Molecular Characterization of *Fusarium oxysporum* f. sp. *niveum*: The Cause of Wilt Disease on Watermelon

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Abstract: The present investigation aimed to add more information on the characterization of *F. oxysporum* f. sp. *niveum* (Fon), the cause of vascular wilt disease on its corresponding host watermelon, *Citrullus lanatus.* Fifty nine isolates of Fon were isolated from vascular wilted watermelon plants collected from different regions in four watermelon producing governorates. Pathogenecity test of the revealed isolates proved to be pathogenic on watermelon Giza 1 cultivar. The pathogenic potential was varied from isolate to isolate and from location to another. Isolates No. 5, 24, 44, 50, 54 caused the highest disease incidence and disease severity, while the least virulent isolates were No. 8, 25, 32, 43, 47, and 51. Six watermelon cultivars were tested towards 4 virulent isolates of Fon. Both cultivars F18463 and F18470 were the most resistant to the tested isolates. However, Giza 1 was the most susceptible one in this respect. The rest of the tested cultivars showed an intermediate response towards the tested isolates of Fon. RAPD technique was used to distinguish between 4 virulent isolates and 2 avirulent ones of the tested Fon. Genetic variation was detected between such virulent and avirulent isolates. Out of five primers, three primers could differentiate between the tested isolates. The resultant dendrogram showed the presence or absence of the genetic relatedness between them. Such genetic variation was clearly discussed in the manuscript.
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1. Introduction

Watermelon *Citrullus lanatus* (Thunb.) Matsum. & Nakai is the third largest vegetable crop in the world. It is one of the most important vegetable crops in Egypt. Watermelon plants are exposed to many fungal diseases which affected the quantity and quality of the crop yield. Wilt disease caused by *Fusarium oxysporum* f. sp. *niveum* (Fon) is one of the most economical diseases in many countries including Egypt (Smith, 1894; Melchers, 1931; Fahmy and El-Menshawy, 1932, Angelov, 1976, Martyn, 1996, Zang *et al*., 2005 and Lin *et al*., 2009). This fungus is very specific and does not infect even closely related plants such as cucumber and muskmelon. (Fon) is soil borne facultative pathogen, which forms resilient spores that can remain viable in the soil for many years. In the last years, (Fon) developed resistant races to specific fungicides used in controlling the disease. *Fusarium* wilt is difficult to manage; long rotations may lessen the survival rate of the fungus spores in the soil and decrease the severity of symptoms. Resistance to *Fusarium* wilt in watermelon cultivars is not complete; that is, some plants within the "resistant cultivar" may be susceptible. However, a susceptible cultivar contains a higher percentage of susceptible plants than resistant cultivar. (Kucharek, *et al*., 2000).

Since more than two decades, interest has been started among plant pathologists in the use of the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique for population and phylogenetic studies in *F. oxysporum* and other filamentous fungi (Grajal-Martin *et al.,* 1993; Shi *et al.,* 1996; Woo *et al.,* 1996). RAPD analysis is based on DNA fingerprinting of the genomes of interest, using PCR and carried out with decamer oligonucleotides as arbitrary primers (Welsh and McClelland, 1990; Williams *et al.,* 1990). Electrophoresis of the amplified DNA regions can display distinct banding-patterns due to differences in a primer-binding site (s) or within the amplified regions of the template DNA. Fungal isolates displaying the same electrophoretic patterns, when tested with several arbitrary primers, have high genetic similarity, indicating that they constitute a genetically distinguishable group (Assigbetse *et al*., 1994). The RAPD-PCR technique, especially in combination with Southern blot analysis, can provide information on the taxonomy and properties of strains, valuable for the monitoring and control of fungal pathogens (Crowhurst *et al.,* 1991). The current work was designed to study the molecular variation of certain isolates of Fon as well as to test the resistance of some watermelon cultivars commonly grown in Egypt to *Fusarium* wilt disease.

