VEGETATIVE COMPATIBILITY OF *VERTICILLIUM DAHLIAE* KLEB. ISOLATES FROM OLIVE IN EGYPT

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ABSTRACT

Verticillium wilt, caused by *Verticillium dahliae*, is the most serious disease in olive cultivation areas in Egypt. Thirty six (36) isolates of *V. dahliae* from olive (*Olea europea* L.) trees originating from eight regions of Egypt were taken for vegetative compatibility analysis using nitrate non-utilizing (nit) mutants. One isolate did not produce a nit mutant. Thirty five (35) isolates yielded nit mutant, they were tested for vegetative compatibility by observing heterokaryon formation among complementary nitrate-nonutilizing (*nit*) mutants. Among 569 chlorate-resistant sectors obtained, only 370 were Nit mutants. Three types of Nit mutants were obtained (Nit1"251", NitM"101" and Nit3"18") on the basis of the fungal phenotype. Nit1 mutants were the most frequent (67.8%), f o l l o w e d b y N i t M (27.3%) a n d N i t 3 (4.9%). Based o n their ability t o form heterokaryons, all 35 olive pathogenic isolates were grouped into a three vegetative compatibility groups. where include VCG1 five isolates "1, 2, 6, 9, 11", VCG2 twenty one isolates" 3, 4, 5, 7, 8, 12, 19, 27, 20, 16, 10, 13, 14, 15, 17, 21, 22, 23, 25, 26, 24" and VCG3 nine isolates "18, 28, 29, 30, 31, 32, 33, 34, 36.
Keywords: *Verticillium dahliae*, Verticillium wilt, Vegetative Compatibility Groups VCGs, *nit* mutants, heterokaryon ,Olive tree, *Olea europea,* Egypt.

INTRODUCTION

Olive (*Olea europaea* L.) is the most important and traditional woody crop in Syria. Olives are cultivated over large areas in Syria. The total number of planted trees is about 80 million, covering 500,000 ha. Syria is sixth in the world ranking of olive and olive oil producers. Al-Ahmad and Mosli, 1993. Klebahn obtained from wilting dahlia in 1913 an isolate of *Verticillium* which he considered sufficiently different from the organism described by Reinke & Berthold to warrant being given specific rank, and named it *V. dahliae.* Isaac.1967. Verticillium wilt of olive, caused by *Verticillium dahliae,* was first reported in 1946 (Ruggieri, 1946). Verticillium wilt caused by *Verticillium dahliae* is a major disease in olive (*Olea europaea* L.) orchards in the Mediterranean region and California,(Al-Ahmad and Mosli,1993. Jimenes-Dias, *et al.* 1998, Thanassoulopoulos,*et al.* 1979, Wilhelm, and Taylor, 1965. Blanco-L'opez *et al.* 1984; L'opez-Escudero and Blanco-L'opez, 2001; Hiemstra and Harris, 1998; Rodr'ıguez-Jurado *et al.* 1993.

Verticillium wilt occurs throughout the range of olive cultivation, reducing the production of olive trees and potentially causing tree death (Jimenez-Diaz *et al.*, 1998). This fungus, which can persist in the soil as microsclerotia for more than 20 years, grows parasitically on the tree, while fungicides, besides having potentially toxic consequences and representing an environmental risk, have little effect on it. Ana, *et al.* 2007. In the last two decades, the Verticillium wilt disease has occurred with increasing frequency

and severity in most olive growing areas of the Mediterranean basin (Jiménez-Díaz *et al.*, 1998). It affects olive trees in the nursery, commercial orchards and landscape plantings. The pathogen is a soil inhabiting fungus and inoculum consists of microsclerotia, which form in the senescing tissues of the diseased plant, Nigro *et al.* 2005. *V. dahliae* exhibits high variability of important traits (e.g. pathogenicity, vegetative compatibility, morphology, etc.) and isolates can be genetically diversified also according to their ecological niches in which many factors may intervene (Roberts *et al.*, 1995; Barbara *et al.*, 1998).

