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Interaction among Egyptian and Syrian Chickpea Cultivars and Isolates of *Fusarium oxysporum* f.sp. *ciceris*

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The reactions of three Egyptian and five Syrian chickpea cultivars to 21 Egyptian isolates of *Fusarium oxysporum* f.sp. *ciceris* (*F.o.c.*) were tested in the greenhouse. Early wilt, late wilt and survivals were determined to evaluate susceptibility of tested cultivars. The Syrian cultivars were less susceptible than the Egyptian ones as their survival plants that ranged from 54.39% to 69.09% and 50.75% to 59.39%, respectively. Analysis of variance (ANOVA) indicated that the main effects of cultivars and isolates as well as their interactions were highly significant sources of variation in the tested parameters. The interaction between *F.o.c.* isolates and the tested chickpea cultivars suggested a physiologic specialization within *F.o.c.* isolates on chickpea and the resistance of the tested cultivars is a mixture of vertical and horizontal resistance. Similarly, pathogenicity of the tested isolates differed in their virulence and aggressiveness to various chickpea cultivars. Three groups of isolates were identified using cluster analysis with no relationship among these groups and their geographical origin. Also, cluster analysis indicated that 80% of Syrian cultivars were included in the same cluster, while a considerable dissimilarity was observed among Egyptian cultivars.

Key words: Chickpea, cluster analysis, *Fusarium oxysporum* f.sp. *ciceris* and interaction.

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop in the world after beans (*Phaseolus vulgaris* L.) (Anonymous, 2005). In addition to its importance as a human food and animal feed, it is valued for its beneficial effects in improving soil fertility and thus sustainability and profitability of production systems. Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend.:Fr. f.sp. *ciceris* (Padwick) Matuo and K. Sato, is one of the most important biotic stresses of chickpea production and has the potential to cause 100% yield losses. An annual loss in chickpea grain yield about 10 to 15% has been reported for this disease (Navas-Cortes *et al.*, 2000). The disease is prevalent in the Ethiopia, Egypt, Indian subcontinent, Mexico, Spain, Syria, Tunisia, Turkey and the United States (Nene *et al.*, 1989; Khattab and Omar, 1992; Halila and Strange, 1996 and Akem *et al.*, 1998).

Management of Fusarium wilt of chickpea is difficult to achieve and no single control measure is fully effective. Currently, the use of resistant cultivars appears to be the most practical and economically efficient control measure for management of Fusarium wilt of chickpea and is also a key component in integrated disease management (IDM) programs (Jiménez-Díaz *et al.*, 1991). Possibly, this control strategy will become increasingly widespread in the coming years in order to satisfy consumers' demand for healthier foods and better environmental quality through new forms of agricultural production, *i.e.* sustainable agriculture, organic agriculture and ecological agriculture. However, the efficiency of resistant cultivars in disease management can be seriously limited by pathogenic variability occurring in pathogen populations, including the existence of pathogenic races. Therefore, knowledge of the evolutionary history and potential of the pathogen population may help to optimize the management of disease resistance genes, irrespective of the breeding strategy used for their development, *i.e.* conventional plant breeding or genetic engineering. (Jiménez-Gasco *et al.*, 2004)

In the present study, biometrical approach was used to study the interactions among Egyptian and Syrian chickpea cultivars and different isolates of *F.o.c.* collected from seven chickpea growing governorates in Egypt.