2. Materials and Methods

2.1- Isolation and Identification of Causal Organism:

Isolation trial was carried-out from naturally infected watermelon plants showing wilt symptoms and collected from watermelon-growing governorates

(Kafr El-Sheikh, Al-Behira, Beni Sueif, Ismailia). Roots of the diseased plants were washed with running tap water to remove any adhering soil particles, cut into small pieces (0.5 cm in length). Tissues of the infected pieces were disinfested using sodium hypochlorite solution (3.0%) for 3 minutes, then rinsed several times in sterilized distilled water and dried using sterilized filter paper. The sterilized pieces were transferred to petri plates containing potato dextrose agar medium (PDA) supplemented with streptomycin sulphate. Plates were incubated at 27°C for 72 hours. The recovered fungal colonies were transferred onto another fresh PDA plates and kept for purification. Pure cultures were obtained by single spore isolation technique grown on water agar. The purified cultures were identified according to their morphological features using the key given by Booth 1971.

2.2- Phathogenicity test:

The pathogenicity of a set of fifty nine isolates of Fon was tested on watermelon, Giza 1 cultivar in a pot experiment in the greenhouse. Clay pots, 30 cm in diameter, were sterilized by immersing in 5% formalin solution for 2 hrs, then left 3 days out door for formalin evaporation. Autoclaved sandy loam soil was served as sowing medium. Inoculum of each isolate was prepared by growing the isolate on autoclaved barley grains in 500 ml bottles for 15 days at 27 °C (Martyn, 1986). Soil infestation was carried-out by adding the pervious inocula to each pot at the rate 5 % of the soil weight. Sterilized barley grains at the same rate were added to a set of pots to serve as control. Pots were watered every other day for 10 days to ensure the establishment of the isolates in the soil. Four seedlings (21 days old) of watermelon (Giza 1) were transplanted in each pot and five pots were used for each isolate. Irrigation was carried-out on 2 days intervals. Data were recorded as percentage of wilted plants as disease incidence (after 60 and 90 days) and disease severity after 90 days from transplanting date. Disease severity on plants was assessed by a scale rating from 0 to 5 according to Egel, 2001 as the following:

0- A healthy plant without wilt signs.
1- A plant with wilt of individual leaves.
2- A plant with individual leaves with necrotic symptoms.
3- A plant with one vine of the plant with wilt symptoms.
4- An entire plant with wilt symptoms.
5- A dead plant.

Disease severity (%) was calculated using the following formula:

Disease severity (%) = Σns/5N x100

Where: n = number of plants in each category, s = numerical values of symptoms category, N= total number of plants, 5 = maxim of numerical values of symptoms categories.

2.3- Reaction of Some Watermelon Cultivars to *Fusarium* Wilt Disease:

Six watermelon cultivars were used, *i.e.*, Aswan hybrid, Giza 1, and Lord hybrid, as commercial cultivars in Egypt and F18463, F18470 and F18485 as new foreign hybrids. Autoclaved soil was infested with inocula of the pathogenic fungal isolates which prepared as mentioned before in the pathogenicity test at the rate of 5% of soil weight (w/w). The sterilized plastic pots (35 cm in diameter) were filled with autoclaved sandy loam soil. Five seedlings (21 days old) from each cultivar were transplanted in each pot. Three pots were served for each cultivar as replicates. Disease incidence was recorded as percentage of wilted watermelon plants after 60 and 90 days from transplanting date.

2.4- Molecular Characterization of Six isolates of (Fon) Using Random Amplified Polymorphic DNA (RAPD) Technique:

Six isolates of (Fon) No.: 5, 24, 44, 54 (highly virulent), 8 and 51 (avirulent) were selected to reveal any molecular variation between them.

2.4.1- Extraction and purification of DNA:

The selected isolates of (Fon) were grown for 10 days at 28±2°C in 250 ml flasks containing 100 ml Czapek´s liquid medium. Mycelium of each isolate was harvested by filtration, washed several times with sterile distilled water and blotted dry. DNA) was isolated and purified from each isolate according to the method given by Bowen *et al.*, 1996.

2.4.2- Polymerase Chain Reaction (PCR) and Amplification Conditions:

Amersham/Pharmacia Ready-to-Go PCR Bead® was used to perform PCR reaction. Five decamer random primers (Table 1) were used (supplied by Metabian Gmb H, Lena-Christ-Strassa 44, d 82 152 Martinsried/Deutschland) to distinguish the molecular variation between the selected Fon isolates.

Table (1): Catalog Number and Nucleotides Sequence of the Tested 5 Decamer Random Primers.