Since *V. dahliae* is a strictly asexually reproducing fungus (Pegg and Brady, 2002), vegetative compatibility is a prerequisite to genetic exchange among different strains of the pathogen. Dervis, *et al.* 2007. Vegetative compatibility has been shown in be a powerful tool to differentiating *V. dahliae* (Joaquim and Rowe, 1990; Strausbaugh *et al.*, 1992; Chen, 1994; Daayf *et al.*, 1995; Watanabe *et al.*, 1997; Bao *et al.*, 1998). Heterokaryosis formation can be obtained using two different techniques: UV-induced microsclerotial colour mutants and spontaneous nitrate non-utilizing mutants. the relationship between the VCG groups and geographical origin of *V. dahliae* strains is still a matter of controversy (Puhalla and Hummel 1983; Joaquim and Rowe, 1990; Korolev and Katan, 1997; Watanabe *et al.*, 1997).

The lack of host specificity among *V. dahliae* isolates (Schnathorst, 1981) makes their classification into sub-species or *formae speciales* difficult. Moreover, characterization of pathotypes by means of pathogenicity tests is problematic. It must be complemented with other criteria such as optimal temperature of mycelium growth, germination of conidia, microsclerotia and conidia production (Wiese, and DeVay.1970). In the case of olive, the greenhouse tests of pathogenicity are considered "delicate" and time consuming (Vigouroux, 1975). Therefore, other techniques such as vegetative compatibility (VC) are preferred. As regards *V. dahliae,* numerous studies have shown that vegetative compatibility analysis may serve as a useful tool to differentiate the strains (Puhalla, 1979; Puhalla and Hummel, 1983; Strausbaugh, 1993; Bao *et al.*, 1998; Elena and Paplomatas, 1998). The identification of vegetative compatibility groups (VCG) allow for the identification of races, genetic diversity and variability of pathogenicity of the population (Chen, 1994). Studies of vegetative compatibility groups (VCGs) using nitrate non-utilizing (Nit) mutants indicated that *V. dahliae* populations were composed of a limited number of VCGs (Rowe, 1995).

Using mutants with coloured microsclerotia, Puhalla and Hummel (1983) classified 83 *V. dahliae* strains into 16 VCGs; using nit-mutants only four VCGs were found (Joaquim and Rowe, 1990). This VCG4A, for instance, was subdivided into six VCG subgroups VCG4A1–VCG4A6 (Strausbaugh, 1993). In Greece, 29% of the *V. dahliae* isolates from olive were assigned to VCG4, 14% to VCG2 and 57% could not be grouped to any VCG (Elena and Paplomatas, 1998). In Morocco, 47% of the isolates from olive were assigned to VCG4B, 32% in VCG2 and 18% could not be classified (Cherrab *et al.*, 2002).

VCG1, VCG2, VCG3 and VCG4 were identified among *V. dahliae* isolates from diverse geographic and plant sources worldwide by using

nitrate-nonutilizing (nit) mutants (Bell 1994; Chandelier *et al.*, 2003; Chen 1994; Daayf *et al.* 1995; Joaquim and Rowe 1991; Korolev *et al.* 2000, 2001; Strausbaugh 1993; Zhengjun *et al.* 1998). Each of VCG1, VCG2 and VCG4 was further divided into two subgroups designated as A and B (Bell 1994; Joaquim and Rowe 1991; Strausbaugh 1993). Recently, VCG6 has been identified among *V. dahliae* isolates infecting pepper in California (Bhat *et al.* 2003). Göre, (2009) was assigned 30 strains of *Verticillium dahliae*, recovered of chrysanthemum in Turkey, to vegetative compatibility groups (VCGs) based on pairings of complementary nitrate-nonutilizing (nit) mutants induced on a chlorate-containing medium. Of these strains, nine were assigned to VCG1, seven to VCG2A, 11 toVCG2B and one to VCG4B. The remaining two strains could not be tested for vegetative compatibility because of their inability to yield nit mutants. Of the 100 *Verticillium dahliae* isolates recovered from wilted cotton plants in Turkey, 49 were assigned to VCG1A, 39 to VCG2B, nine to VCG2A and three to VCG4B.using nitrate non-utilizing mutants and reference tester strains of vegetative compatibility groups. (Dervis *et al.* 2008). Rataj-Guranowska, 2006. was assigned 31 isolates of *Verticillium dahliae*, , to vegetative compatibility groups (VCGs) where 15 isolates were assigned to VCG2, and 10 isolates to VCG4, while 6 isolates could not assigned to any VCGs.