Materials and Methods

Twenty one isolates of *Fusarium oxysporum* f.sp. *ciceris* (*F.o.c.*) were isolated from diseased chickpea samples collected from different governorates in Egypt (Table 1). Purification and identification of these isolates were done during a course of Ph.D. study of Mr. I.M. Al-Mohamed (unpublished data). Maintenance of collected Fusarium isolates was done on potato-dextrose agar (PDA) slants in a refrigerator (5°C). Substrate for growth of isolated *F.o.c.* was prepared in 500-ml glass bottles; each one contained 100g sorghum grains, 50g sand and 90ml tap water. Contents of each bottle were autoclaved for 30 minutes. Isolates inoculum, taken from one-week-old culture on PDA, was aseptically transferred into the bottle and allowed to colonize sorghum for three weeks. Batches of autoclaved clay loam soil were separately infested with inoculum of each isolate at the rate of 50 g/kg of soil. Infested soil was dispensed into 20-cm-diameter clay pots, which were then planted with 10 seeds/pot for each of the tested cultivars, *i.e.* Ghab 1, Ghab2, Ghab 3, Ghab4 and Ghab5 (taken from ICARDA) as well as Giza1, Giza195 and Giza531 (taken from Field Crops Res. Inst., ARC, Giza, Egypt). In the control (check) treatments, autoclaved sorghum grains were thoroughly mixed with soil at the rate of 50 g/kg soil. Pots were randomly distributed on greenhouse benches under temperature regime ranging from 13±2 to 25±2°C. Three pots were used as replicates for each treatment. Data of early wilt was recorded 25 days after planting; meanwhile late wilt and survivals were recorded 60 days after planting.

Statistical analysis:

The randomized complete block design, with three replicates, was used in this study. Duncan's multiple range test was used to compare treatment means. Percentage data were subjected to the appropriate transformation before carrying out ANOVA to produce approximately constant variance. The results were expressed as

Table 1. Geographical sources of *Fusarium oxysporum* f.sp. *ciceris* isolates used in this study

Geographic origin	Isolate No.*
Assiut	7
Assiut	11
Assiut	12
Beheira	1
Beheira	2
Beheira	3
Beni-Suef	8
Beni-Suef	9
Beni-Suef	10
Gharbia	16
Gharbia	17
Gharbia	18
Giza	4
Giza	5
Giza	6
Menia	13
Menia	14
Menia	15
Sharkia	19
Sharkia	20
Sharkia	21

* Tested isolates were collected from diseased chickpea plants grown at different locations during 2003-2004 growing season.

a phenogram (Joseph *et al.*, 1992). Cluster analysis, based on three pathological parameters, *i.e.* early wilt, late wilt and plant survival, was performed with a computerized program (Porta-Puglia *et al.*, 1996).

Results

Twenty one isolates of *Fusarium oxysporum* f.sp. *ciceris* derived from seven governorates in Egypt, were tested for level of aggressiveness on two-month-old greenhouse-grown plants of eight Egyptian and Syrian chickpea cultivars. ANOVA show highly significant effects of cultivar, isolate and/or interaction of cultivar × isolate with all tested parameters (Table 2).

Table 2. Analysis of variance of the interaction between chickpea cultivars and isolates of *Fusarium oxysporum* f.sp. *ciceris* in greenhouse

Parameter	Source of variance	Sum of squares	D.F.	Mean square	F. value	P> F
Early wilt	Replication	498.175	2	249.088	1.576	0.208
	Isolate	73476.112	21	3498.862	22.135	0.000
	Cultivar	4213.577	7	601.940	3.808	0.001
	Cultivar × Isolate	40366.276	147	274.601	1.737	0.000
	Error	55325.334	350	158.072		
Late wilt	Replication	162.527	2	81.264	1.047	0.352
	Isolate	23158.336	21	1102.778	14.203	0.000
	Cultivar	6078.443	7	868.349	11.183	0.000
	Cultivar × Isolate	24389.617	147	165.916	2.137	0.000
	Error	27176.093	350	77.646		
Survival	Replication	373.227	2	186.613	1.899	0.151
	Isolate	91472.035	21	4355.811	44.333	0.000
	Cultivar	7948.228	7	1135.461	11.556	0.000
	Cultivar × Isolate	37340.005	147	254.014	2.585	0.000
	Error	34388.565	350	98.253		

Isolate was the most important factor for determining the variation in early wilt and survival (Table 3). Meanwhile, isolate × cultivar interaction was the second factor while cultivar was the last important one in this concern. Moreover, isolate × cultivar was the most important factor for determining the variation in late wilt, meanwhile isolate was the second factor, while cultivar was the least important one. It was possible to assess the relative contribution of each of cultivar, isolate and cultivar × isolate interaction in the explained (model) variation (Table 3). Horizontal resistance of cultivars accounted for 3.57, 11.33, and 5.81% of the explained variation in early wilt, late wilt incidence and survival, respectively. Moreover, isolate aggressiveness accounted for 62.23, 43.18, and 66.88% of the explained variation in early wilt, late wilt incidence and survival, respectively.