Ser. No.	Cat. No.	Sequence
1	AB1-05	5´- CTGAGACGGA-3′
2	AB1-03	5´- AGGACACTGC-3′
3	AB1-08	5´- AAGGATCAGG-3′
4	AB1-11	5´- CAGGCCCTTC-3′
5	AB1-09	5´- ACCACCTGGC-3′

Ten µl containing 40 ng DNA / reaction were used as template to determine the molecular variation between the tested six isolates of (Fon). Then, a volume 5 µl of each random primer containing 12 ng

were added to each **Ready-to-Go PCR Bead®** reaction tube. The final work volume was completed to 25 μl using sterile distilled water. The amplification protocol was carried out according to Qiagen, 2000.

2.4.3 -PCR Product Analysis:

The amplified DNAs were electrophorated using Hoefer HE 99 X Max submarine electrophoresis unit on 2 % agarose and 1X TBE buffer at consistent 100 Volt for about three hrs. The different bands size were determined using 100 bp ladder from Boehringer Mannheim. The developed bands were stained with Ethedium Bromide solution (10 mg/ml) and documented using Polaroid Instant camera provided with a UV Transeliminator.

2.4.4- Phylogenetic Tree Construction:

The banding patterns generated by RAPD-PCR analysis were compared to determine the genetic relatedness of (Fon) isolates. The amplified fragments were scored either as present (1) or absent (0). Bands of the same mobility were scored as identical. The similarity coefficient (F) between two isolates was defined by the formula of Nei and Li (1979), $F= 2N_{XY}/N_X+N_Y$, where: N_{XY} is the number of common bands between the isolates, and N_X and N_Y are the number of bands in isolates X and Y respectively. A dendrogram was derived from the distance by the unweighted paired-group method of arithmetic mean (UPGMA) algorithm contained in the computer program NTSYS PC 2.0 (Rohlf, 2000).

3. Results

3.1-Isolation and Identification of Fon:

Fifty nine isolates of (Fon) were isolated from diseased watermelon plants showed vascular wilt symptoms. Diseased watermelon plants were collected from four different locations in four watermelon growing governorates, (Kafr El-Sheikh, Al- Behira, Beni Sueif and Ismailia). The recovered isolates were 19, 20, 10, 10 from Kafr El-Sheikh, Al-Behira, Beni Sueif, and Ismailia respectively. Each of these isolates was identified as Fon according to the morphological features described by Booth, 1971.

3.2-Pathogenicity Test:

An experiment was carried out in order to test the pathogenic potential of the Fon isolates using watermelon common commercial cultivar Giza 1. Data were recorded as percentage of wilted plants (% disease incidence) after 60 and 90 days and (% disease severity) after 90 days from transplanting date. Data in Table 2 indicated that the tested isolates of (Fon) were pathogenic to the tested watermelon cultivar (Giza 1). They caused significant increase in disease incidence (after 60 and 90 day from transplanting date). Data also showed that isolate 5 from Kafr El-Sheikh, isolate 24 from Al-Behira, isolate 44 from Beni Sueif and isolates 50 and 54 from Ismailia were the most aggressive and gave the highest percentage of disease incidence and severity without significant differences among them in most cases. However, the lowest percentage of disease incidence and severity were resulted by isolates 8, 25, 32, 43, 47 and 51 after 60 and 90 days from transplanting date. The rest of isolates had an intermediate position between the highest and the lowest values of disease incidence and severity after 60 and 90 days from transplanting date with significant differences between them. In addition, the obtained data revealed also that the isolates from the same governorate significantly varied in their pathogenic properties. For example, isolates from 1 to 19 obtained from Kafr El-Sheikh governorate varied in disease incidence and severity at both dates after 60 and 90 days from planting date. Similary, the twenty isolates from Al-Behira governorate caused 12.5 - 62.5% disease incidence after 60 days from transplanting date and 25.0 - 77.5 % disease severity after 90 days from the transplanting date.

In conclusion, all isolates of (Fon) were pathogenic and significant variations were detected in their pathogenic properties even between those isolated from diseased plant materials collected from the same governorate (s). In addition, isolates 5, 24, 44, 50 and 54 were the most virulent in this respect.

Table (2): Pathogenicity test of diffrent (Fon) isolates on watermelon (Giza 1).

