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Fusarium oxysporum f.sp. *cumini*, the Causal of Cumin Wilt in Egypt: Vegetative Compatibility Groups, Virulence and Geographical Distribution

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One hundred and eighty eight nitrate non-utilizing (*nit*) mutants were recovered from 52 isolates of *F. oxysporum* f.sp. *cumini*(F.O.C.) cultured on three media: potato-dextrose chlorate (PDC), potato sucrose chlorate (PSC) and minimal chlorate (MMC). Out of these mutants, 159, 17 and 12 were *nit* 1, Nit M and *nit* 3, respectively. The majority (80.32 %) of *nit* mutants were generated on PSC medium compared with 14.89 and 4.78 % on PDC and MMC, respectively. Not all three phenotypes classes were recovered from all isolates and the frequency of occurrence of each mutant type varied from isolate to another. "n*it"* mutants were used to force heterokaryon formation to determine vegetative compatibility groups (VCGs) and their relation to virulence and geographic origin of (F.O.C.). Twelve distinct VCGs were identified among 25 isolates of (F.O.C.)and the other 27 isolates were incompatible with any of the identified VCGs, reflecting the higher genetic diversity among the isolates of (F.O.C.). There was no definite relationship between VCG groups, virulence and geographic origin among isolates. According to the available literature, these are the first notes on the genetic diversity of *F. oxysporum* f.sp. *cumini*,the causal of vascular wilt disease of cumin in Egypt.

Keywords: Cumin, *F. oxysporum f.sp. cumini*, Genetic diversity,*nit* mutants and Vegetative compatibility groups.

Fusarium wilt of cumin (*Cuminum cyminum* L.), caused by *F. oxysporum* f.sp. *cumini*, was recorded in young and mature plants throughout cumin-growing regions in southern Egypt (Arafa, 1985,Hilal*et al*., 1993, Tawfik and Allam, 2004 a, b and Baiuomy *et al*., 2008). This disease is one of the major limiting factors for stable production of cumin, since resistant cultivars have not been developed yet and

effective control measures are not available. Cumin fields were heavily infested through the continuous planting of the crop in the same field. A clear understanding of the pathogenic population of the fungus is required to allow plant breeders to effectively screen for *Fusarium* resistance in cumin and enhance the potential to manage the disease. Heterokaryosis or vegetative compatibility analysis provides the opportunity to study the genetic diversity of several fungal genera, including *Fusarium* (Leslie, 1996;Mofrad*et al*., 2005 and Mohammadi and Mofrad, 2009).

Puhalla (1985) found a correlation between vegetative compatibility group (VCG) and formae specials of *F. oxysporum*, *i.e.* members of a VCG belong to the same forma specials. He suggested that when the sexual stage and meiotic recombination in *F. oxysporum* were lost, the loci that determine vegetative incompatibility (*vic* or *het* loci) and virulence became closely linked. Therefore, VCG within forma specials significantly associated with the virulence level of the isolates in *F. moniliforme*, *F. oxysporum* f.sp. *cucumerinum* and *F. oxysporum* f.sp. *melonis* (LaMondia and Elmer, 1989,Ahn*et al*., 1998 andAhn and Lee, 2000). These results suggest that VCG analysis could be used as an alternative marker for virulence variations of fungal populations within *Fusarium* species. In addition, VCGs have been used to study the origins and affinities among fusaria as plant pathogenic fungi (Ploetz and Shepard, 1989). Isolates of *F. oxysporum* in the same VCG are supposed to be a clone, even if they are geographically separated from each other (Leslie, 1990).

The objectives of this study were to investigate genetic diversity within the population of *F. oxysporum* f.sp. *cumini* in Egypt with respect to the vegetative compatibility and to determine the relationship between VCGs, virulence and geographic distribution.

Materials and Methods

Recovery of nitrate non-utilizing (nit) mutants:

Fifty-two isolates of *F. oxysporum* f.sp. *cumini*, obtained from different cumin field areas in many regions of Middle and Upper Egypt in Beni-Sueif, Minya and Assiut governorates (Ghebrial, Eman, 2013), were used in this experiment. Each isolate was grown on minimal medium (MM) for 7-10 days at 25^{o}C. A mycelial transfer (5-mm MM disc) of each isolate was put in the center of a petriplate (6 cm. diam.) containing either minimal medium chlorate (MMC), potato dextrose chlorate (PDC) or potato sucrose chlorate (PSC) (Puhalla, 1985 and Correll *et al*., 1987). The plates were incubated at 25^{o}C and examined periodically for the appearance of fast-growing sectors from the initially restricted colony. The concentration of $KClO_3$ was increased to 3-5% when needed for isolates that were not restricted with 1.5% $KClO_3$ (Jacobson and Gordon, 1988). Growth of wild-type isolates is restricted on $KClO_3$, presumably because it is reduced by nitrate reductase to highly toxic chlorite. Nitrate non-utilizing mutants are unable to reduce chlorate to chlorite and hence are chlorate-resistant. All sectors were transferred to MMand those showing a thin growth with no aerial mycelia were considered *nit* mutants, indicated that the sectors were also unable to reduce nitrate. Isolates that reverted to dense aerial

mycelia on MM were considered chlorate-resistant, nitrate-utilizing isolates and discarded.

Nit mutant phenotypes:

The *nit* mutants were assigned to different phenotypic classes based on their growth on media containing one of five different nitrogen sources (1) Nitrate, (2) Nitrite, (3) Hypoxanthine, (4) Ammonium tartarate and (5) Uric acid. To determine the physiological phenotype, a mycelial transfer (5-mmMM disk of 7 days old culture) of the *nit* mutant was put on each of the five media. The plates were incubated at 25^{o}C and the colony morphology was scored relative to the wild-type parent after 4 days. Mutant was designated *nit* 1, *nit* 3 or Nit M according to Correll *et al*. (1987) following the suggestions of Yoder *et al*. (1986). A mutant was designated as:

- *nit* 1: if it showed a thin growth on nitrate but wild-type growth on the othernitrogen sources.
- *nit* 3: if it showed a thin growth on both sodium nitrate and nitrite and wild-type growth on ammonium tartarate, hypoxanthine and uric acid.
- Nit M: if it showed a thin growth on both sodium nitrate and hypoxanthine and wild-type growth on the other nitrogen sources.

