IDENTIFICATION OF FUSARIUM WILT CAUSED BY FUSARIUM OXYSPORUM AND PATHOGEN VARIABILITY IN FABA BEAN, LENTIL, AND CHICKPEA CROPS IN EGYPT

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Abstract

Isolation was made from wilted plants of faba bean, lentil, and chickpea; collected from different regions of Egypt. Isolates of *F.oxysproum* collected from each crop were grouped depending on their habit characters. Four isolates from faba bean representing four groups of isolates, 6 from lentil, and 6 from chickpea were used in this study. Morphological characters, pathogenicity and pathogenic variability of each crop isolates were studied.

The results proved that, all isolates from faba bean were F.oxysporum f.sp.fabae, isolates from lentil were F.oxysporum f.sp. Lentis and isolates from chickpea were F.oxysporum.sp. ciceri. Morover, results showed clear variation among isolates of each crop, hence we roughly subdivided F.oxysporum f.sp. fabae into 3 types and F.oxysporum f.sp. Lentis into 4 types. F.oxysporum f.sp. ciceri showed clear variation among the tested isolates in their habit characters.

INTRODUCTION

F.oxysporum is considered an important pathogen causing vascular wilt disease in faba bean, lentil and chickpea in Egypt and throughout the world. Regular surveys in Egypt indicate that wilt diseases usually cause a considerable yield losses in legume crops annually (Abdel Rehim 1962, El-Awadi 1993, El-Garhy 1994 and Abou-Zeid et al 1995).

F.oxysporum has about 80 formae specialse and several are subdivided into races. Identified specialized formae that cause wilt disease on legume crops are

F.oxysporum f. sp. fabae on faba bean, F.oxysporm f.sp. lentis on lentil, and F.oxysporum f.sp. ciceri on chickpea (Booth 1971 and Armstrong and Armstrong 1981).

On the basis of pathogenicity studies, *F.oxysporum f.sp.ciceri* has been separated into 7 races designated 0-6, four of which were in India (Haware and Nene 1982) and three in Spain (Cabrera de la Colina *et al.* 1985 and Jimenez - Diaz *et al.* 1989). Also, *F.oxysporum* f.sp. *lentis* was subdivided into eight strains (Khare *et al.* 1975) or seven strains (Kannaiyan and Nene, 1978).

This work amied to confirm the identity of fusaria isolated from diseased plants of faba bean, lentil and chickpea, and to in define the extent of variability in morphological and pathogenic properties.

MATERIALS AND METHODS

Isolation and Identification:

A regular survey of diseases infected faba bean lentil and chickpea plants was conducted in all growing areas in Egypt during 95/1996 season. Plants showing wilt symptoms were collected from different fields for laboratory studies. Isolation was made from roots and basal stem of each wilted plant on PDA medium. Emerged fungi were isolated, transferred to PDA plates, and purified using single-spore technique as described by Toussoun and Nelson (1968). Isolated fungi were identified according to their morphological characters according Booth (1971) and Nelson *et al.* (1983) .

Morphological Characters of F.oxysporum Isolates:

Morphological characters of the isolated *F.oxysporum* cultures grown on PDA medium for 7 days were studied. The characters included colony colour, type of mycelium, colour of substratum, presence and size of micro-and macroconidia, zonation, and presence and type of chlamydospores. Average radial growth per day was calculated from colony diameters measured 3 and 7 days after inoculation.

Representative cultures were preserved in sand tubes as follows: 2 ml of conidial suspension placed on 10g of autoclaved fine riverbed sand in a glass tube and stored at 5° C (Haware and Nene 1982).