Isolate No	Governorate	Disease Measures and Plant Age		
		Disease Incidence (60 days)	Disease Incidence (90 days)	Disease Severity (90 days)
1	Kafr El-Sheikh	18.75	25.00	26.25
2		12.50	31.25	25.00
3		37.50	50.00	38.75
4		43.75	68.75	81.75
5		62.50	100.00	95.25
6		25.00	43.75	38.75
7		12.50	18.75	20.00
8		06.25	12.50	11.75
9		18.75	25.00	23.75
10		06.25	18.25	20.50

Table (2): (Continued)

Isolate No	Governorate	Disease Measures and Plant Age		
		Disease Incidence (60 days)	Disease Incidence (90 days)	Disease Severity (90 days)
11	Kafr El-Sheikh (Cont.)	31.25	56.25	37.00
12		37.50	43.75	34.50
13		12.50	31.25	28.75
14		31.25	50.00	71.25
15		43.75	62.50	57.50
16		37.75	56.25	32.50
17		43.75	62.50	71.25
18		06.25	31.25	16.25
19		37.50	56.25	58.75
20	Al-Beheira	43.75	68.75	63.75
21		31.25	43.75	37.75
22		43.75	62.50	32.50
23		12.50	31.25	27.50
24		62.50	100.00	96.75
25		18.75	37.50	30.00
26		31.25	50.00	36.25
27		43.75	62.50	65.00
28		12.50	43.75	36.25
29		37.50	68.75	61.25
30		18.75	34.75	57.50
31		25.00	68.25	58.75
32		12.50	31.25	25.00
33		18.75	37.50	31.25
34		18.75	37.50	32.50
35		43.75	68.75	68.75
36		50.00	68.75	71.25
37		06.25	81.25	77.50
38		31.25	56.25	57.00
39		43.75	68.75	65.00
40	Beni Sueif	37.50	56.25	58.75
41		31.25	50.00	42.50
42		18.75	37.50	28.75
43		12.50	43.75	37.50
44		62.50	100.00	96.50
45		25.00	56.25	36.25
46		18.75	37.50	21.25
47		12.50	43.75	27.50
48		18.75	43.75	38.75
49	Ismailia	50.00	68.75	72.50
50		62.00	93.75	91.25
51		12.50	25.00	21.25
52		18.75	37.50	33.75
53		31.25	50.00	35.00
54		68.75	100.00	88.75
55		31.25	56.25	68.75
56		37.50	56.25	53.75
57		31.25	43.75	46.25
58		31.25	62.50	62.50
59		37.50	56.25	47.50
Control		00.00	00.00	00.00

3.3- Reaction of some watermelon cultivars To *Fusarium* wilt Disease:

Six watermelon cultivars namely, Giza 1, Aswan hybrid, Lord hybrid, F18463, F18470 and F18485 were tested for their reactions to infection with four aggressive isolates (5, 24, 44 and 54) of Fon. Data presented in Table 3 showed that there were significant differences between the responses of each of the tested cultivars towards the tested Fon isolates. Both cultivars F18463 and F18470 were the most resistant to the tested Fon isolates. However, Giza 1 was the most susceptible one in this respect. The rest of the tested cultivars had an intermediate response towards the tested isolates of Fon.

Table (3): Susceptibly of six watermelon cultivars under artificial inoculation of four different isolates of Fon.

Cultivar	Isolate 5		Isolate 24		Isolate 44		Isolate 54	
	60 days	90 days	60 days	90 days	60 days	90 days	60 days	90 days
	Disease incidence %	Disease severity%	Disease incidence%	Disease severity %	Disease incidence%	Disease severity%	Disease incidence%	Disease severity%
Aswan hybrid	55.00	99.40	50.00	88.00	25.00	42.00	25.00	47.00
Giza 1	75.00	100.00	95.00	92.00	45.00	87.00	60.00	95.20
Lord hybrid	50.00	100.00	65.00	66.00	45.00	77.00	15.00	46.00
F18463	45.00	100.00	25.00	22.00	10.00	32.00	05.00	21.00
F18470	20.00	75.00	30.00	29.00	10.00	21.00	25.00	37.00
F18485	30.00	100.00	70.00	67.00	30.00	57.00	55.00	87.00

3.4- Relationship between six isolates of (Fon) using Random Amplified Polymorphic DNA (RAPD) technique.

