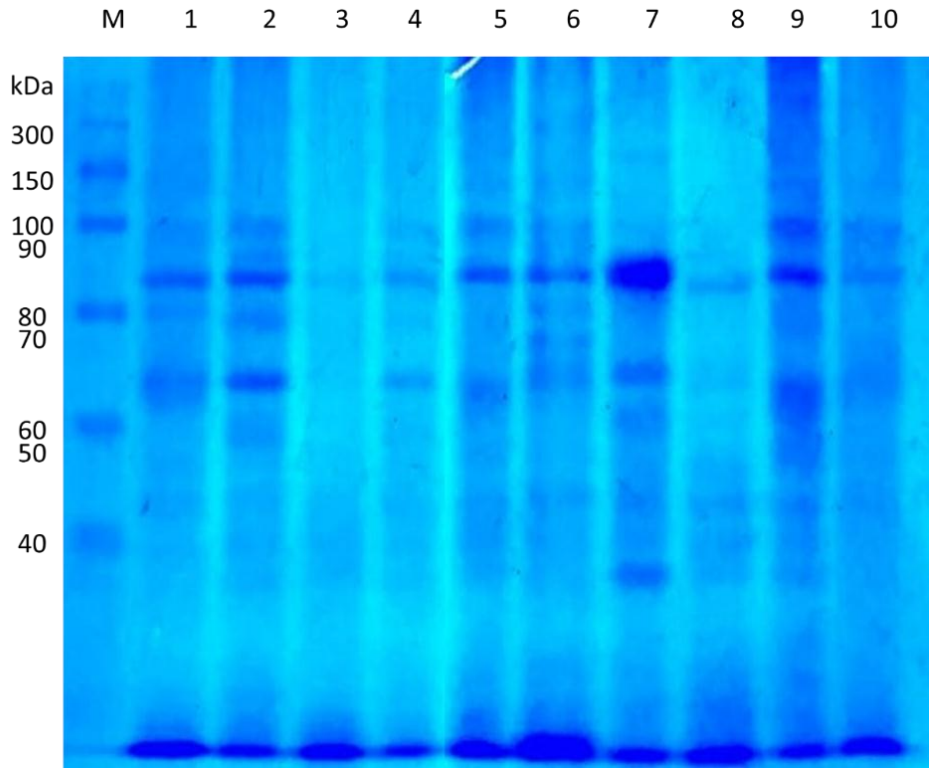

Figure 3: SDS-PAGE total protein band patterns on a Coomassie blue-stained gel for *Fusarium oxysporum f. sp. frageria* isolates. (M: protein ladder, 1 – 10: fungal isolates).