Pathogenicity and Pathogen Variability Tests:

Faba bean and lentil *F.oxysporum* isolates. Pathogenic potential and pathogen variability of 4 faba bean and 6 lentil *F.oxysporum* isolates were tested using the spore suspension technique as descirhed by Wensley adn Mckeen (1962). Inoculum of each tested isolate was prepared from infested sand by sprinkling a small amount on PDA plates and incubation at 25°C for 7 days. Conidial suspensions were obtained by flooding cultures of *F.oxysporum* grown on PDA plates (7 day-old) with sterile water and filtering through three layers of cheese-cloth. The suspensions were adjusted, using a haemocytometer, to a concentration of 10⁶ conidia/ml.

Seeds were sown in autoclaved soil in 15-cm sterilized plastic pots at the rate of 5 seeds/pot. The seeds were disinfested by immersion in a 2% solution of sodium hypochlorite for 3 min and rinsed in distilled water before planting. The pots were kept under greenhouse conditions for 15 days then inoculated with 50 ml spore suspension.

Chickpea *F.oxysporum* isolates: Pathogenic potential and pathogen variability of 6 chickpea *F.oxysporum* isolates were determined by pot-inoculation technique developed by Haware and Nene (1982). Inoculum of each tested isolate was prepared from infested sand by sprinkling an amount on PDA plates and incubated at 25°C for 7 days. A small agar disk cut from the edge of the colony was transfered to 500 ml bottle containing 150g autoclaved sand-sorghum medium. The inoculated bottles were incubated at 25°C for three weeks. Autoclaved soil and riverbed sand mixture (1:1) was mixed thoroughly with the inoculum at the rate of 20:1 (w/w). Infested soil was potted in 15-cm sterilized plastic pots and left for 4 days before transplanting the tested seedlings.

Chickpea seeds were germinated in autoclaved sand in sterilized trays for 7 days before transplanting in the pots containing the infested soil. Five seedlings were planted in each pot.

Four cultivars of faba bean i.e. Giza 461, Giza 402, Giza 716 and Giza 3; six cultivars of lentil i.e. Flip 92-43 L, Precoze, Giza 9, Giza 370, Flip 89-16 L, and Pant 192; and four cultivars of chickpea i.e. Giza 1, Giza 88, Giza 195 and Giza 531were used in this experiment. All seeds were obtained from Food Legume Res. Dept., FCRI, ARC, Giza, Egypt.

Four replicates were prepared for each treatment. Uninoculated checks were

kept for each cultivar. Pots of each plant species were arranged in a randomized complete block design on benches in the greenhouse where the temperature was 28±2 during the day and 20±2°C at night, and kept under investigation for 40 days.

Data were recorded after 40 days from planting and scored as described by Haware and Nene (1982) as follows:

R: Resistant 0-20% wilted plants

M : Moderately susceptible 21-50% wilted plants

S : Susceptible >50% wilted plants

The whole experiment was repeated twice.

RESULTS

Isolation, Identification and Characterization of F.oxysporum Isolates:

The number of F.oxysporum isolates collected from different wilted plant samples during 1995/96 season were 20 isolates from faba bean diseased samples, 10 isolates from lentil, and 10 from chickpea (Table 1). Distribution of these isolates in the legume growing areas in Egypt is also given. F.oxysporum isolates of each crop were classified into group depending on their habit characters. Isolates obtained from faba bean, lentil, and chickpea diseased samples were classified into 4,6, and 6 groups. One isolate from each group was used in this study.

As shown in Table (2) there are obvious differences among F.oxysporum isolates of each crop in density, shape, colour and growth rate of aerial mycelium. Also, there are clear differences in colour of substratum, texture and zonation among isolates of each crop (Table 2 and Fig. 1).

Microconidia of all faba bean isolates (Table 3), were moderate to abundant and varied in length and width except isolates No 63 and 76 which were similar in width, and isolates No 53 and 76 which were similar in length. All lentil isolates produced abundant microconidia with almost similar width and length except for the isolates No 211 and 212. Both isolates No 211 and 212 were similar to each other in width and length, but different from the rest of the lentil isolates. Meanwhile, microconidia in F.oxysporum isolates of chickpea were moderate to abundant. Isolates No. 22 and 23 were similar to each other in length and width, while the others were varied.