Polymerase chain reaction (PCR) was carried out using five decameric random primers in order to determine the genetic relatedness between the six isolates of (Fon). Three decameric primers (AB 1-03, AB 1-05, AB 1-11) produced polymorphic bands between the tested Fon isolates, while the other two did not. The obtained results showed that each primer generated distinct RAPD pattern differed than the others (Figures 1, 2, 3). The recovered phylogenetic tree (dendrogram) (Figure 4) according to the RAPD-PCR bands showed that there were genetic distances between the studied Fon isolates. The phylogenetic tree was divided into two main clusters. The first cluster contained isolates 8, 51, and 54. Both isolates 51 and 54 were genetically similar. Their similarity coefficient was 68.4%. However, isolate 8 was genetically far distant from isolates 51 and 54. The similarity coefficient between those three isolates was 57.5%. The second cluster contained isolates 5, 24, and 44. Both isolates 5 and 24 were genetically similar. Their similarity coefficient was 74%. However, isolate 44 was genetically highly far distant from the other two isolates in this respect. The similarity coefficient between those three isolates was 54.6 %.

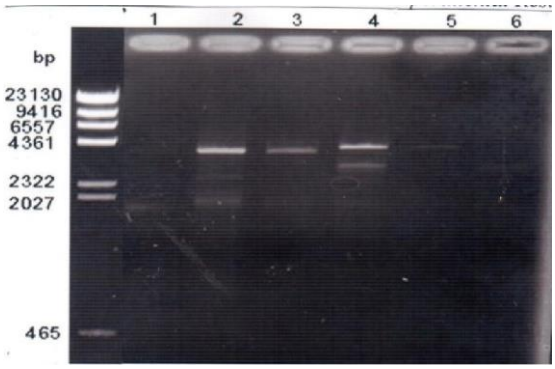

Fig 1: Random Amplified DNA polymorphisms of six isolates of (Fon) with primer AB 1-03 (5´-AGGACACTGC-3´): DNA bands were separated on a 1.5% agarose gel and stained with ethidium bromide 1= isolate 8; 2= isolate 5; 3= isolate 24; 4 = isolate 44; 4= isolate 54 and 6= isolate 51.

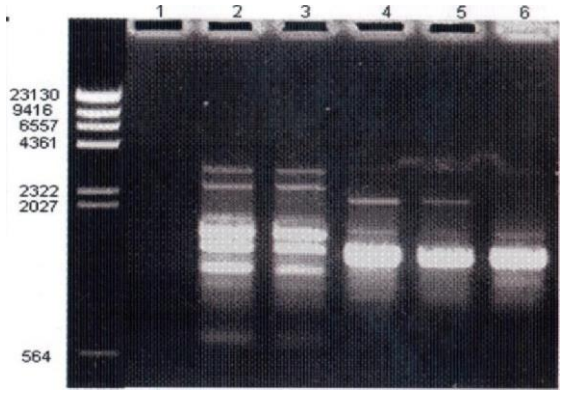

Fig.2: Random Amplified DNA polymorphisms of six isolates (Fon) with primer AB 1-05 (5´-CTGAGACGGA-3´): DNA bands were separated on a 1.5% agarose gel and stained with ethidium bromide 1= (Fon) isolate 8; 2=isolate 5; 3= isolate 24; 4= isolate 44; 5= isolate 54 and 6= isolate 51

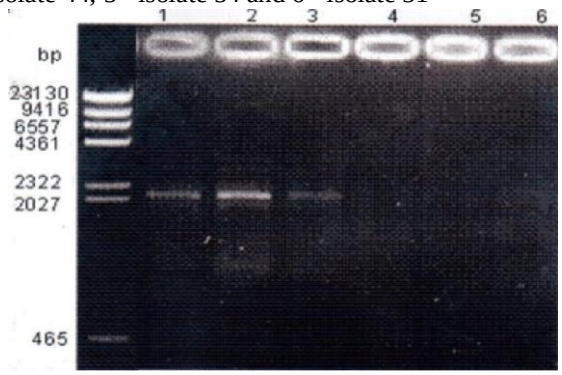

Fig.3: Random Amplified DNA polymorphisms of six isolates of (Fon) with primer AB 1-11 (5´-CAGGCCCTTC-3´): DNA bands were separated on a 1.5% agarose gel and stained with ethidium bromide 1= isolate 8; 2= isolate 5; 3= isolate 24; 4 = isolate 44; 4= isolate 54 and 6= isolate 51.