Table 3. Relative contribution of Egyptian and Syrian chickpea cultivars, *Fusarium oxysporum* f.sp. *ciceris* isolates and their interaction to variation in incidence of chickpea wilt

Source of variation	Relative contribution [a] to variation in		
	Early wilt incidence	Late wilt incidence	Survival
Cultivar (C)	3.57	11.33	5.81
Isolate (I)	62.23	43.18	66.88
C × I	34.19	45.48	27.30

[a] Calculated as percentages of sum of squares tabulated in Table 2.

Vertical resistance of cultivars or virulence of isolates accounted for 34.19, 45.48, and 27.30 of the explained variation in early wilt, late wilt incidence and survival, respectively (Vanderplank, 1984).

Due to the highly significant effect of cultivars × isolate interaction on early wilt incidence, a least significant difference (LSD) was calculated to compare isolates means (arc-sine transformed values) within each cultivar (Table 4) these comparisons showed that the differences in early wilt incidence between isolates and the control were not the same for each cultivar, that is, cultivars responded differently to the isolates. Thus Ghab 1, Ghab 2, Ghab 3, Ghab 4, Ghab 5, Giza 1, Giza 195 and Giza 531 were susceptible to 8, 13, 7, 8,8,8,2, and 8 isolates, respectively. Also, cultivars Ghab2, Ghab3, Ghab5, Giza1 and Giza 195 were completely resistant to isolates 11,4,1,6, and 7, respectively in spite of these isolates were able to infect other cultivars, it was found that the magnitude of the differences between isolates differed from one cultivar to another. For example, the difference between isolate 3 and 4 was highly significant on Ghab 1, while it was non-significant on Ghab 2. Similarly, in respect of late wilt incidence, the difference between isolate 2 and 3 was significant within cultivar Giza 1, whereas the difference between the two isolates was non-significant in case of cultivar Ghab 1 (Table 5), *i.e.* isolates of *F.o.c.* responded differently with the different cultivars. The same trend observed in survival (Table 6).

To represent the relationship between isolates, a cluster analysis was performed, three groups of similar isolates were found (isolates 18, 19, 16, 20, 1, 15, 9; isolates 6, 12, 11, 14, 10, 7, 5, 4, 3 and isolates 2, 8, 13, 21, 17) (Fig. 1). Taking into account the geographical origin of the isolate, they seem to be randomly distributed among the groups. No associations were observed between virulence of isolates and their geographical origin. Also, a cluster analysis was performed to identify the relationship between cultivars, data in Fig.(2) indicate that four of five (80%) of Syrian cultivars were included in the same cluster, *i.e.* cultivars Ghab2, Ghab4, Ghab1, Ghab5, but Ghab3 was found in another cluster. A considerable dissimilarity was observed among Egyptian cultivars. In general, the Syrian cultivars were less susceptible than the Egyptian ones as the percentage of the survival plants was 54.39–69.09% in the Syrian, while it was 50.75–59.39% in the Egyptian ones.