The identification of vegetative compatibility groups (VCGs) has proven to be an effective approach for studying the genetic structure of anamorphic populations of soil borne wilt and root rot pathogens such as *Fusarium oxysporum* (Katan *et al.*, 1991; Marlatt *et al.*, 1996) and *Verticillium* spp. (Correll *et al.* 1988; Joaquim and Rowe, 1991).

However, the vegetative compatibility of olive isolates has not been studied in Egypt. Thus the object of the present study was to investigate genetic diversity in a population of *V. dahliae* isolated mainly from olive.

MATERIALS AND METHODS

Isolation of *V. dahliae* from olive plants

The isolates originating from 12 sites in 8 Regions in Egypt were collected from olive trees with wilt symptoms between 2003 and 2006. Pieces of vascular tissue from branch segments (1.5-2 cm long) were dipped in 70% ethanol, surface-sterilized with 1% NaOCl for 1 min, rinsed in sterile distilled water and dried on sterile filter paper. Each segment was then placed on Selective medium described by Ausher *et al.* (1975), Cultures Were incubated at 24 C in the dark and examined after 5-7 days for growth of *V. dahliae*.

Verticillium dahliae was identified on the basis of its morphological features according to the description of Smith (1965). Barnet and Hunter (1995). Watanabe (2002).

Monoconidial isolation of the fungus was performed using the method described previously (Bell 1992). A total of 36 single-spored isolates of *V. dahliae* each of which was obtained from different sites was stored on PDA at 4°C and used for VCG characterization.

Generation of nitrate-non-utilizing (nit) mutants

Water agar-chlorate medium (WAC) was used to select nit mutants. This medium was based on water agar (2%) amended with 0.02% glucose and 2.5–5% potassium chlorate (Korolev and Katan 1997). Mycelial plugs (about 2-3 mm2) cut from the edge of the monoconidial cultures were placed on WAC in 9 cm Petri dishes, and incubated at 24°C for up to 4 weeks. Chlorate resistant sectors, which appear as thin fast- growing mycelial sectors or as fan-like sectors at the colony perimeter after 10–28 days, were transferred to Czapek-Dox agar (CDA) Petri dishes (9-cm diameter) and allowed to grow for 5-7 days at 24 °C. Only sectors that grew on CDA as colonies with a thin, expansive mycelium were considered nit mutants.fig1 (a,b).

Phenotype characterization of nit mutants

CDA amended with sodium nitrite (0.5 g l−1) or hypoxanthine (0.2 g l−1) was used for partial phenotyping of the nit mutants Mutants that grew profusely (similar to wild-type) on CDA with nitrite or hypoxanthine were classified as nitl. Fig1 (d), Mutants that grew sparsely on CDA with hypoxanthine were classified as NitM.Fig1 (e) Mutants that grew profusely on hypoxanthine and sparsely on nitrite were classified as nit3. These partially phenotyped nit mutants were labelled and stored for future use. Table (2). (Correll *et al.*, 1987).