Table 1. Number of *F.oxysporum* isolates collected from different wilted food legume samples during 1995/96 season, and their distribution.

Governorate	District	Faba bean	Lentil .	Faba bean
Sharqiya	Abo-Hammad W	s were Smilar in	esteles to to	en edition are taken
Kaliobia	Kaliob	chickpea isolate		and diginal at estate over the food figures
	Tokh	ate No. 23 while	lozi_towns	each other as with
Fayoum	Fayoum	1 resent in any c	510 of 100 of	- amydoed
	Etsa	(7 days 2 after s	non-denice	le juit vilt is earc
Ismailia	Ismailia has	oorum <mark>r</mark> isolat	18 EVENS	a distantionis
Beni-Suef	Beni-Suef	1 is vitoineuo 9as	-	- Linning Liopa
Beheira	Kafr El-Dawar			They seemed to
	Wady El-Natron	are to the particle of the National St.	other scotter	migration and about or tradition and fall
	Nubaria	2	2	2
	Delengat	om Vallovi sets	(or = 107 o)	1 - 1 - 1
Giza	Giza	1	1	1
Gharbia	Gemmeiza	being the second	Lin Link to	
Kafr El-Sheikh	Kafr El-Sheikh	Ozar Speed on 21	atvetjus jesti	2
North Sinai	Rafah	Fisolates No.	orit q i dinasa	m em _ 9(-11) gr
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	Dairout .	4) show results of the continued Giza 1		1 2
	Abnoub	vas moderateis eru stoanwhile,	noviture 58 s	endifer a series
Total	of freedom box	20	10	10

Macroconidia of faba bean isolates were moderate with 2 to 4 septa and almost similar in width, but very variable in length. Macroconidia of lentil isolates were moderate to moderately abundant with 2 to 4 septa for all isolates. Isolates No. 212 and 213 were similar in length and width, but different from the rest of isolates. All the rest of isolates were similar in width, but showed clearly differences in length. Macroconidia of chickpea isolates were moderate with 2 to 4 septa except for the isolate No. 22 where they were absent. All isolates were similar to each other in width except isolate No. 23 which produced macroconidia narrower than the others.

No clamydospores were present in any of the tested isolates of all legume crops at the time of examination (7 days after subcultures).

Pathogenicity of *F.oxysporum* Isolates and Pathogen Variability in Legume Crops :

Faba bean: Results of pathogenicity and pathogen variability tests of 4 *F.oxysporum* isolates with 4 faba bean cultivars showed clear differences in resistance and susceptibility of cultivars to the pathogen isolates (Table 4). Cultivar Giza 461 was resistant to the isolates No. 76 and 67, but susceptible to the isolates No. 53 and 63. Cultivar Giza 402 was resistant to the isolate No. 76, but not to other isolates. Meanwhile, cultivar Giza 716 was susceptible to all isolates. Also, cultivar Giza 3 was resistant to the isolates No. 67 and 76 but susceptible to the other 2 *F.oxysporum* isolates.

Lentil: Six *F.oxysporum* isolates were tested on 6 lentil cultivars. Data in Table (4) indicate that, both cultivars Flip 92-43 L and Precoze were susceptible to all tested isolates. Also, cultivar Giza 9 was susceptible to the isolates 211, 212, 215 and 216, and moderatley susceptible to 213 and 214. On the contrary, cultivar Flip 89-16 L was resistant to the 4 isolates No. 211, 212, 213 and 214; and moderately susceptible to the isolates No. 215 and 216.

Chickpea: Data in Table (4) show results of testing 6 *F.oxysporum* isolates with 4 chickpea cultivars. Both cultivars Giza 1 and Giza 195 were susceptible to the six isolates. Giza 88 cultivar was moderately susceptible to isolates No. 23 and 55, but susceptible to the others. Meanwhile, cultivar Giza 531 was moderately susceptible to the isolates No. 22, 55, and 173 and susceptible to the other 3 isolates.