Complementation tests:

Vegetative compatible *nit* mutants may complement one another by forming a heterokaryon on MM, *i.e.* dense aerial growth develops where mycelia of the two *nit* mutant colonies come in contact, anastomose and form a heterokaryon. A portion of the Nit M mutants derived were placed individually on MM plates (9 cm. diam.) and paired against *nit* 1 and *nit* 3 mycelium from other strains, which placed at an equal distance from the Nit M colony forming a triangle configuration. Complementation between *nit* 1 and *nit* 3 mutants occurs less frequently than complementation between one of these mutants with a Nit M mutant (Loffler and Rumine, 1991). Each Nit M was paired with a *nit* 1 and a *nit* 3 from other strains in all possible combinations. At least three plates were prepared for each pairing. Pairings were incubated at 25^{o}C, examined periodically for 7-14 days, and then scored for complementation. Heterokaryons were usually evident within 7 days. Some pairs of mutants reacted faster (4-6 days), whereas few required up to 14 days to form visible heterokaryons (Katan and Katan, 1988). In addition, all of the *nit* mutants recovered from the same parent were paired with one another to test for self-incompatibility. Also, some *nit* mutants of the same phenotype were paired (Correll *et al*., 1987).

Complementation was evident by the formation of a dense aerial growth similar to that of the wild type where two mutants had met and formed a heterokaryon, while mycelia outside the anastomosed region remained thin. Absence of wild-type growth at the contact zone between two *nit* mutants of the same parent isolate indicated allelic, overlapping or otherwise noncomplementary mutation or vegetative self-incompatibility. On the other hand, absence of wild-type growth at the contact zone between *nit* mutants from different parent isolates indicated either

non-complementary or an inability to form heterokaryons due to a lack of vegetative compatibility. When mutants of different isolates formed a heterokaryon, their parent isolates were assigned to the same VCG (Katan *et al*., 1994).

Results

1. Recovery of nitrate non-utilizing(nit) mutants:

Spontaneous chlorate resistant sectors were readily recovered from all 52 isolates of *F. oxysporum* f.sp. *cumini* when cultured on three media, *i.e.* MMC, PDC and PSC containing chlorate as a toxic analogue of nitrate. Initially, growth of the isolates on these media were highly restricted compared to un-amended media but, after 7-10 days, fast growing sectors developed from the fungal colonies (one to four per colony from most isolates). The majority of the chlorate-resistant sectors recovered was unable to utilize nitrate as a sole of nitrogen source and consequently grew as thin expansive colonies with no aerial mycelium on (MMC) (Fig. 1).These sectors were designated *nit* mutants. A few sectors were recovered from some isolates that were chlorate-resistant but able to utilize nitrate. The frequency at which chlorate-resistant sectors were produced depended upon the isolate, type of medium and the amount of chlorate in the medium, and generally, *nit* mutants were easily generated from most isolates. A total of 188 nitrate non-utilizing (*nit*) mutants were recovered from the 52 isolates of *F. oxysporum* f.sp. *cumini* on the three media amended with 1.5% $KClO_3$ (Table 1). There were also great differences in sectoring frequency of each isolate. In general, mutants yield was higher on PSC than PDC and MMC media. Nearly 151 (80.32%) of *nit* mutants were generated on PSC, while PDC and MMC generated 28 (14.89%) and 9 (4.78%) mutants, respectively. All *nit* mutants grew as wild type when transferred to PDA; but when they were again sub-cultured from PDA to minimal medium with nitrate, their growth remained appressed. Observation of the plates no correlation was found between the number of chlorate-resistant sectors and the geographic origin or aggressiveness of isolates.

Table 1. Frequency of nitrate non-utilizing (*nit*) mutantsrecovered from 52 isolates of *F. oxysporum* f.sp. *cumini* on three chlorate media

Chlorate media	No. of *nit* mutants in media amended with 1.5 % $KClO_3$	Percentage of *nit* mutants
Potato sucrose chlorate (PSC)	151	80.32
Potato dextrose chlorate (PDC)	28	14.89
Minimal media chlorate (MMC)	9	4.78
Total	188	-

1- *"nit"*mutants phenotypes identification:

The *nit* mutants could be divided into three phenotypic classes: *nit* 1, *nit* 3 and *nit*M. Growth was assessed as being wild type (+) or starved: thin expansive colonies with no aerial mycelium (-) (Table 2). All the *nit* mutants had wild type morphology on medium containing an ammonium salt and produced thin growth on nitrate medium. The *nit* mutants that produced wild type growth on both nitrite and hypoxanthine-containing media were classified as *nit* 1; those with wild type growth on medium with hypoxanthine or nitrite as the sole N source were identified as *nit* 3 and *nit*M, respectively (Fig. 2).

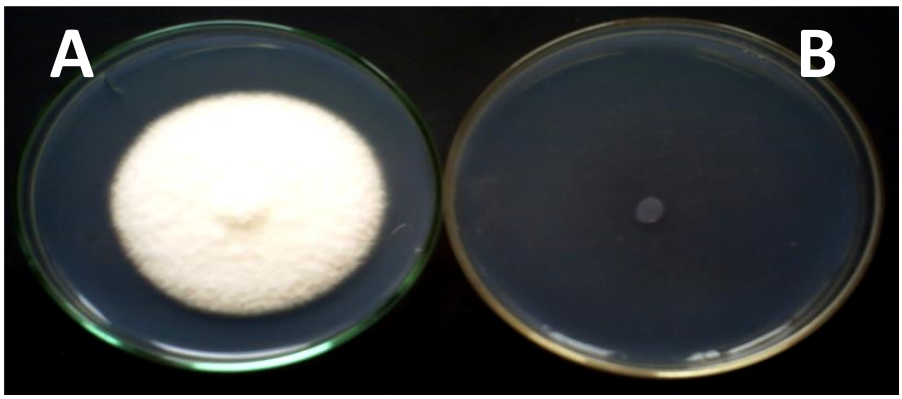

Fig. 1. Growth of wild-type parental strain of *F.oxysporum* f.sp. *cumini* on minimal medium (A) and nitrate nonutilizing (*nit*) mutant; note normally expansive, but very thin growth (B) after 7 days of incubation at 25°C.