Complementation and vegetative compatibility

Vegetative compatibility groups (VCGs) were determined for egyptian *V: dahliae* isolates, using complementary *nit* mutants. A mycelial plug was cut from the edge of a *nit* mutant grown on CDA at 24°C, and transferred to a plate containing BM amended with sodium nitrate (CDA medium). The nit1 and NitM mutants from each isolate were paired with each other to test for heterokaryon self-compatibility. Because the success rate is higher than Nit1 * Nit3 or Nit3 * NitM.Complementation was tested on CDA. Generally, each 9-cm diameter Petri dish was inoculated with three mutants, 1.5cm apart and incubated for 28 days at 24°C in the dark. Pairings were scored for prototrophic growth 7–28 days after inoculation. Complementation was indicated by the formation of a dense, aerial growth (similar to wild type isolate growth). In this case, the 2 isolates crossed are considered to belong to the same compatibility group. If the opposite is true (no apparent complementation), and if the strain is not self-incompatible, checks were made to see whether crossing in the opposite direction (NitM from A crossed with Nit1 from B if the first cross was between Nit1 from A and NitM from B) leads to complementation, before concluding that the two strains tested belong to different compatibility groups. (Puhalla, 1985; Correll *et al.*, 1987, Bellahcene, *et al.*, 2005). Fig1(f,g).
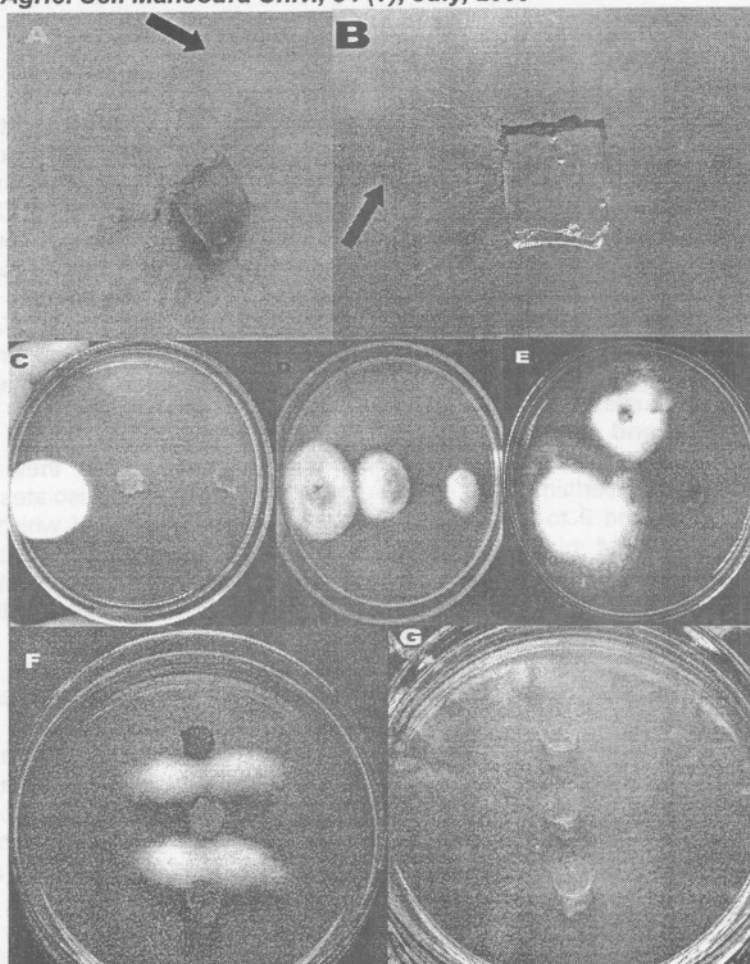
Fig.1: A: Growth of isolate 13 on water agar chlorate medium (WAC) after 21 days of incubation at 24°C.

B: Growth of *nit* mutants from isolate 5 on CDA medium after 7 days of incubation at 24°C.

C: Growth of isolate 17(left) *nit*M mutants (middle) of isolate 17 and *nit*1 mutant (right) of isolate 17 on nitrate medium (CDA). after 7 days of incubation at 24°C.

