Morphological Variability and Races of *Fusarium oxysporum* f.sp. *ciceris* Associated with Chickpea (*Cicer arietinum*) Crops

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Abstract: Problem statement: Mexico is the third largest producer and exporter of chickpea (*Cicer arietinum*), with the states of Sinaloa and Sonora accounting for 70 and 20% of Mexico's production, respectively. The most damaging disease affecting this species is caused by *Fusarium oxysporum* f.sp. *Ciceris* (FOC), which causes losses of up to 60% in Sonora. The objective of this study was to isolate and characterize the phenotype and genetics of FOC collected from affected chickpea plants in northwestern Mexico and to identify the abiotic factors that allow it to develop. **Approach:** Sampling focused on affected plants from 12 crops in Sonora and Sinaloa. Based on 355 isolated strains, using Polymerase Chain Reaction (PCR) 161 were positive for FOC. **Results:** Of the 161 strains, 91 were identified as races previously recorded for the Americas: Yellowing (R0 (41%), R1B/C (15%) and wilting (R5 (14%) and R6 (28%) reflecting the symptoms observed in the areas sampled. The other 70 isolates could be nonpathogenic, or could be races yet to be recorded for the Americas. **Conclusion:** Morphological variability in FOC was high in the main chickpea producing regions in northwestern Mexico and was not a function of the physical and chemical properties of the soil, nor of the geographic location of the cropfields. This is the first report of races of FOC in Mexico.

Key words: Phenotype, root rot, vascular wilt, *Fusarium oxysporum*, pathotypes, characteristics, cropfields microscopic northwestern, temperature, chemical, macroconidia, potassium, Polymerase Chain Reaction (PCR), FOC, *Cicer arietinum*

INTRODUCTION

Chickpeas (*Cicer arietinum* L.) are grown during winter under different agroclimate conditions in the northwestern states of Sinaloa, Sonora and Baja California in Mexico. Annual production is about 200,000 ton most of which is destined for the international market (Manjarrez-Sandoval *et al*., 2004; Padilla-Valenzuela *et al*., 2008). Chickpea crop yield varies depending on the cultivar, climate conditions and disease; among the latter are root rot caused by a complex of fungi such as *Fusarium*, *Rhizoctonia*, *Macrophomina* and *Sclerotium*, leaf diseases caused by *Botrytis cinerea* Pers., *Peronospora* sp., *Uromyces ciceris-arietini* (Grognot) Jacz. and G. Boyer and wilting caused by *Fusarium oxysporum* f.sp. *ciceris* Matuo and K. Sato (FOC) (Padilla-Valenzuela *et al*., 2008). FOC is quickly dispersed, attacks the root of the plant and causes hydric stress by blocking the xylem vessels and also causes chlorosis, necrosis and abscission (Di Pietro *et al*., 2001; Gupta *et al*., 2009).

There are two pathotypes of the disease caused by FOC: yellowing and wilting. The first produces yellowing of the leaves and vascular discoloration and the plant dies 40 d after inoculation with the pathogen, while in the second, wilting, severe chlorosis, flaccidity and vascular discoloration occur and the plant dies 20 d after inoculation (Jimenez-Gasco *et al*., 2004). In addition to the symptomology that FOC produces in chickpea, eight pathogenic races are known (0, 1A, 1B/C, 2, 3, 4, 5 and 6) and can be identified by the differential reactions they produce in the plant: yellowing is produced by 0 and 1B/C, while the others cause wilting. According to reports from different studies, the races have specific geographic distributions since 2, 3 and 4 have only been reported for India while 0, 1B/C, 5 and 6 have mainly been found in the Mediterranean region and in the United States of America (California). In contrast, race 1A has a broader distribution and has been found wherever it has been looked for (Jimenez-Gasco *et al*., 2004).

In the Mexican Bajio region, FOC isolates belonging to both pathotypes were obtained; their genetic

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variability was determined using PCR-RAPD, but there was no consistent information for distinguishing between strains by origin or plant symptomology (Luna-Paez *et al*., 2004). In northwestern Mexico the disease is diagnosed based on the plant's symptoms. Chickpea wilt has been detected in 60% of the soils of La Costa de Hermosillo, Sonora, while in Sinaloa losses of 20% have been recorded (Manjarrez-Sandoval *et al*., 2004) and losses of 10 to 90% have been reported for other countries (Landa *et al*., 2006; Sharma and Muehlbauer, 2007).