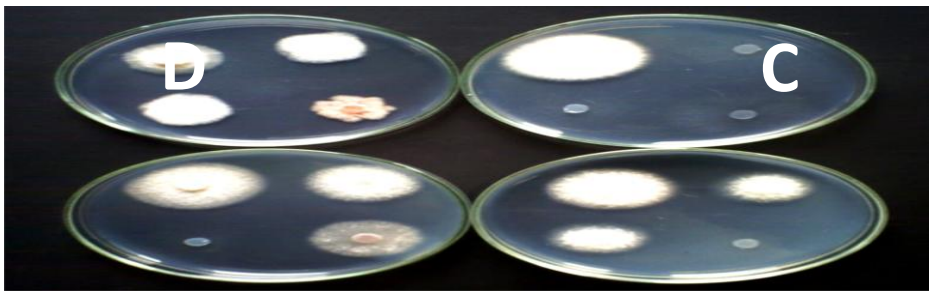

Fig. 2.Growth of wild-type parental strain of *F. oxysporum* f.sp.*cumini* and 3 nitrate non-utilizing (*nit*) mutant phenotypes on different nitrogen source media. On each plate: upper left, wild-type; upper right, *nit* 1; lower right, *nit* 3; lower left, *nit* M. A: Ammonium medium, B: Nitrate medium with thin growth of mutants, C: Nitrite medium with thin growth of *nit* 3

Table 2. Identification of nitrate non-utilizing (*nit*) mutants recovered from 51 fungal isolates by growth on different nitrogen sources

Mutation[a]	Mutant designation	Growth on nitrogen sources[b]				
		Nitrate	Nitrite	Ammonium tartarate	Hypoxanthine	Uric acid
None	Wild-type	+	+	+	+	+
Nitrate reductase structural locus	*nit* 1	-	+	+	+	+
Pathway-specific regulatory locus	*nit* 3	-	-	+	+	+
Molybdenum cofactor loci	*nit*M	-	+	+	-	+

a- According to Garrett & Amy (1978) and Marzluf (1981).

b- *Growth on minimal medium with various nitrogen sources:*(+) Dense (typical wild-type growth); (-) Thin, transparent growth without aerial mycelium.

The three *nit* mutant classes were not recovered with equal frequency with the majority of *nit* mutants recovered being *nit* 1 irrespective of which medium was used. Among the 188 *nit* mutants, there were 159 classified as *nit* 1; 12 as *nit* 3 and 17 as*nit*M (Table 3). The frequency of *nit* 1 mutants was higher on PSC (82.39%) than on PDC (13.21%) or MMC (4.40%). The same trend was observed with *nit* 3 and *nit*M, where the frequency of *nit* 3 and *nit*M was higher on PSC (83.33% & 58.82%) than those on PDC and MMC which recorded 8.33% & 35.29% and 8.33% & 5.88%, respectively. In addition, *nit* 1 was recovered from 50 isolates, while *nit* 3 from 11 isolates and 12 isolates yield *nit*M mutants (Table, 4).

Table 3. Frequency of phenotypic classes of nitrate non-utilizing (*nit*) mutants recovered from 52 fungal isolates grown on three chlorate-media

		Chlorate media					
Phenotypic classes	Total number	PSC		PDC		MMC	
		nit mutants (No.)	*nit* mutants (%)	*nit* mutants (No.)	*nit* mutants (%)	*nit* mutants (No.)	*nit* mutants (%)
nit 1	159	131	82.39	21	13.21	7	4.40
nit 3	12	10	83.33	1	8.33	1	8.33
*nit*M	17	10	58.82	6	35.29	1	5.88
Total	188	151	-	28	-	9	-

Table 4. Phenotypes of *nit* mutants recovered from 52 fungal isolates

No. of isolates (A)	No. of *nit* mutants examined (B)	classes of *nit* mutants			(A)	(B)	classes of *nit* mutants		
		nit 1	*nit* 3	*nit*M			*nit* 1	*nit* 3	*nit*M
F_1	3	3	0	0	F_{27}	3	3	0	0
F_2	3	1	0	2	F_{28}	2	1	1	0
F_3	4	2	0	2	F_{29}	2	1	1	0
F_4	8	6	0	2	F_{30}	2	2	0	0
F_5	6	5	0	1	F_{31}	3	3	0	0
F_6	6	5	0	1	F_{32}	7	6	1	0
F_7	2	2	0	0	F_{33}	4	4	0	0
F_8	4	4	0	0	F_{34}	1	1	0	0
F_9	2	2	0	0	F_{35}	5	5	0	0
F_{10}	2	2	0	0	F_{36}	5	5	0	0
F_{11}	1	1	0	0	F_{37}	1	0	1	0
F_{12}	2	1	1	0	F_{38}	4	3	1	0
F_{13}	3	2	1	0	F_{39}	3	2	0	1
F_{14}	3	3	0	0	F_{40}	2	2	0	0
F_{15}	2	1	1	0	F_{41}	6	5	1	0
F_{16}	3	3	0	0	F_{42}	2	2	0	0
F_{17}	10	9	0	1	F_{43}	4	3	0	1
F_{18}	3	3	0	0	F_{44}	2	0	0	2
F_{19}	5	4	1	0	F_{45}	5	5	0	0
F_{20}	2	2	0	0	F_{46}	4	1	2	1
F_{21}	2	2	0	0	F_{47}	2	2	0	0
F_{22}	4	4	0	0	F_{48}	1	1	0	0
F_{23}	4	4	0	0	F_{49}	2	2	0	0
F_{24}	2	2	0	0	F_{50}	11	10	0	1
F_{25}	2	2	0	0	F_{51}	10	8	0	2
F_{26}	4	4	0	0	F_{52}	3	3	0	0
Total	-	-	-	-	Total	188	159	12	17

2- Complementation analysis:

Physiological complementation among *nit* mutants with different phenotypes was indicated by the development of dense aerial growth where the mycelia of the *nit* mutant colonies came in contact and anastomosed to form a heterokaryon, *i.e.*vegetatively compatible isolates formed a zone of wild-type growth (aerial mycelium) where the borders of expanding *nit* mutant colonies met, while vegetatively incompatible isolates did not (Fig. 3). Several *nit* mutants which derived from 52 fungal isolates were selected from each isolate and paired in all possible intra and inter-isolate combinations (4,880 combinations) to determine complementation within and between isolates (Table 5).

Table 5. Summary of complementation tests between nitrate non-utilizing (nit) mutants of *F.oxysporum* f.sp. *cumini* isolates.