D: Growth of isolate 17(left) *nit*M mutants (middle) of isolate 17 and *nit*1 mutant (right) of isolate 17 on nitrite medium after 8 days of incubation at 24°C.

E: Growth of isolate 23(top) *nit*M mutants (right) of isolate 23 and *nit*1 mutant (left) of isolate on hypoxanthine medium . after 16 days of incubation at 24°C.

F: A pairing between the complementary mutants among different isolates: *nit*1 mutant (top) of isolate 23 and *nit*1 mutant (down) of isolate 24 pairing with *nit*M mutant (middle) of isolate 22 in plates after 10 days of incubation at 24°C. G: A pairing between the complementary mutants among different isolates: *nit*1 mutant (top) of isolate 31 and *nit*1 mutant (down) of isolate 32 pairing with *nit*M mutant (middle) of isolate 19 in plates after 20 days of incubation at 24°C.

RESULTS

Isolation of *V. dahliae* from olive plants

The thirty six (36) isolates originated from 12 sites at 8 locations in Egypt. In all, nine (9) isolates (1,2,3,4,5,6,9,25,27) were obtained from diseased olive trees from 3 sites in Matruh province, nine (9) isolates (28,29,30,31,32,33,34,35,36) from one site in North Sinaa province, five (5) isolates (7,8,14,17,26) from two sites in Behiera province, two (2) isolates(23,24) from one site in Ismailia province, one isolate(10) from one site in Alexandria province, three (3) isolates (11,20,21) from one site in Menoufyia province, three (3) isolates (12,13,19) from one site in Fayoum province and four (4) isolates (15,16,18,22) from two sites in Giza province (Table 1). In all, 36 single-spore isolates of *V. dahliae* were obtained and used in VC studies.

Generation and characterization of *nit* mutants

Chlorate-resistant sectors appeared infrequently but their visual distinction was certain. In 5 to 20 replications, each of 35 isolates of *V. dahliae* produced 9 to 32 chlorate-resistant sectors, 6 to 17 of which were phenotyped as *nit* mutants. Whereas isolate 35 did not give any *nit* mutant. Some mutants had a tendency to revert back to the wild type. Mutants from the isolates were usually characterized within 10 days after collecting them from the chlorate-amended medium.

Phenotype characterization of *nit* mutants

Most *nit* mutants grew profusely (similar to wild type) on CDA with nitrite or hypoxanthine were classified as *nit1* (251 *nit* mutant, 67.8%). Some of the *nit* mutants (101, 27.3%) grew sparsely on CDA with hypoxanthine and were classified as nitM. Few *nit* mutants grew profusely on hypoxanthine and sparsely on nitrite medium (18, 4.9 %) and were classified as *nit3*.Table (2). While some of the *nit* mutants grew profusely on CDA with hypoxanthine and did not grow on nitrite medium, their phenotype was not clear. All *nit* mutants showed wild-type growth on PDA.

Complementation of *Nit* mutants and VCG determination

Complementation between nit mutants was tested on Czapek-Dox agar (CDA) Medium. All posibilities of complementations between the *NitM* of the 35 isolates and *Nit1* of the 35 isolates were done. The results showed that isolates of *V. dahliae* obtained from different Geographical regions in Egypt were belong to three vegetative compatibility groups include VCG1 five isolates "1, 2, 6, 9, 11", VCG2 twenty one isolates" 3, 4, 5, 7, 8, 12, 19, 27, 20, 16, 10, 13, 14, 15, 17, 21, 22, 23, 25, 26, 24" and VCG3 nine isolates "18, 28, 29, 30, 31, 32, 33, 34, 36. (Table 3). (Fig. 2).