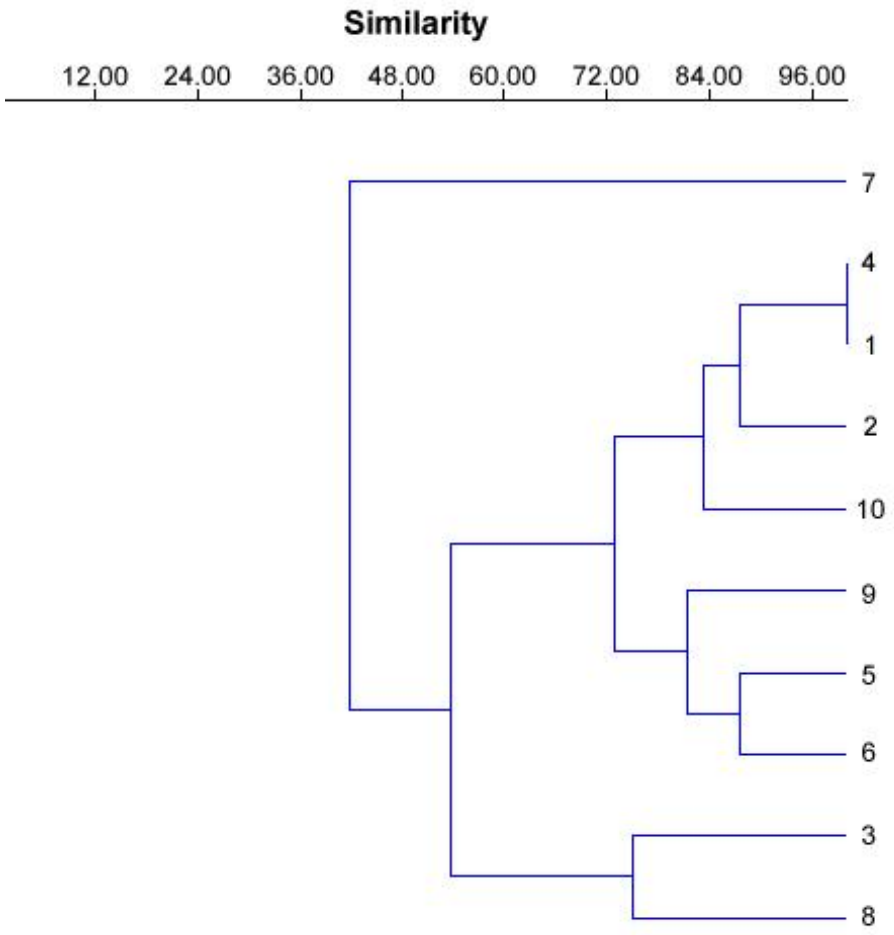

Figure 4: Dendrogram of protein analysis conducted with ten different *Fusarium oxysporum f. sp. frageria* isolates.

These studies suggest that protein analysis can be a useful tool for identifying and characterizing *Fof* isolates. However, more research is needed to determine if there is a correlation between protein variation and pathogenicity.

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التقييم الجزيئى والكيمائى لفطر الفيوزاريوم باستخدام دلائل الحمض النووي متعدد الأشكال المضخم العشوائي والبروتين على الفراولة

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الملخص العربى

يمكن استخدام تعدد الأشكال الجيني لدراسة التنوع الجيني للمجموعات الفطرية، وكذلك العلاقة بين العزلات والسلالات الفطرية المختلفة. هدفنا في هذه الدراسة هو استخدام تحليل الحمض النووي متعدد الأشكال العشوائي وتحليل البروتين لتوصيف ١٠ عزلات من الفيوزاريوم تم الحصول عليها من خمسة مواقع مصرية مختلفة. أظهرت النتائج أن نسبة التعدد الأشكال تراوحت بين ٦٢.٥ إلى ٩١.٧، بمتوسط ٨١.١ لكل بادئ. من حيث محتوى المعلومات متعدد الأشكال تراوحت مداها من ٠.٣ الى ٠.٤١ بمتوسط ٠.٣٥. فيما يتعلق بقدرة التمييز تراوحت من ١.٦ الى ٦.٦ بمتوسط ٣.٦ لكل بادئ. انتج البادئ الثانى اعلى قيمة لنسبة التعدد الفعال ١٠.١ ، بينما اعطى البادئ الخامس اقل قيمة ٣.١٢ ، بمتوسط ٦.٣٩ بكل بادئ. تراوحت قيمة مؤشر الدليل من ٠.٧٢ الى ٤.١٤ بمتوسط ١.٩٦ لكل بادئ. فيما يتعلق بقيم مؤشر التنوع لكل نطاق متعدد الاشكال فإنها تراوحت من ٠.٠٨ الى ٠.٣٨ لكل من البادئ السادس والثانى على التوالى. قيم التشابه الوراثى تراوحت من ٠.٦٢ الى ٠.٩٢ بمتوسط ٠.٧٥ . قسمت العضر عزلات الى مجموعتين رئيسيتين. وضعت العزلات ارقام ١، ٨، ١٠، ٩، ٣، ٢ فى المجموعة الاولى، بينما العزلات ارقام ٥، ٦، ٤، ٧ وضعت فى المجموعة الثانية. أظهرتا العزلتان رقم ٨، ١٠ اعلى مؤشر تشابه بقيمة ٠.٩٢%. بينما العزلتان رقم ٤، ٧ اظهرتا اقل نسبة تشابه ٠.٧٥. فيما يتعلق بقيم نتائج حزم البروتين نجد انها قسمت العزلات تحت الدراسة الى مجموعتين رئيسيتين الاولى تحوى العزلة رقم ٧ فقط مع درجة تشابه ٤٤ % مع باقى العزلات والمجموعة الثانية تحوى باقى العزلات بدرجة تشابه ٥٤% والتى بدورها انقسمت الى قسمين، القسم الاول يضم العزلتان رقم ٣ ، ٨ بينما القسم الثانى تفرع الى تحت قسمين، الاول يضم العزلات رقم ١، ٤، ٢، ١٠ والاخر يضم العزلات رقم ٥، ٦، ٩. تراوحت قيم مؤشر التشابه بين ٤٤% الى ٩٦%.

يمثل التباين الوراثى الواسع بين العزلات تحديا كبيرا بتطوير استراتيجيات فعالة لمكافحة الامراض. لذلك، استخدمت دراستنا دلائل الحمض النووي متعدد الأشكال المضخم العشوائي والبروتين لتقييم التنوع الوراثى لفطر الفيوزاريوم والتى يمكن ان تكون أدوات مفيدة لفهم العلاقة بين العزلات المختلفة كقاعدة لدراسة العلاقة بين التنوع الوراثى والقدرة المرضية لتطوير برنامج مناسب ضد مختلف الانماط المرضية فى المستقبل.