No. of isolates	Phenotypic classes	F1	F2	F5	F6	F7	F11	F17	F22	F23	F25	F30	F31	F34	F35	F38	F39	F41	F43	F45	F46	F48	F49	F50	F51
		nit1	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit1*	*nit3*	*nit1*	*nit1*	*nit1*	*nit1*
F_2	*nit*1 Nit M		+++	+++	+++			+++																	
F_4	*nit*1 Nit M																						+		
F_5	*nit*1 Nit M	++		++				+++																	
F_6	*nit*1 Nit M	+++	+++	+++	+++			++																	
F_7	*nit*1					+																			
F_{17}	*nit*1 Nit M	+++	+++		+++			+++																	
F_{22}	*nit*1								+																
F_{23}	*nit*1									+++															
F_{25}	*nit*1 *nit3*																	+++							
F_{39}	*nit*1 *nit3*																	+++							
F_{41}	*nit*1																				+				
F_{57}	*nit*1 *nit3*																							+	+
F_{39}	*nit*1 Nit M					++										+++	++								
F_{43}	*nit*1 Nit M		++	++	+++			+++											+++						
F_{46}	*nit*1 *nit3* Nit M																			+++	+++ +++				
F_{50}	*nit*1 Nit M																					+		+ +	
F_{51}	*nit*1 Nit M						|																	++

+++ Strong heterokaryotic reaction.
++ Moderate heterokaryotic reaction.

+ **Weak heterokaryotic reaction.**

Complementary pairs of mutants were differed in the speed and vigor of heterokaryon formation. Mutants deficient in the molybdenum cofactor (*nit*M) rapidly formed robust, heterokaryotic growth with mutants from other complementation groups (*nit* 1 or *nit* 3). Nit M was more vigorous when pairing with *nit* 1, *nit* 3 mutants in all possible combinations and the majority of complementation groups were identified among the *nit*M mutants. Heterokaryon formation was recorded for up to 15 days, the reactions could usually be clearly defined as either positive (complementation) or negative (no complementation). The intensity of heterokaryon growth could also be different between *nit* 1 x Nit M combinations of a given isolate. Pairing between *nit* 1 mutants and *nit* 3 mutants, on the other hand, developed heterokaryotic growth more slowly. Frequently, even after 3 weeks, the complementation reaction was weak (very little aerial mycelium). Some *nit* 1 mutants were able to complement one another, as could some *nit* 3 and *nit*M mutants.

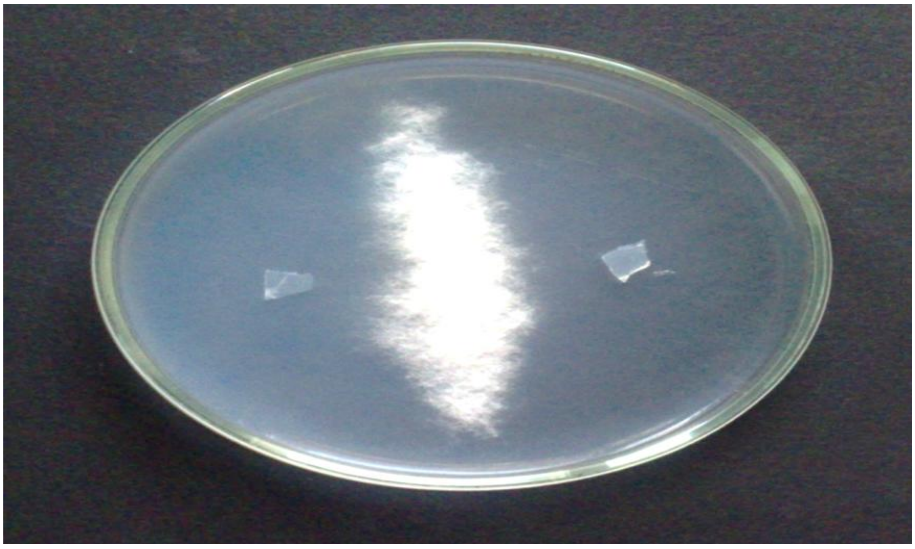

Fig. 3. A pairing between the complementary mutants among two isolates*:* *nit*1 mutant of isolate F_{17} (Right) paired with *nit*M mutant of isolate F_{43} (Left) on minimal medium after 11 days of incubation at 25 ^{o}C. The heavy, white line of growth where the two mutants colonies contact indicates heterokaryon formation

Based on pairing complementary *nit* mutants of all isolates, mainly with *nit* 1 and *nit* M; *nit* 1 and *nit* 3 and *nit* 3 and *nit* M, 25 out of the 52 isolates were compatible with each other to some extent and grouped into 12 VCGs. No complementation was found among the 27 rest isolates (Table 6). The vast majority (6 isolates) were

arbitrarily designated as VCG-A including 4 isolates (F_1, F_2, F_5 and F_6) from Beni-Sueif and 2 isolates (F_{17} and F_{43}) of Minya. Also 2 VCGs groups, each contained three isolates from Minya, were assigned as VCG-B & VCG-C and 4 other VCGs each contained two isolates as follows: VCG-D and VCG-F which comprised F_{20}, F_{41} and F_{48}& F_{50} from Minya, respectively.

Table (6): Vegetative compatibility groups (VCGs) of *F. oxysporum*f.sp. *cumini* isolates and their comparison with geographic origin and virulence

Isolate	Geographic origin	Virulence	VCG group	Isolate	Geographic origin	Virulence	VCG group
F_1	Beni-Sueif	HV	A	F_8	Minya	HV	NC
F_2	Beni-Sueif	HV	A	F_9	Minya	HV	NC
F_5	Beni-Sueif	HV	A	F_{10}	Minya	LV	NC
F_6	Beni-Sueif	HV	A	F_{12}	Minya	V	NC
F_{17}	Minya	HV	A	F_{13}	Minya	HV	NC
F_{43}	Minya	HV	A	F_{14}	Minya	HV	NC
F_{11}	Minya	HV	B	F_{15}	Minya	HV	NC
F_{38}	Minya	HV	B	F_{16}	Minya	HV	NC
F_{39}	Minya	HV	B	F_{18}	Minya	V	NC
F_{34}	Minya	HV	C	F_{19}	Minya	HV	NC
F_{35}	Minya	HV	C	F_{21}	Minya	V	NC
F_{37}	Minya	MV	C	F_{24}	Minya	V	NC
F_{20}	Minya	HV	D	F_{25}	Minya	MV	NC
F_{41}	Minya	HV	D	F_{26}	Minya	HV	NC
F_4	Beni-Sueif	HV	E	F_{27}	Minya	HV	NC
F_{49}	Minya	MV	E	F_{28}	Minya	HV	NC
F_{48}	Minya	HV	F	F_{29}	Minya	HV	NC
F_{50}	Minya	MV	F	F_{32}	Minya	HV	NC
F_{30}	Minya	V	G	F_{33}	Minya	V	NC
F_{51}	Assiut	HV	G	F_{36}	Minya	HV	NC
F_7	Beni-Sueif	V	H	F_{40}	Minya	HV	NC
F_{22}	Minya	HV	I	F_{42}	Minya	HV	NC
F_{23}	Minya	HV	J	F_{44}	Minya	HV	NC
F_{31}	Minya	V	K	F_{45}	Minya	HV	NC
F_{46}	Minya	HV	L	F_{47}	Minya	HV	NC
F_3	Beni-Sueif	HV	NC	F_{52}	Assiut	HV	NC

V: Virulent. LV: Low virulent. MV: Moderately virulent.