Table (1): Isolates of *V. dahliae* from olive trees, their host and geographic origin, year of isolation

Isolate of V.dahliae	Host	Year of isolation	Geographic origin		
			Country	Region	Site
1	Olive	2003	Egypt	Matrouh	Barrani
2	Olive	2003	Egypt	Matrouh	Siwa oasis
3	Olive	2006	Egypt	Matrouh	Siwa oasis
4	Olive	2006	Egypt	Matrouh	Siwa oasis
5	Olive	2003	Egypt	Matrouh	El – Dabaa
6	Olive	2006	Egypt	Matrouh	Siwa oasis
7	Olive	2003	Egypt	Beheira	West Noubaria
8	Olive	2006	Egypt	Beheira	West Noubaria
9	Olive	2006	Egypt	Matrouh	Siwa oasis
10	Olive	2003	Egypt	Alexandria	El - Ameria
11	Olive	2003	Egypt	Menoufyia	El – Khatatba
12	Olive	2003	Egypt	Fayoum	Sennoris
13	Olive	2006	Egypt	Fayoum	Sennoris
14	Olive	2006	Egypt	Beheira	West Noubaria
15	Olive	2003	Egypt	Giza	El – Saff
16	Olive	2006	Egypt	Giza	El – Saff
17	Olive	2006	Egypt	Beheira	El-Boustan
18	Olive	2003	Egypt	Giza	Embaba
19	Olive	2006	Egypt	Fayoum	Sennoris
20	Olive	2006	Egypt	Menoufyia	El – Khatatba
21	Olive	2006	Egypt	Menoufyia	El – Khatatba
22	Olive	2006	Egypt	Giza	El – Saff
23	Olive	2006	Egypt	Ismailia	El – Ismailia
24	Olive	2006	Egypt	Ismailia	El – Ismailia
25	Olive	2006	Egypt	Matrouh	Siwa oasis
26	Olive	2006	Egypt	Beheira	El-Boustan
27	Olive	2006	Egypt	Matrouh	Barrani
28	Olive	2003	Egypt	North Sinai	El – Arish
29	Olive	2006	Egypt	North Sinai	El – Arish
30	Olive	2006	Egypt	North Sinai	El – Arish
31	Olive	2006	Egypt	North Sinai	El – Arish
32	Olive	2006	Egypt	North Sinai	El – Arish
33	Olive	2006	Egypt	North Sinai	El – Arish
34	Olive	2006	Egypt	North Sinai	El – Arish
35	Olive	2006	Egypt	North Sinai	El – Arish
36	Olive	2006	Egypt	North Sinai	El – Arish

Table (2): Number of each *Nit* mutant type selected on characterization media.

Isolate code	Nit1		Nit m		Nit3		total
	No.	%	No.	%	No.	%	
1	6	75	2	25	0	0	8
2	7	87.5	1	12.5	0	0	8
3	6	66.6	3	33.3	0	0	9
4	7	70	3	30	0	0	10
5	9	75	3	25	0	0	12
6	7	77.7	2	22.2	0	0	9
7	10	83.3	2	16.6	0	0	12
8	8	61.5	3	23.1	2	15.4	13
9	6	66.6	3	33.3	0	0	9
10	9	69.2	4	30.8	0	0	13
11	7	63.6	4	36.4	0	0	11
12	6	50	4	33.3	2	16.7	12
13	9	60	2	13.3	4	26.7	15
14	8	66.6	4	33.3	0	0	12
15	10	71.4	4	28.6	0	0	14
16	6	75	2	25	0	0	8
17	6	50	3	25	3	25	12
18	6	54.5	3	27.3	2	18.2	11
19	13	76.5	4	23.5	0	0	17
20	10	66.6	4	26.7	1	6.7	15
21	9	75	3	25	0	0	12
22	7	63.6	3	27.3	1	9.1	11
23	10	83.3	2	16.7	0	0	12
24	6	75	2	25	0	0	8
25	7	70	3	30	0	0	10
26	7	77.8	2	22.2	0	0	9
27	6	75	2	25	0	0	8
28	6	60	3	30	1	10	10
29	8	66.6	4	33.3	0	0	12
30	7	70	3	30	0	0	10
31	5	71.4	2	28.6	0	0	7
32	6	60	4	40	0	0	10
33	2	28.6	3	42.8	2	28.6	7
34	5	62.5	3	37.5	0	0	8
35	0	0	0	0	0	0	0
36	4	66.6	2	33.3	0	0	6
total	251	67.8	101	27.3	18	4.9	370