Table 4. Reaction of some Egyptian and Syrian chickpea cultivars to *Fusarium oxysporum* f.sp. *ciceris* regarding to early wilt of chickpea in greenhouse

Isolate	Early wilt incidence (%) 25 days after planting								
	Ghab1	Ghab2	Ghab3	Ghab4	Ghab5	Giza1	Giza195	Giza 531	Mean
1	6.147 *	30.000	8.853	17.707	0.000	28.777	18.440	21.147	16.384
2	45.00	50.853	35.217	38.853	41.070	46.923	51.147	39.147	43.526
3	48.93	17.217	15.000	21.930	36.930	26.070	28.287	19.923	26.786
4	17.70	12.293	0.000	6.147	12.293	32.217	15.000	21.147	14.600
5	13.07	25.777	30.000	8.853	6.147	38.853	26.560	36.930	23.275
6	6.14	8.853	6.147	8.853	8.853	0.000	21.930	46.923	13.463
7	8.85	26.070	39.230	13.077	21.930	21.147	0.000	23.853	19.270
8	52.777	45.293	31.923	61.223	54.783	50.853	51.147	51.147	49.893
9	12.293	6.147	8.853	15.000	17.707	21.147	21.147	26.560	16.107
10	19.223	8.853	32.300	17.707	30.000	42.700	30.783	17.707	24.909
11	8.853	0.000	21.930	25.777	26.560	30.993	21.147	30.293	20.694
12	26.153	0.000	8.853	17.707	8.853	30.293	26.070	17.707	16.955
13	28.077	52.777	30.993	53.070	36.930	45.000	28.777	25.777	37.675
14	8.853	8.853	26.153	30.000	26.153	37.223	26.560	26.560	23.795
15	0.000	15.000	21.147	0.000	23.853	28.777	23.853	23.363	16.999
16	8.853	6.147	17.707	17.707	8.853	8.853	6.147	15.000	11.158
17	34.223	40.860	57.000	50.853	34.923	21.930	50.770	31.923	40.310
18	6.147	8.853	8.853	17.707	0.000	26.070	21.147	12.293	12.634
19	15.000	17.217	15.000	8.853	18.440	6.147	12.293	17.707	13.832
20	8.853	0.000	17.707	12.293	0.000	6.147	12.293	12.293	8.698
21	33.000	31.923	23.853	43.077	52.777	36.847	28.077	38.853	36.051
Check	0.000	8.853	0.000	0.000	0.000	0.000	12.293	15.000	4.518
Mean	18.553	19.175	20.760	22.109	21.230	26.680	24.267	25.966	22.342
LSD (P<0.05) for: Isolate (I)=7.166; cultivar(C)= 4.324 and C x I interaction= 20.317.									

* Percentage data were transformed into arc sine angles before carrying out the analysis of variance.

Table 5. Reaction of some Egyptian and Syrian chickpea cultivars to *Fusarium oxysporum* f.sp. *ciceris* regarding to late wilt of chickpea in greenhouse

Isolate	Late wilt incidence (%) 60 days after planting *								
	Ghab1	Ghab2	Ghab3	Ghab4	Ghab5	Giza1	Giza195	Giza 531	Mean
1	15.000 **	12.293	18.440	26.560	26.070	15.000	33.210	18.440	20.627
2	26.070	23.853	33.210	35.007	41.070	25.370	12.293	43.077	29.994
3	28.777	30.293	23.853	21.147	30.293	46.923	28.077	15.000	28.045
4	32.300	33.000	19.223	30.783	38.853	45.000	45.000	48.930	36.636
5	12.293	28.287	26.560	26.560	28.777	37.140	33.000	28.777	27.674
6	15.000	12.293	12.293	26.070	30.783	15.000	12.293	23.363	18.387
7	26.560	30.783	17.707	26.070	35.007	47.007	30.993	12.293	28.303
8	21.147	33.000	23.853	21.147	21.147	28.077	28.077	6.147	22.824
9	21.930	21.147	6.147	15.000	19.923	28.777	39.230	21.147	21.663
10	21.147	30.783	21.147	26.560	30.000	26.070	36.930	28.777	27.677
11	26.070	23.853	33.000	23.853	35.217	23.853	37.223	12.293	26.920
12	37.140	36.847	12.293	28.777	28.077	21.147	23.363	18.440	25.760
13	21.147	28.777	12.293	23.853	38.070	39.063	35.217	30.783	28.650
14	21.930	33.000	6.147	17.217	34.923	6.147	33.000	18.440	21.350
15	12.293	30.783	12.293	23.853	11.070	21.147	26.070	18.440	19.494
16	26.070	30.783	18.440	21.930	33.000	35.217	18.440	32.217	27.012
17	35.217	33.000	26.070	28.287	36.930	39.147	26.560	21.147	30.795
18	26.070	28.777	12.293	21.147	17.217	22.140	11.070	28.777	20.936
19	18.440	19.923	15.000	23.363	21.147	23.363	15.000	28.777	20.627
20	23.853	28.777	12.293	23.853	37.223	23.363	30.783	21.147	25.162
21	30.783	42.700	18.440	33.000	21.147	23.853	36.930	21.147	28.500
Check	0.000	0.000	0.000	0.000	0.000	0.000	12.293	0.000	1.537
Mean	22.693	26.952	17.318	23.820	27.997	26.946	27.502	22.616	24.480