DISCUSSION

In case of faba beaan, pathogenicity tests indicated that all tested isolates were *F.oxysporum f.sp. fabae*. It could also be observed that both isolates No 53 and 63 gave the same reaction with the tested cultivars, while isolates No 67 and 76 gave different reactions from each others and from the first 2 isolates. It is obvious from the habit characters and from the pathogen variability tests that there are three different types of *F.oxysporum* f.sp. fabae. Type 1, isolates no 53 and 63; type 2, isolate No. 67; and type 3, isolate No. 76. This means that, F.oxysporum f.sp. fabae might have different races, and it is possible to subdivide the formae speciales of the pathogen into races based on the pathogenic potential of these isolates to certain culivars of faba bean. In this respect, several formae speciales were reported for *F.oxysporum f.sp. pisi* (Krafr and Hagland 1978) and for *F.oxysporum f.sp. entis* (Kare et al 1975 and Kanaiyan and Nene 1978).

In case of lentil, the results of pathogenicity tests proved that all tested isolates were *F.oxysporum f.sp. lentis*. Pathogenic variability and morphological characters showed clear variation among the tested isolates. According to the obtained data the tested isolates could be into 4 types. The first type is isolate No. 211, the second is isolate No. 212, the third are isolates No. 213 and 214, and the fourth are isolates No. 215 and 216. It is well known that *F.oxysporum* f.sp. *lentis* has several races. Khare *et al.*, (1975) reported eight strains; Kannaiya and Nene (1978) reported seven. Moreover, Kannaiyan and Nene (1976) found that, culivars Pusa 3 and Pant L 234 were very promising, while JL 500 and JL 674 were resistant to five out of seven strains of the fungus tested in infested soil .

Reaction of chickpea cultivars (Table 4) to the tested isolates indicated that all tested isolates were *F.oxysporum* f.sp. *ciceri*. Although morphological characters of the 6 isolates (Tables 2 and 3) showed clear variation among the tested isolates, pathogenic variations (Table 4) were not clear. All the tested cultivars did not exhibit resistance to the tested isolates. According to the characters, the tested isolates might belong to different races. The existence of pathogenic races of *F.oxysporum* f.sp. *ciceri* is now well established. Haware and Nene (1982) were the first to show the occurrence of races of the pathogen, namely races 1,2,3 and 4 based on the differential interaction in the artificial inoculation of 10 chickpea lines with isolates of the pathogen from India. Three additional races (0,4 and 6) were identified (Jimenez - Diaz *et al.*, 1989) in spain.

Table 2. Cultural characteristic of Fusarium Oxysporum isolates, from differen legume crops.

crop	solate	Aerial mycelium	colour of	Texture	Zonation	RGR/D*
	No.		substratum			(mm)
Faba	53	Scanty. Semi-submerged. Dark mauve in colour. Moderate growth.	Dark mauve, olive in the center.	Fluffy	Sometimes present	0.9
	63	Moderate to abundant. White to light mauve. Fast growth.	Light mauve, dark mauve in the center.	Cottony	Present	0.7
	29	Abundant. White. Fast growth.	Gray white.	Cattony	Absent	7.0
-	92	Scanty. Semi-submerged. Very light	Gray white to very	Appressed to	Absent	5.0
		mauve. Moderate growth.	§	nui y	Abcont	7.0
Lentil	211	Moderate to abundant. Sometimes semisubmerged. White and very light mauve in the center. Fast growth	Olive, sometimes brounish	Cottony	Absent	i dica c
	212	Moderate. Semi-submerged. Gray white to	Brownish, sometimes	Cottony	Sometimes	0.9
		light mauve. Moderate growth.	olive.	Clueter	Dracent Dracent	9
	213	Moderate, Light mauve, Moderate growth.	Dark mauve.	FIULTY	Pleseill	0.0
	214	Moderate. Light mauve. Moderate gowth.	Light mauve.	Cottony	Absent	0.9
	215	Moderate. Semi-submerged. White to light mauve. Fast growth.	Light mauve.	Appressed	Sometimes	0.9
	216	Moderate. Sometimes semi-submerged.	Light cream to gray	Cottony	Absent	5.5
Chickpea	22	Moderate to abundant. White. Not cover the	Light cream and gray	Cottony	Absent	2.0
		whole plate. Moderate growth.	white in the center.		415-14	0
	23	The same as 22.	The same as 22.	Cottony	Absent	0.0
	55	Very scanty or absent. Semi-submerged. Light mauve colour around center. Fast growth.	Light cream to light mauve.	Appressed	Absent	6.5
	173	Moderate. White to light mauve. Fast growth.	Light mauve.	Fluffy	Sometimes present	7.0
	174	Scanty. Semi-submerged. Light cream.	Orange cream to light			6.5
		Fast growth.	mauve	Appressed	Present	
	179	Scanty. Semi-submerged. Dark mauve.	Very light mauve.	Fulffy	Present	7.0
	224435.2	Fast growth.				