HV:Highly virulent. NC: Not compatible with any other isolates.

Isolates F_4 (Beni-Sueif) and F_{49} (Minya) were compatible with each other and designated as VCG-E, while isolate F_{30} (Minya) and F_{51} (Assiut) were able to form weak heterokaryon and grouped into VCG-G. However, isolates F_7 (Beni-Sueif), F_{22}, F_{23}, F_{31} and F_{46} (Minya) were self-compatible but vegetatively incompatible with all other isolates tested. All are described as distinct VCGs (Tables 5 and 6). Complementary*nit* M testers (F_2, F_6, F_{17} & F_{43}), which have demonstrated the ability to form strong heterokaryons with many other mutants, were chosen as local representative tester isolates. The pattern of complementary heterokaryon formation revealed the presence of highly genetic diversity among Egyptian isolates of *F. oxysporum* f.sp. *cumini* as indicated by multiple VCGs.

On the other hand, isolates within a VCG included some that belonged to a different geographic origin, virulence and vice versa. This indicated that vegetative compatibility has no definite relationship with pathogenic behavior and geographic distribution.

3- Heterokaryon self-incompatibility (HSI):

Results in Tables (4and 6) demonstrate that no complementation between any *nit* mutants of F_3 (2 *nit* 1 and 2 Nit M), F_8 (4 *nit* 1), F_9 (2 *nit* 1), F_{10} (2 *nit* 1), F_{12} (1 *nit* 1 and 1 *nit* 3), F_{13} (2 *nit* 1 and 1 *nit* 3), F_{14} (3 *nit* 1), F_{15} (1 *nit* 1 and 1 *nit* 3), F_{16} (3 *nit* 1), F_{18} (3 *nit* 1), F_{19} (4 *nit* 1 and 1 *nit* 3), F_{21} (2 *nit* 1), F_{24} (2 *nit* 1), F_{25} (2 *nit* 1), F_{26} (4 *nit* 1), F_{27} (3 *nit* 1), F_{28} (1 *nit* 1 and 1 *nit* 3), F_{29} (I *nit* 1 and 1 *nit* 3), F_{32} (6 *nit* 1 and 1 *nit* 3), F_{33} (4 *nit* 1), F_{36} (5 *nit* 1), F_{40} (2 *nit* 1), F_{42} (2 *nit* 1), F_{44} (2 Nit M), F_{45} (5 *nit* 1), F_{47} (2 *nit* 1) and F_{52} (3 *nit* 1) was observed, even after repeated attempts. Two types of mutants of themselves were obtained from F_3, F_{12}, F_{13}, F_{15}, F_{19}, F_{28}, F_{29} and F_{32}. They were considered to be heterokaryon self-incompatible. However, with F_8, F_9, F_{10}, F_{14}, F_{16}, F_{18}, F_{21}, F_{24}, F_{25}, F_{26}, F_{27}, F_{33}, F_{36}, F_{40}, F_{42}, F_{44}, F_{45}, F_{47} and F_{52} only one type of mutant was recovered, either *nit* 1, *nit* 3 or Nit M despite different chlorate concentration and different media being used. When no complementation occurred between the mutants of these isolates in themselves, they were also considered as showing heterokaryon self-incompatibility (HSI). These isolates were recovered from Minya except F_3 from Beni-Sueif and F_{52} from Assiut governorates as well as differed in their virulence, so HSI is not exclusive to one location or distinct virulence pattern.

Discussion

Spontaneouschlorate resistant mutantsfrom *F. oxysporum* f.sp. *cumini*were recovered on the three media: MM, PSA and PDAsupplemented with chlorate. The *nit* mutants have also been generated from a number of*Fusarium* species (Puhalla, 1985,Correll *et al.*, 1987, Bowden and Leslie, 1992,Ahn *et al.*, 1998, AhnandLee, 2000,Altierand Groth, 2005,He, 2007 and Mohammadi and Mofrad, 2009). The chlorate-resistant strains (CRSs) grew as thin expansive colonies with no aerial mycelium (*nit* mutants) because they were unable to utilize nitrate present in the

media. In this regard, Kim *et al.* (2005) reported that $KClO_3$ showed toxic against *F. oxysporum* according to restoration by the nitrate reductase. At this time, the apex hyphae separated from the chlorate-resistant sector, which was created from chlorate cultural media, could not form aerial mycelium after transfer the apex hyphae to the minimum culture medium. The *nit* mutant was separated from these since this chlorate-resistant strain could not restore the nitrite from nitrate.

The tested *F.oxysporum* f.sp. *cumini* isolates varied widely in their ability to generate *nit* mutants (1 to 15). Some isolates could not produce thin growth mycelia at 1.5% chlorate, whichinsufficiently inhibited the growth of wild type either after repeated inoculations on chlorate media. In this case, chlorate concentration gradually increased from 1.5 to 4.0% until obtained mutants from these isolates. In this concern, sectoring frequency has been shown to be heritable and to vary among isolates (Klittich and Leslie, 1988). Differences between loci in susceptibility to mutation could be related to the physical size of the gene, with larger genes representing a larger target. Alternatively, some loci may contain sequences, which are mutational "hot spots", rendering them more susceptible to mutation. The wide range of sectoring frequency in plant pathogenic fungi on different concentrations of chlorate has also been suggested as a selective advantage for rapid adaptation to environmental stresses such as fungicides and host resistance (Klittich and Leslie, 1988). Chlorate media with different $KClO_3$ concentrations ranging from 1.5 to 5.0% used by various researchers (Chulze *et al.*, 2000,AltierandGroth, 2005, Chen *et al.*, 2007,Zainudin*et al.*, 2008 and Mohammadi and Mofrad, 2009).