LSD (P<0.05) for: Isolate(I)= 5.022, Cultivar (C)= 3.031 and C x I interaction= 14.239.

* Late wilt percentages were calculated according to the following equation:
Late wilt (%)= [(S–E)/T]×100
Whereas: S= number of survived plants, E= number of early wilted plants and T= total tested plants.

** As described in Table (4) footnote.

Table 6. Reaction of some Egyptian and Syrian chickpea cultivars to *Fusarium oxysporum* f.sp. *ciceris* regarding to survival of chickpea in greenhouse

Isolate	Survival (%) 60 days after planting								
	Ghab1	Ghab2	Ghab3	Ghab4	Ghab5	Giza1	Giza195	Giza 531	Mean
1	72.293 *	51.847	66.637	54.993	63.930	54.993	50.770	61.223	59.586
2	33.000	28.077	37.223	30.783	21.147	26.153	34.923	17.707	28.627
3	26.070	50.853	59.007	55.077	36.930	30.993	46.923	59.217	45.634
4	48.930	52.777	70.777	57.000	47.007	21.930	39.147	33.000	46.321
5	64.630	45.000	43.077	59.217	59.007	27.293	45.000	39.147	47.796
6	72.293	72.783	75.000	59.007	54.993	75.000	60.000	33.000	62.760
7	59.217	46.923	43.077	55.077	43.077	34.923	59.007	61.223	50.315
8	28.777	21.930	46.923	15.000	26.560	23.363	23.853	36.930	27.917
9	59.707	66.637	75.000	68.070	57.000	52.777	43.077	54.783	59.631
10	57.293	54.993	49.223	54.993	38.853	34.923	37.223	52.777	47.535
11	59.007	66.147	44.707	49.140	43.077	48.847	45.000	54.993	51.365
12	37.140	53.153	72.783	52.777	57.000	50.853	53.070	61.713	54.811
13	49.223	17.707	54.993	26.070	28.077	15.000	41.153	43.077	34.413
14	63.847	52.777	57.700	47.007	38.153	50.853	45.000	56.790	51.516
15	77.707	52.777	63.930	66.147	59.217	52.777	52.777	59.217	60.568
16	59.007	57.000	61.713	54.993	52.777	50.937	68.853	50.853	57.017
17	34.630	26.153	18.440	23.853	33.000	38.853	26.560	48.930	31.303
18	61.223	57.000	72.783	59.007	72.783	51.147	61.923	57.000	61.608
19	63.930	57.000	64.630	61.713	61.223	64.630	70.077	53.070	62.034
20	61.223	61.223	63.930	61.223	52.777	64.630	55.077	63.930	60.502
21	41.153	28.777	59.007	28.777	28.777	42.783	39.230	43.077	38.948
Check	90.000	81.147	90.000	90.000	90.00	90.00	72.293	75.000	84.805
Mean	55.468	50.122	58.662	51.360	48.426	45.621	48.679	50.757	51.136
LSD (P<0.05) for: Isolate (I)= 5.649, Cultivar (C)= 3.409 and I x C interaction= 16.018									

* As described in Table (4) footnote.