* RGR / D (mm) = Radial growth rate / day (mm).

For breeding and screening programs for resistant cultivars in wilt diseases of faba bean, lentil and chickpea, it is inevitable to define the races of *F.usarium* formae speciales, that attack each crop and their distribation in the county.

Table 3. Presence, size and septation of micro-and macroconidia of *Fusarium oxy-sporum* isolates from different legume crops .

		(4))	Microconidia				
crop	Isolate No.	Pre- sence	Size (μ)	Pre- sence	Sept- ation	Size (μ)	
Faba bean	53	++	3.7-5.0x5.0-12.5	27 +	2-4	5.0-6.5x22.5-32.	
57	63	+	2.5-4.0x5.0-10.0	+ 21	2-4	5.0-7.5x22.5-27.	
	67	+++	2.5-3.7x3.5-12.5	+	2-4	5.0-6.5x27.5-37.5	
	76	+++	2.5-4.0x5.0-12.5	22 + 1	2-4	5.0-6.5x17.5-22.5	
Lentil	211	+++	2.5-3.5x5.0-7.5	++	2-4	5.0-6.5x17.5-27.5	
	212	+++	2.5-3.5x5.0-7.5	+	2-4	2.5-5.0x15.0-20.0	
	213	+++	2.5-5.0x5.0-11.5	+	2-4	2.5-5.0x15.0-20	
coops I	214	+++	2.5-5.0x5.0-13.0	++	2-4	5.0-6.5x15.0-30.0	
	215	+++	2.5-5.0x5.0-11.5	++	2-4	5.0-6.5x17.5-30.0	
54.	216	+++	2.5-5.0x5.0-11.5	No ++	2-4	5.0-6.5x17.5-25.0	
Chickpea	22	++	.2.5-3.7x5.0-10.0		-	-	
	23	+++	2.5-3.7x5.0-10.0	+ 12	2-4	2.5-5.0x15.0-20.0	
	55	++	2.5-4.0x5.0-12.5	+	2-4	5.0-6.5x20.0-30.0	
	173	+++	3.5-5.0x7.5-15.0	+	2-4	5.0-6.5x17.5-32.5	
	174	+	3.5-5.x8.0-14.0	+	2-4	5.0-6.5x17.5-30.0	
g (a)	179	+++	2.5-4.0x3.5-10.0	+	2-4	5.0-7.5x15.0-25.0	

^{- :} Absent, + : Moderate, ++ : Moderately abundant, +++ : Abundant

Table 4. Reaction of faba bean, lentil and chickpea cultivars to isolates of F.oxysporum.