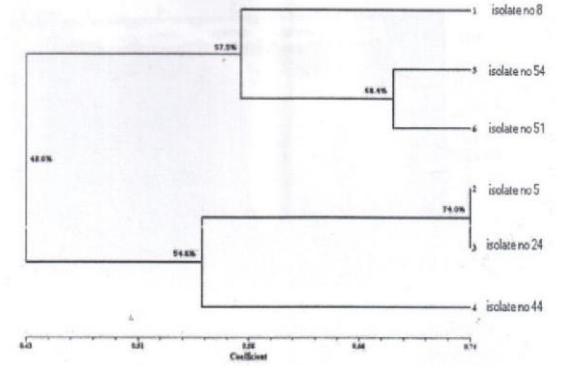

Fig.4: Dendrogram showing polymorphisms of DNA six isolates of (Fon) 1= isolate 8; 2= isolate 5; 3= isolate 24; 4= isolate 44; 5= isolate 54; 6= isolate 51 revealed by UPGMA sub cluster analysis of jaccard genetic similarity coefficients calculated from RAPD (primers 1, 2, 4).

4. Discussion

Fusarium wilt caused by *F. oxysporum* f.sp. *niveum* (E. F. Smith) Snyder & Hansen (Fon) is an important watermelon (*Citrullus lanatus*) disease in watermelon growing countries worldwide. The disease can cause complete yield loss when susceptible cultivars are grown (Egel and Martyn, 2013). Disease symptoms include vascular discoloration, especially around crown and upper taproot and withering and wilting leaves followed by death of either a vine or whole plant (Armstrong and Armstrong, 1978 and Martyn, 1996). In the current studies, four (5, 24, 44, 54) out of 59 isolates collected from four watermelon growing Egyptian governorates were proved to be highly virulent on the Egyptian Giza 1 cultivar. In addition, disease resistance of 5 watermelon cultivars was tested towards the former 4 isolates. Both cultivars F18463 and F18470 were the most resistant to the tested Fon isolates. However, Giza 1 was the most susceptible one in this respect. The rest of the tested cultivars had an intermediate response towards the tested isolates of Fon. RAPD analysis was a useful tool for characterizing genetic variation among the six isolates of Fon including four virulent isolates (5, 24, 44, 54) as well as two avirulent isolates (8, 51). Genetic variation was observed among such isolates using five random primers. Out of five primers, three primers differentiate only some of the studied isolates. Results of primers 1, 2, and 4 were statistically analyzed together into one dendogram. Such results were supported by Turner *et al*., 1998 who reported that the RAPD analysis was able to recognize between *F. avenaceum* and *F. tricinctum*. Also, Vakalounakis and Fragkiadaki, 1999 confirmed the current work by differentiating between 106 isolates of *F. oxysporum* using RAPD analysis. They reported that RAPD technique was effective in distinguishing isolates of *F. oxysporum* f.sp. *radicis cucumerinum* from those of *F. oxysporum* f. sp. *cucumerinum.* Similar results were obtained by Saleh 1997 who could distinguish between avirulent and virulent isolates of *F. oxysporum* f.sp. *lycopersici* using RAPD technique. He also, could recognize between race1 and race2 as well as some Egyption and American isolates of *F. oxysporum* f. sp. *lycopersici*.

In conclusion, the differentiation of isolates of vascular wilt pathogen of watermelon based on pathogenicity and molecular characterization can ultimately help in our endeavor of managing this economic disease.