Complementary *nit* mutants recovered from each isolate were categorized into one of the several phenotypic classes by their relative growth on phenotypic media containing different nitrogen sources. These classes of *nit* mutants presumably reflect mutations at a nitrate reductase structural locus (*nit* 1). These mutants cannot grow on nitrate, but can grow on nitrite, hypoxanthine and ammonium as sole of N sources; *nit* 3 mutants were mutated in a locus which was probably involved in the regulation of both nitrate and nitrite reductase, consequently these mutants cannot grow on nitrate or nitrite as sole N sources, but can grow on hypoxanthine or ammonium. Nit M mutants were mutated in loci (at least five) that affect the assembly of a molybdenum-containing co-factor, which is necessary for both the reduction of nitrate and the hydroxylation of hypoxanthine. These mutants cannot grow on nitrate or hypoxanthine as the only N source, but they will grow on nitrite or ammonium (Puhalla, 1985,Klittich *et al.*, 1986 and Correll *et al.*, 1987). Among the188 *nit* mutants,*nit* 1 was recovered at the highest frequency (84.57%), followed by Nit M (9.04%) and *nit* 3 (6.38%). There are two plausible explanations for these results. First, the efficiency of recovering these complementary mutants is low and isolate dependent (average 0.13 per inoculum plug, range 0-0.25; Puhalla, 1985). Therefore, a large number of inoculum plugs per isolate should have been plated on different media supplemented with different concentrations of $KClO_3$ to recover more *nit* 3 and Nit M mutants. Secondly, some isolates could have been heterokaryon self-incompatible (HSI), as defined by Correll *et al.* (1987), though

HSI strains usually occur at low frequency in *F. oxysporum* populations (1-2%, Leslie, 1993; 4 %, Steinberg *et al.*, 1997).

Twelve distinct VCGs were identified among 25 isolates of *F.oxysporum*f.sp. *cumini*. The collection of isolates in this study, although not necessarily representing, the entire population of the pathogen in Egypt, contained seven widespread VCGs and five minor VCGs. Mofrad *et al.*(2005) classified 50 isolates of *F. oxysporum* f.sp. *cumini* into six VCGs: VCG-A comprised 44 isolates (93.6%), while each of the remaining VCGs represented by a single isolate. Also, Mohammadi and Mofrad (2009) grouped 19 isolates of *F. solani*from cumin into 15 VCGs groups: A, B and C have 2, 3 and 2 isolates, respectively, while the others have a single isolate.

The fact that additional 27 isolates of *F. oxysporum* f.sp. *cumini* were incompatible with any of the identified VCGs is evidence for the existence of additional VCGs. These results indicate more genetic diversity in *F. oxysporum* f.sp. *cumini*. It is possible that future work with isolates from other geographic origins may add more VCGs, because the distribution of the VCGs reported in this study was limited to particular sites. Vegetative compatibility groups are considered to be genetically isolated populations (Kistler, 1997). Each VCG may be a single clone (Anderson and Kohn, 1995). Multiple VCGs in *F.oxysporum* f.sp. *cumini* indicate the existence of several clonal lineages. Strains within a VCG are usually more similar genetically than strains distributed across a number of VCGs (Elmer and Stephens, 1989), often share pathological and physiological traits as well as geographical origins (Swift *et al.*, 2002). These attributes can be seen in various species of *F. oxysporum* isolates in which the VCGs were grouped according to the symptoms and locations (Latiffah*et al.*, 2008).

The isolates of one geographical area sometimes exhibits several VCGs, *i.e.* isolates of Beni-Sueif were distributed in VCG-A, E and H, while those of Minya distributed in all VCGs except VCG-H. In addition, VCG-G compressed one isolate (F_{51}) from Assiut, which was compatible with isolate (F_{30}) from Minya. The recovery of isolates in each VCG from diverse locations indicates that this genetically homogeneous population is widespread among geographically separated regions. Similar results have been obtained by Bosland and Williams (1987), Woo *etal.* (1996) and Swift *et al.* (2002), who reported that *F. oxysporum* isolates from crucifers did not exhibit a correlation between VCG and geographical origin. It is presumed that the isolates in the same VCG to be clones even if the isolates are geographically isolated (Leslie, 1990). The occurrence of a VCG in more than one region could be explained by an initial broad distribution of a compatibility group or more recent spread of a VCG from a single geographic origin through seeds, seedlings, debris and human activities (Ahn*et al.*, 1998).

It is interesting to note that the isolates of *F. oxysporum* f.sp. *cumini* characterized by different levels of virulence in the same VCG. Thus, it seems that

there is no relationship between the VCG and level of virulence. VCG-A, B, D, I, J and L were characterized by highly virulent isolates, while the other six VCG groups contained moderate to virulence and highly virulence isolates. These results are in agreement with those of Venter *et al.* (1992), Fiely*et al.* (1995) and Harveson and Rush (1998).

The lack of complementation between phenotypically distinct *nit* mutants recovered from some isolates, even after repeated attempts lead to the designation of these strains as heterokaryon self-incompatibility (HSI). Isolates or strains carrying mutations that prevent them from fusing to form heterokaryons, even with themselves, have been identified in field population of *Fusarium* species such as *F. oxysporum* (Correll, 1991, Katan *et al.*, 1994, Harveson and Rush, 1997, Vakalounakis and Fragkiadakis, 1999 and Latiffah *et al.*, 2008). The difference in frequency of self-incompatibility might be due to the existence or absence of gene (s) or mutations controlling heterokaryon self-incompatibility (Correll *et al.*, 1987 and Klittichand Leslie, 1988). Genetic exchange by parasexual recombination or formation of hybrids is usually reported only between vegetatively compatible isolates. Vegetative incompatibility in fungi may be an evolutionary adaptation to prevent transfer between strains of deleterious or incompatible genetic elements in their cytoplasms (Nauta and Hoekstra, 1994).

Although heterokaryosis is controlled by vegetative compatibility, the growth of complementary heterokaryons is also affected by the growth habit, branching pattern and anastomosis frequency of individual isolates, as well as the biochemical nature of their *nit* mutants (Katan and Katan, 1999). In this study, all the tester isolates representing the different VCGs belonged to Nit M phenotypic class (F_2, F_6, F_{17} and F_{43}) which have demonstrated the ability to form strong heterokaryons with many other mutants. In studies of *F. oxysporum* population, each VCG is represented by a pair of complementary *nit* testers (Puhalla, 1985 and Correll *et al.*, 1987). Since not all *nit* mutants are equally capable of forming complementary heterokaryons, there are two requirements that reliable testers must fulfil. First, at the biochemical level, they must be complementary with all the mutants to be tested in order to avoid false-negative results. The second requirement of reliable testers is a strong capacity to anastomose with other isolates of their VCG. As testers, Nit M mutants derived from strongly anastomosing isolates should serve better than those derived from weakly or slowly anastomosing isolates. Thus, phenotype alone is not sufficient for selecting the most reliable testers (Woudt *et al.*, 1995). For this purpose, candidate mutants should be compared by multiple pairings, and the rarest Nit M mutants, which effectively complement the broadest range of mutants, should be identified.