Table (3): Results of pairings on a Czapek - Dox agar (CDA) Medium between complementary mutants generated from isolates of *V. dahliae* from olive trees.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	36
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
3			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
4				+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
5					+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
6						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
7							+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
8								+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
9									-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
10										+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
11											-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
12												+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
13													+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
14														+	+	+	+	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
15															+	+	+	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
16																+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
17																	+	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
18																		+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
19																			+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
20																				+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
21																					+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
22																						+	+	+	-	-	+	-	-	-	-	-	-	-	-
23																							+	+	-	-	+	-	-	-	-	-	-	-	-
24																								+	-	-	+	-	-	-	-	-	-	-	-
25																									-	-	+				-	-	-	-	-
26																									+	+					-	-	-	-	-
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31																															+	+	+	+	+
32																																+	+	+	+
33																																	+	+	+
34																																		+	+
36																																			+

+: Formation of heterokarotic mycelium in the contact zone. - : No complementation between the mutants tested.
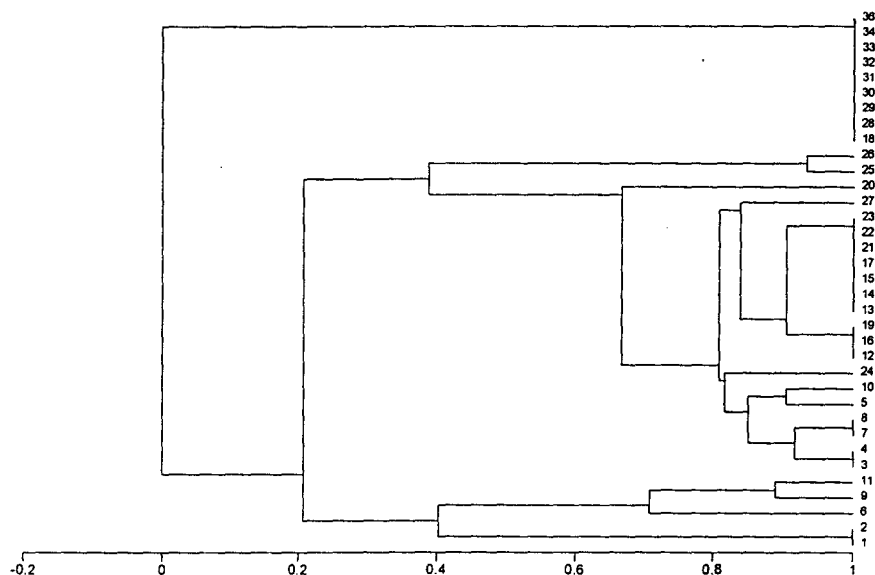
Fig.(2) A comparison of *Verlicillium dahliae* isolates from Egyptian olive based on Jaquard's similarity coefficient.

DISCUSSION

Verticillium wilt of olive in Egypt is caused by *V. dahliae*, as indicated in a previous study by Radwan and Hilal (1994). Vegetative compatibility analysis using *nit* mutants yielded interesting results on genetic diversity and pathogenicity within the *V. dahliae* population. In the present study, vegetative compatibility of 36 *V. dahliae* isolates from Egypt was assessed using *nit* mutants. Some isolates produced *nit* mutants readily, whereas others produced a few mutants.one isolate did not yield any nit mutants. *nit*3 mutants were rarely produced in this study and the vast majority of mutants were of the *nit*1 phenotype mutants. Some *nit*3 mutants could not be distinguished from *nit*1 because they did not grow on nitrite medium. Similar results were also reported by several researchers (Korolev and Katan . (1997), Korolev *et al.* (2000). Bellahcene, *et al.* (2005). Dervis, and Bicici, (2005), Dervis *et al.* 2007). While Rataj-Guranowska, 2006 was obtained 84–92% of *nit* 1/*nit* 3, and 8–16% of *nit* M mutants.