References

1. Angelov, D. 1976. *Fusarium* wilt of watermelon, a new disease in Bulgaria. (Abstr.). Rev. Plant Pathol. 55: 5406.

2. Armstrong, G. M. and Armstrong, J. K. 1981. Formae specials and races of *Fusarium oxysporium* causing wilt disease of the *Cucurbitacea*. Phytopathology 68: 19-28.
3. Assigbetse, K. B., Femandez, D., Duhois, M.P. and Geiger, J. P. 1994. Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. Phytopathology 84: 622-626.
4. Booth, C. 1971. The genus *Fusarium*. The Eastern Press, London, pp.147-149.
5. Bowen, J. K., Franicevic, S. C., Crowhurst, R. N., Templeton, M. D., Stewart, A. 1996. Differentiation of a specific Trichoderma biological control agent by restriction fragment length polymorphism (RFLP) analysis. New Zealand Journal of Crop and Horticultural Science 24: 207-217.
6. Crowhurst, R. N., Hawthorne, B. T., Rikkerink, E. H. A. and Templeton, M. D. 1991.
7. Differentiation of *Fusarium solani* f.sp. *cucurbitae* 1 and 2 by random amplification of polymorphic DNA. Current Genetics 20:391-396.
8. Egel, D. S. 2001. Evaluation of fumigants for the control of *Fusarium* wilt and root-knot nematode of watermelon. Fungicide and Nematicide Test Report N19.
9. Egel, D. S. and Martyn, R. D. 2013. *Fusarium* wilt of watermelon and other cucurbit crops. Online Publication. Plant Health Instruct. doi:10.1094/PH-I-2007-0122-01.
10. Fahmy, T. and El-Menshawy, I. 1932. Wilt of watermelon. Report of Agriculture Research, Ministry of Agriculture, Egypt.
11. Grajal-Martin M.J., Simon, C.S., and Muehlbauer, F. J. 1993. Use of random amplified polymorphic DNA (RAPD) to characterize race 2 of *Fusarium oxysporum* f. sp. *pisi*. Phytopathology 83: 612-614.
12. Kucharek, T., Jones, J. P., Hopkins, D. L. and Strandberg, J. 2000. Some diseses of vegetable and agronomic crops caused by *Fusarium* in Florida. Circle-1025. Florida Cooperative Extension Service, IFAS, University of Florida.
13. Lin, Y., Chen, K., Huang, J. and Chang, P. 2009. Development of a molecular method for rapid differentiation of watermelon lines resistant to *Fusarium oxysporum* f. sp. *niveum*. Botanical Studies 50: 273-280.
14. Martyn, R. D. 1986. A new race of *Fusarium* watermelon wilt fungus. HortScience 21: 941-943.
15. Martyn, R. D. 1996 *Fusarium* wilt of watermelon. In: Zitter, T. A., Hopkins, D. L., Thomas, C. E. (eds) Compendium of Cucurbit Diseases. The American Phytopathological Society, St. Paul, pp 13-14.
16. Melchers, L.E. 1931. A check list of plant diseases and fungi occurring in Egypt. Tranaction of Kansas Acad. Sci. 34 Qiagen. 2010. *Taq* PCR handbook. www.qiagen.com.
17. Rohlf, F. J. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 2.1. Setauket (NY): Exeter software.
18. Saleh, A. A. 1997. Genetic variability within *Fusarium* oxysporum f.sp. lycopersici. M. Sc. Thesis. Faculty of Science, Cairo University.
19. Shi, Y. L., Loomis, P., Christian, D., Canis. L.M. and Leung, H. 1996. Analysis of the genetic relationship among the wheat bunt fungi using RAPD and ribosomal DNA markers. Phytopathology 86: 311-318.
20. Smith, E. F. 1894. The watermelon disease of the south. Proc. Amer. Assoc. Adv. Sci. sec. G43: 289-290.
21. Turner, A. S., Lees, A. K., Rezanoor, H. N. and Nicholson, P. 1998. Refinement of PCR-detection of *Fusarium avenceum* and evidence from DNA marker studies for phonetic relatedness to *Fusarium tricinctum*. Plant Patholgy 47:278-288.
22. Vakalounakis, D. J. and Fragkiadakis, G. A. 1999. Genetic diversity of *Fusarium oxysporum* isolates from cucumber: differentiation by pathogenicity, vegetative compatibility and RAPD fingerprinting. Phytopathology 89:161-168.
23. Welsh, J. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18: 7213-7218.
24. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, J. V.1990.
25. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.
26. Woo, S. L, Zoina, A., del Sorbo, G., Lorito, M., Nanni, B., Scala, F. and Noviello, C. 1996. Characterization of *Fusarium oxysporum* f. sp. *phaseoli* by pathogenic races, VCGs, RFLPs, and RAPD. Phytopathology 86: 966-973.
27. Zhang, Z., Zhang, J., Wang, Y. and Zheng, X. 2005. Molecular detection of *Fusarium oxysporum* f. sp. *niveum* and *Mycosphaerella melons* in infected plant tissues and soil. FEMS Microbiol. Lett. 249: 39-47.

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