In conclusion, the multiple VCGs of *F. oxysporum* f.sp. *cumini*, the cause of wilt disease of cumin in Egypt, indicated the existence of substantial genetic diversity, with no definite relationship between VCGs groups, pathogenic behavior and geographic origin.

References

Ahn, I.P., Chung, H.S. and Lee, Y.H. 1998. Vegetative compatibility groups and pathogenicity among isolates of *Fusarium oxysporum* f.sp. *cucumerinum*. *Plant Dis*.,**82**: 244-246.

Ahn, P. and Lee, Y.H.2000. Vegetative compatibility groups and pathogenicity variation among isolates of *Fusarium oxysporum* f.sp. *melonis*. *Plant Pathol. J.*,**16**: 227-230.

Altier, N.A. and Groth, J.V. 2005. Characterization of aggressiveness and vegetative compatibility diversity of *Fusarium oxysporum* associated with crown and root rot of birdsfoot trefoil. *Lotus Newsletter*,**35**: 59-74.

Anderson, J.B. and Kohn, L.M.1995. Clonality in soil-borne plant-pathogenic fungi. *Annu. Rev. Phytopathol.*,**33**: 369-391.

Arafa, M.K.M. 1985. Studies on Fusarium wilt of cumin. M.Sc. Thesis, Fac. Agric., Assiut University, Egypt.

Baiuomy, M.A.M., Nada, M.G.A. and El-Morsy, S.A. 2008. Management of cumin wilt disease using some safe means. *Egypt. J. Appl. Sci*.,**23**(7): 67-85.

Bosland, P.W. and Williams, P.H. 1987. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility and geographical origin. *Can. J. Bot*., **65:** 2067-2073.

Bowden, R.L. and Leslie, J.F. (1992). Nitrate-nonutilizing mutants of *Gibberellazeae* (*Fusarium graminearum*) and their use in determining vegetative compatibility. *Exp. Mycol*.,**16:** 308-315.

Chen, Y.; Wang, J.X.; Zhou, M.G.; Chen, C.J. and Yuan, S.K. 2007. Vegetative compatibility of *Fusarium graminearum* isolates and genetic study on their carbendazim-resistance recombination in China. *Phytopathology*,**97:** 1584-1589.

Chulze, S.N.; Ramirez, M.L.; Torres, A. and Leslie, J.F. 2000. Genetic variation in *Fusarium* section *Liseola* from no-till maize in Argentina. *Appl. Environ. Microbiol.*,**66**: 5312-5315.

Correll, J.C. 1991. The relationships between formaespeciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. *Phytopathology*,**81**: 1061-1064.

Correll, J.C.;Klittich, C.J.R. and Leslie, J.F. 1987. Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology*, **77**: 1640-1646.

Elmer, W.H. and Stephens, C.T. 1989. Classification of *Fusarium oxysporum* f.sp. *asparagi* into vegetatively compatible groups. *Phytopathology*, **79**: 88-93.

Fiely, M.B.;Correll, J.C. and Morelock, T.E. 1995. Vegetative compatibility, pathogenicity, and virulence diversity of *Fusarium oxysporum* recovered from spinach. *Plant Dis.*, **79**: 990-993.

Garrett, R.H. and Amy, N.K. 1978. Nitrate assimilation in fungi. *Adv. Microb. Physiol.*,**18**: 1-65.

Ghebrial, Eman W.R. 2013. Pathological and physiological studies on wilt disease of cumin caused by *Fusarium oxysporum* f.sp. *cumini* and its control.Ph.D. Thesis, Fac. of Sci., Cairo Univ., Egypt, 321p.

Harveson, R.M. and Rush, C.M. 1997. Genetic variation among *Fusariumoxysporum* isolates from sugar beet as determined by vegetative compatibility. *Plant Dis*., **81**: 85-88.

Harveson, R.M. and Rush, C.M. 1998. Characterization of Fusarium root rot isolates from sugar beet by growth and virulence at different temperatures and irrigation regimes. *Plant Dis.*, **82**:1039-1042.

He, X.H. 2007. Bio-control of root rot disease in vanilla. Ph.D. Thesis, Wolverhampton University, China. pp. 46-83.

Hilal, A.A.;Zayed, A.A. and Harridy, I.M.A. 1993. Fusarium wilt of cumin (*Cuminumcyminum* L.) in Egypt: Occurrence and chemical control in relation to fruit and volatile oil yields. Proc. 5th Nat. Conf. of Pests & Dis. of Veg. & Fruits in Egypt, Ismailia, pp.467-480.

Jacobson, D.J. and Gordon, T.R. 1988. Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f.sp. *melonis*. *Phytopathology*, 7**8**: 668-672.

Katan, T. and Katan, J. 1988. Vegetative-compatibility grouping of *Fusariumoxysporum*f.sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. *Phytopathology*,**78**: 852-885.

Katan, T. and Katan, J. 1999. Vegetative compatibility grouping in *Fusarium oxysporum*f.sp. *radicis-lycopersici* from the UK, the Netherlands, Belgium and France. *Plant Pathol.*,**48**: 541-549.

Katan, T.; Katan, J.; Gordon, T.R. and Pozniak, D. 1994. Physiological races and vegetative compatibility groups of *Fusarium oxysporum* f.sp. *melonis* in Israel. *Phytopathology*,**84**: 153-157.

Kim, S.H.; Kim, J.T.; Jang, C.S.;Yoo, S.J. and Kim, H.G. 2005. Vegetative compatibility group of *Fusarium oxysporum* f.sp. *radicis-lycopersici* in Korea. *J.Plant Pathol*.,**21**: 214-220.

Kistler, H.C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum. Phytopathology*,**87**:474-479.

Klittich, C.J.R. and Leslie, J.F. 1988. Nitrate reduction mutants of *Fusariummoniliforme* (*Gibberellafujikuroi*). *Genetics*,**118**: 417-423.

Klittich, C.J.R.;Leslie, J.F. and Wilson, J.D.1986.Spontaneous chlorate-resistant mutants of *Gibberellafujikuroi (Fusarium moniliforme)*.(Abstr.). *Phytopathology*,**76**: 1142.

LaMondia, J.A. and Elmer, W.H. 1989. Pathogenicity and vegetative compatibility among isolates of *Fusarium oxysporum*and *Fusarium moniliforme* colonizing asparagus tissues. *Can. J. Bot.*,**67**: 2420-2424.