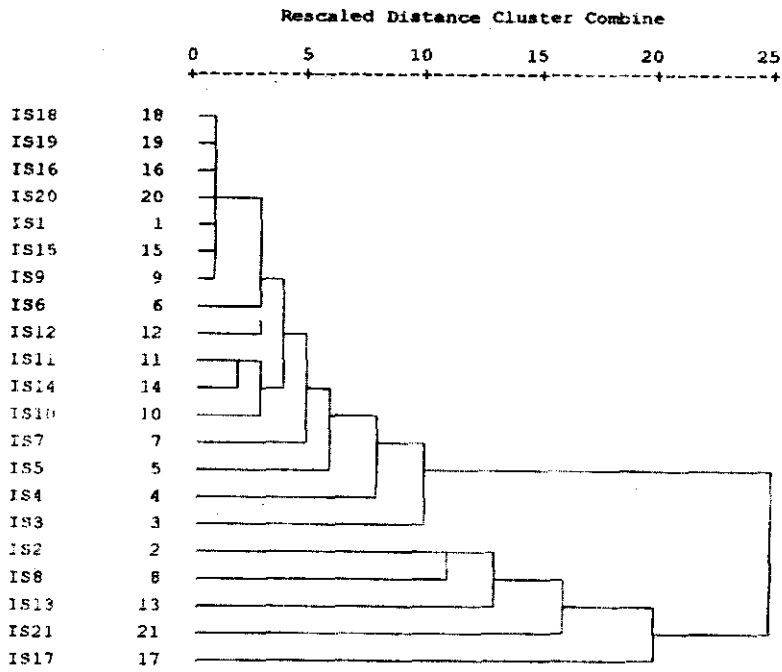

Fig.1. Phenogram drawn from cluster analysis of 21 isolates of *F.o.c.* tested on a set of eight Egyptian and Syrian chickpea cultivars.

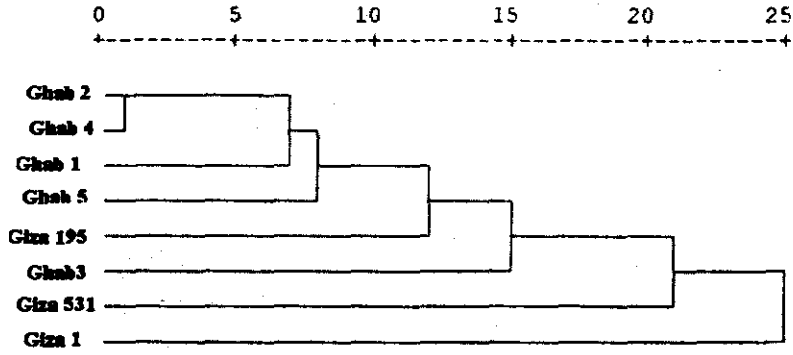

Fig.2. Phenogram drawn from cluster analysis showing interaction of some Egyptian and Syrian chickpea cultivars to *F.o.c.*

Discussion

Limited understanding of the population structure of pathogens may account for the limited success in disease management (Martin and English 1997), the use of resistant cultivars appears to be the most practical and economically efficient control measure for management of Fusarium wilt of chickpea, but, its efficiency in disease management is limited by pathogenic variability in *Fusarium oxysporum* f.sp. *ciceris* populations. Thus, research on the genetic diversity within populations of the *F.o.c.* will improve our understanding of the factors involved in the breakdown of wilt resistance.

Specificity in host-pathogen relationships is often indicated by significant isolate × variety interaction in the analysis of variance (ANOVA) of an experiment where a number of pathogen isolates are tested in all possible combinations on a set of host genotypes. Unspecificity is identified by lack of such interaction (Vanderplank, 1984).

Analysis of variance has been widely used to detect quantitative host-pathogen specificity in many pathosystems (Jenns *et al.*, 1982; Faris *et al.*, 1983; Porta-Puglia *et al.*, 1996).