				Plant	Reaction		
	Isolate no.	53	63	67	76		
	Replicates	1234	1234	1234	1234		
- aba bean cultivars	Giza 461	SSSS	SSSS	RRRR	RRRR		
-aba bean cultivals	Giza 402	SSSS	SSSS	SSSS	RRRR		
	Giza 716	SSSS	SSSS	SSSS	SSSS		
	Giza 3	SSSS	SSSS	RRRR	RRRR		
	isolate no.	211	212	213	214 .	215	216
Lentil cultivars	Replicates	1234	1234	1234	1234	1234	1234
	Flip 98-43 L	SSSS	SSSS	SSSS	SSSS	SSSS	SSSS
	Precoze	SSSS	SSSS	ssss	SSSS	SSSS	ssss
	Giza 9	SSSS	SSSS	мммм	MSMS	SSSS	ssss
	Giza 370	SSMS	SSMS	мммм	RMMM	SSSS	ssss
	Flip 89-16 L	RRRR	RRRR	RRRR	RRRR	мммм	RMMN
	Pant 162	SMMM	SSMM	мммм	мммм	SMSS	SSSM
	Isolate no.	22	23	55	173	174	179
Chickpea cultivars	Replicates	1234	1237	1234	1234	1234	1234
	Giza 1	SSSS	SSSS	SSSS	SSSS	ssss	SSS
	Giza 88	SSS	SSS	MMSM	SSSM	ssss	SSS
	Giza 195	SSS	SSS .	SSS	SSSS	SSSS	SSS
	Giza 531	мммм	ММММ	мммм	MMSM	SSSS	SSM

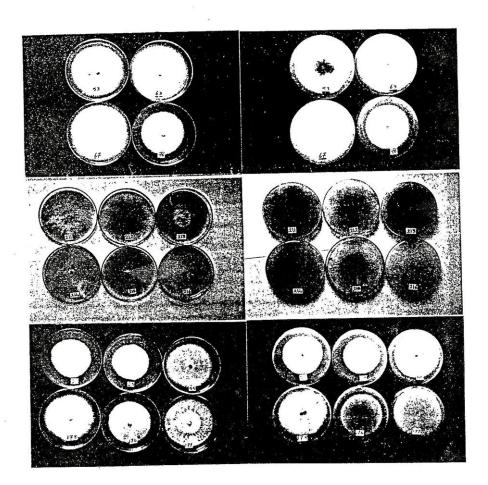


Fig. 1. Growth of the tested isolates of Fusarium oxysporum on PDA plates, 7 day-old.

Upper: F.oxysporum f.sp. fabae (4 isolates).

Middle: F.oxysporum f.sp. Lentis (6 isolates).

Bottom: F.oxysporum f.sp. ciceri (6 isolates).

Left: Upside plates, aerial mycelium.

Right: Downside plates, Substratum colour.

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الاختلافات المورفولوجية والمرضية في عزلات الفطرفيوز اريوم اوكسيسبورم المسبب لمرض الذبول الفيوز اريومي في مصلول الفول البدى ، العدس ، والحمص في مصر

جمعه عنتر المرسى ، ناجى محمد ابو زيد ، احمد محمد حسنين

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اتضح من الدراسة ان كل عزلات الفطر المعزولة من الفول البلدى هي F.oxysporum f.sp. lentis وايضا العزلات sp.fabae وايضا العزلات المعزولة من العدس هي F.oxysporum f.sp. ciceri وايضا العزلات المعزولة من الصمص هي F.oxysporum f.sp. ciceri من ناحية اخرى اظهرت الدراسة المورقولجية والمرضية وجود اختلافات واضحة بين عزلات الفطر لكل محصول ، وعلى ذلك تم تقسيم عزلات الفول البلدى الى ٣ انواع وايضا عزلات العدس الى ٤ انواع بالرغم من وجود اختلافات مورقولوجية واضحة بين عزلات القطر المعزولة من نباتات الحمص المصابة بالذبول الا ان قدرتها المرضية على الاصناف المختلفة كانت متشابهه ، وعلى ذلك لم يكن من السهل تقسيمها الى مجاميع مرضية مختلفة.