Latiffah, Z.; Nur Hayati, M.Z.;Baharuddin, S. and Maziah, Z. 2008. Vegetative compatibility group of *Fusarium* species associated with root and stem rot of Orchid. *Malaysian J. Microbiol.*,**4**: 49-52.

Leslie, J.F. 1990. Genetic exchange within sexual and asexual populations of the genus *Fusarium*. *In Fusarium wilt of banana*, ed. R.C. Ploetz, pp. 37-48. St. Paul, MN: APS Press. 140 p.

Leslie, J.F. 1993. Fungal vegetative compatibility. *Ann. Rev. Phytopathol*., **31**: 127-150.

Leslie, J.F. 1996. Fungal vegetative compatibility-promises and prospects. *Phytoparasitica*,**24**:3-6.

Loffler, H.J.M. and Rumine, P. 1991. Virulence and vegetative compatibility of Dutch and Italian isolates of *Fusarium oxysporum* f.sp. *lilii*. *J. Phytopathol*.,**132**: 12-20.

Marzluf, G.A. 1981. Regulation of nitrogen metabolism and gene expression in fungi. *Microbiol. Rev.*,**45**: 437-461.

Mofrad, N.N.;FarrokhiNejad, R. and Alizadeh, A. 2005. Genetic diversity in populations of *Fusarium oxysporum*f.sp. *cumini*, the causal agent of cumin wilts in Khorasan using vegetative compatibility groups. *Iranian J. Plant Pathol.,* **41**: 437-453.

Mohammadi, A. and Mofrad,N.N. 2009. Genetic diversity in population of *Fusarium solani* from cumin in Iran. *J. Plant Prot. Res.*, **49**: 283-286.

Nauta, M.J. and Hoekstra, R.F. 1994. Evolution of vegetative incompatibility in filamentous ascomycetes. I. Deterministic models. *Evolution*, **48:** 979-995.

Ploetz, R.C. and Shepard, E.S. 1989.Fusarial wilt of banana in Florida. *Mycol. Res.*,**93**:242-245.

Puhalla, J.E. 1985, Classification of strains of *Fusarium oxysporum*on the basis of vegetative compatibility. *Can. J. Bot*., **63**: 179-183.

Steinberg, C.; Edel, V.; Gautheron, N.; Abadie, C.;Vallaeys, T. and Alabouvette,

C.1997.Phenotypic characterization of natural populations of *Fusarium oxysporum*in relation to genotypic characterization.*FEMS Microbiol. Ecol.*,**24**: 73-85.

Swift, C.E.; Wickliffe, E.R. and Schwartz, H.F. 2002. Vegetative compatibility groups of *Fusarium oxysporum*f.sp. *cepae*from onion in Colorado. *Plant Dis.*,**86**: 606-610.

Tawfik, A.A.and Allam, A.D.A. 2004a. Improving cumin production under soil infestation with Fusarium wilt pathogen:I-Screening of biocontrol agents. *Assiut Univ. Bull. Environ. Res.*, **7**: 35-45.

Tawfik, A.A.and Allam, A.D.A. 2004b. Improving cumin production under soil infestation with Fusarium wilt pathogen: II- Field trial of different landraces and seed treatments. *Assiut Univ. Bull. Environ. Res.*,**7**: 47-64.

Vakalounakis, D.J. and Fragkiadakis, G.A. 1999. Genetic diversity of *Fusariumoxysporum* isolates from cucumber: Differentiation by pathogenicity, vegetative compatibility and RAPD fingerprinting. *Phytopathology*,**89:** 161-168.

Venter, S.L.; Theron, D.J.; Steyn, P.J.; Ferreira, D.L. and Eicker, A. 1992. Relationship between vegetative compatibility and pathogenicity of isolates of *Fusarium oxysporum*f.sp. *tuberosi*from potato. *Phytopathology*,**82**: 858-862.

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.

Woudt, L.P.; Neuvel, A.; Sikkema, A.; van Grinsven, M.Q.J.M.; de Milliano, W.A.J.;Campbell, C.L. and Leslie, J.E. 1995. Genetic variation in *Fusarium oxysporum*from cyclamen. *Phytopathology*, **85**: 1348-1355.

Yoder, O.C.; Valent, B. and Chumley, F. 1986. Genetic nomenclature and practice for plant pathogenic fungi. *Phytopathology*, **76**: 383-385.

Zainudin, N.A.I.M.;Sidique, S.N.M. and Salleh, B. 2008. Genetic diversity of *Fusarium fujikuroi* isolated from bakanae disease of rice on the basis of vegetative compatibility. *Pertanika J. Trop. Agric. Sci.*,**31**: 271-278.

مجموعات التوافق الخضرى لفطر *Fusarium oxysporum* f.sp. *cumini* المسبب لمرض ذبول الكمون فى مصر، القدرة المرضية والتوزيع الجغرافي

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أمكن الحصول على عدد 188 طفرة مستغلة لأيون النترات من عدد 52 عزلة لفطر فيوزاريوم أوكسيسبورام كيوميني عند تنميتها عل ى ثلاثة أنواع من بيئات الزرع المتخصصة وهي (MMC)، (PDC) و (PSC). وقد أعطت بيئة البطاطس والسكروز والكلورات (PSC) أعلى نسبة من الطفرات (80.32 %) بالمقارنة ببيئة البطاطس والدكستروز والكلورات وبيئة الحد الأدنى المزودة بالكلورات (14.89 %، 4.78 %) على التوالى . تم تقسيم الطفرات الغذائية إلى ثلاث مجموعات وهي *nit* 1, *nit* 3 and Nit M مع أعلي نسبة تواجد للنوع *nit* 1 (159) حيث قدرت بحوالي 84.57 ٪ بينما كانت نسبة تواجد الطفرة *nit* 3 (17) حوالي 6.38 % و 9.04 % على التوالي. لم يتم الحصول Nit M,(12) على الأنواع الثلاثة من الطفرات من جميع العزلات حيث تنوعت نسبة الحصول من عزلة لاخرى. تم تعريف إثني عشر مجموعة من مجموعات التوافق الخضري من بين 25 عزلة فقط من عزلات الفيوزاريوم المختبرة أما باقي العزلات (27 عزلة) فوجد أنها لا تتوافق مع أي من المجموعات المعرفة مما يعكس التنوع الجيني الكبير بين عزلات الفطر المنتشرة في مصر. أظهرت النتائج أنه لا توجد علاقه واضحة بين مجموعات التوافق الخضري والشدة المرضية والتوزيع الجغرافي للعزلات.