It has been suggested that the presence of a significant cultivar × isolate interaction in the analysis of variance is an evidence for a differential (vertical) host-pathogen relationship (Vanderplank, 1984). Lack of significant interaction is taken to indicate that the association is nondifferential (horizontal), implying that differences in cultivar susceptibility are consistent relative to one another, regardless of pathogen isolates. In any host-pathogen relationship the two type of resistance may act together in determining the outcome of the association between the host, and the pathogen (Vanderplank, 1984).

In the present study ANOVA showed that the main effects of cultivars, isolates or interaction of cultivar × isolate were a highly significant source of variation in all the tested parameters. Statistically, significant interaction between chickpea cultivars and isolates in this study suggests that physiologic specialization exists within *F.o.c.* isolates pathogenic on chickpea. Thus, chickpea cultivars should be tested by using as many isolates of *F.o.c.* as possible, as this will improve the chance of identifying chickpea cultivars having resistance against several isolates of *F.o.c.* Also, promising cultivars must be tested under different agroecological systems, representing chickpea-producing regions, to ensure their susceptibility to many populations of *F.o.c.*

According to Vanderplank (1984), the ANOVA in the present work implies that the resistance of the tested cultivars is a mixture of both vertical and horizontal resistance and there are significant differences among cultivars in both types of resistance. Similarly, pathogenicity of the tested isolates is also mixture of virulence and aggressiveness. Moreover, the isolates significantly varied in both types of pathogenicity.

The application of cluster analysis has been suggested for assessing similarity and/or dissimilarity in gene-for-gene host-parasite relationships (Lebeda and Jendrulek, 1987; Priestley *et al.*, 1984). Thus, cluster analysis was used to study genetic similarity among 17 isolates of *Pyrenophora tritici-repentis* (Died.) Drech. (Schilder and Bergstrom, 1990), 41 isolates of *Ascochyta rabiei* (Pass.) Labrousse (Porta-Puglia *et al.*, 1996). and 20 isolates of *Macrophomina phaseolina* (Omar, 2005).

In this study, cluster analysis proved to be useful in determining the similarity of *F.o.c.* isolates, based on their virulence on eight chickpea. Several reasons, *e.g.* isolates represent different agroecological zones of chickpea-producing regions in Egypt, planting chickpea in small dispersal areas changing annually and the introduction or selection of the promising cultivars from different international ancestors, may contribute to the considerable variability of Egyptian *F.o.c.* populations and the lack of homogeneity between Egyptian chickpea cultivars.

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التفاعل بين أصناف الحمص المصرية والسورية وعزلات الفطر فيوزاريوم أوكسيسبورم

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تم اختبار رد الفعل لثلاثة أصناف مصرية وخمسة سورية من الحمص من حيث القابلية للإصابة بإحدى وعشرين عزلة مصرية من الفطر *Fusarium oxysporum* f.sp. *ciceris*، المسبب لمرض الذبول، تحت ظروف الصوبة. قدرت النسبة المئوية للإصابة المبكرة والمتأخرة والنباتات المتبقية (الحية) لتقييم تلك الأصناف للإصابة بهذا الفطر. تبين أن الأصناف السورية أقل قابلية للإصابة من المصرية، تراوحت نسبة النباتات الحية من ٥٤,٣٩ إلى ٦٩,٠٩ %، بينما كانت في الأصناف المصرية من ٥٠,٧٥ إلى ٥٩,٤%. أظهر تحليل التباين وجود معنوية عالية بين الأصناف و العزلات وتفاعل الأصناف مع العزلات، كذلك وجود تخصص فسيولوجي بين عزلات الفطر، وان مقاومة أصناف الحمص للفطر هي خليط من المقاومة الرأسية والأفقية. كما أن، القدرة المرضية لعزلات الفطر هي خليط من القدرة المرضية المتخصصة والغير متخصصة. وباستخدام التحليل العنقودي تم تقسيم العزلات إلى ثلاث مجاميع، لم يكن بينها ترابط من حيث الموقع الجغرافي، كما تبين وجود نسبة تجانس عالية بين الأصناف السورية، وعدم تجانس في الأصناف المصرية.