This is the first study of vegetative compatibility of *V. dahliae* isolates from olive in Egypt. Based on results pairing of *nit* mutants, we identified three VCGs from Egyptian *V. dahliae* isolates from olive. This study demonstrated that 21 isolates (60% of the characterized isolates) were assigned to VCG2 and nine isolates (25.7 % characterized isolates) were

assigned to VCG3. Whereas five isolates (14.3 % characterized isolates). were assigned to VCG1

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التوافق الجسدي لعزلات الفطر *Verticillium dahliae* المعزولــة مــن الزيتون في مصر

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يعتبر مرض الــذبول الفيرتيــسليومي المتــسبب عــن الفطــر .Kleb *Verticillium dahliae* من أخطر الأمراض التي تهدد توسع زراعة الزيتون في مصر وقد أجريت هذه الدراسة بهدف تحديد مدى التنوع الوراثي لعزلات الفطر *V. dahliae* من خلال تحديد مجاميع التوافــق الجسدي "Vegetative Compatibility Groups. VCGs" لعزلات الفطر المعزولــة مــن مناطق زراعة الزيتون المختلفة على مستوى الجمهورية.

وأوضحت نتائج الدراسة أنه تم الحصول على "36" عزلة من الفطر *V. dahliae* من ثمان مناطق جغرافية مختلفة في مصر وتم تحديد مجاميع التوافق الجسدي باستخدام تقنية الطفرات التي فقدت القدرة على الاستفادة من النترات كمصدر للنتروجين وقد أمكن الحصول على طفــرات من 35 عزلة من مجمل العزلات البالغة 36 عزلة حيث لم تعط العزلة رقم 35 أية طفرات ، وقد تم الحصول على 569 قطاع ميسيليومي مقاوم للكلورات حدد منها 370 طفرة فقدت القدرة علــى الاستفادة من النترات وقد صنفت هذه الطفرات إلى ثلاث أنماط بناءً على مــدى قــدرتها علــى الاستفادة من مصادر النتروجين المختلفة حيث تم تحديد "251" طفرة من الــنمط Nit1 و"101" طفرة من النمط NitM و 18 طفرة من النمط Nit3 ، حيث كان الــنمط Nit1 هــو الأكثــر تكراراً بنسبة 67.8 % من إجمالي الطفرات المتحصل عليها تلاه النمط NitM بنسبة 27.3 % وأخيراً النمط Nit3 بنسبة 4.9% ، وبعد إجراء عمليــة الاقتــران بــين الطفــرة مــن الــنمط Nit1والطفرة من النمط NitM للعزلات المختلفة وبكل الاحتمالات وتسجيل نتيجة الاقتران أمكــن تصنيف عزلات الفطر *V. dahliae* المسبب لمرض الذبول الفيرتيسليومي للزيتون في مصر إلى ثلاث مجاميع رئيسية متمايزة وراثياً حيث ضمت المجموعة الأولى خمس عــزلات " 11،9،6 ، 1,2"بينما شملت المجموعة الثانية إحدى وعشرين عزلة " 3, 4, 5, 7, 8, 12, 19, 27, 20, 16, 10 , 13, 14, 15, 16, 17, 21, 22, 23, 25, 26, 24 " في حين احتوت المجموعــة الثالثة على تسع عزلات " 36 , 34 ,33 ,32 ,31 ,30 ,29 ,28 ,18" وتظهــر هــذه النتــائج التنوع الوراثي لعزلات الفطر *V. dahliae* المعزولة من مناطق جغرافية مختلفة في مصر ولــم يكن هناك ارتباط بين المجموعة التي تنتمي إليها العزلات والمناطق الجغرافية التي عزلت منها.