There have been different attempts to control this disease, such as adjusting the sowing date and using resistant varieties of chickpea (Padilla-Valenzuela *et al*., 2008). Among the factors that favor the development of FOC are excess soil humidity, particularly clayey soil with poor drainage, along with a deficiency of potassium and excess sodium. Among the factors that contribute to pathogenesis is temperature, with 25-28°C optimal for the development of FOC (Bhatti and Kraft, 1992). However, an increase from 24-27°C is sufficient to change the resistance of some varieties of chickpea and make them susceptible to this pathogen (Landa *et al*., 2006).

The aim of this study was to isolate *F. oxysporum* f.sp. *ciceris*, characterize its phenotype and determine its pathogenic race and their relationship to abiotic factors where this fungus is found.

MATERIALS AND METHODS

Sampling sites: Based on the occurrence of chickpea yellowing and wilt, four locations were selected for sampling: in Sonora, La Costa de Hermosillo and Valle del Yaqui and in Sinaloa, Los Mochis and Guamúchil (Table 1). Three fields were sampled at each location with three quadrats per field. Samples were taken from plants showing signs of the disease. Ten plants were collected from each quadrat for a total of 360 chickpea root samples. Each plant was analyzed in triplicate, by placing five root sections in each Petri dish for a total of 5400 root fractions analyzed. All sampling sites were georeferenced using a geopositioning device (Garmin GPSmap 76S, Kansas, USA).

Strain isolation: FOC strains were obtained from the rhizoplane of the four most commonly grown varieties of chickpea in northwestern Mexico: Blanco Sinaloa 92, Aura, Teki Blanco and Costa 2004.

The roots of each one of the plants sampled were washed under running water, excess water was removed and the roots were cut into 1-2 cm sections. To

Table 1: Cropfields sampled, their location, chickpea variety and the percentage of roots infected with FOC

Cropfield[a]	N. Latitude	W. Longitude	Chickpea variety[b]	Percentage of roots with FOC± S.D
Sinaloa: Los Mochis				
LM1	25º50'04.6"	109º01'07.7"	BS	18±16
LM2	25º49'14.2"	108º55'47.8"	BS/A	32±15
LM3	25º44'32.7"	109º00'46.4"	BS	22±10
Guamúchil				
G1	25º10'41.3"	108º04'06.5"	BS	20±30
G2	25º25'26.4"	108º13'46.8"	BS	26±33
G3	25º17'48.6"	108º08'59.8"	BS	29±13
Sonora: La Costa de Hermosillo				
CH1	28º42'39.5"	111º33'20.6"	BS	28±13
CH2	28º47'40.9"	111º36'13.6"	TB/C	40±6
CH3	28º45'52.5"	111º20'09.9"	BS	29±5
Valle del Yaqui				
VY1	27º29'37.4"	110º09'26.9"	BS	48±8
VY2	27º29'22.3"	110º09'48.9"	BS	43±23
VY3	27º30'31.9"	110º10'56.8"	BS	56±15

[a]: Cropfield names: LM1 = Compuertas, LM2 = Las Noventas, LM3 = Ejido Flores Magón, G1= Rafael Buelna, G2 = Penjamo, G3 = La Ilama, CH1 = Santa Lucia, CH2 = La Esperanza, CH3= La Tinajita, VY1 = Block 213, VY2 = Block 215, VY3 = Block 217. [b] Chickpea variety: BS: Blanco Sinaloa 92; A: Aura; TB: Teki Blanco; C: Costa 2004

eliminate surface tension from the roots, they were left in 50% ethyl alcohol for 30 s and then superficially disinfected by immersion in 2% sodium hypochlorite for 2 min. The chlorine was removed by washing the roots four times in sterile distilled water. Excess moisture was removed and five root sections were placed in each Petri dish which contained Potato Dextrose Agar (PDA; Difco) acidified to 1.4 with 10% tartaric acid and 500 mg L^{-1} chloramphenicol. These were incubated at 25°C for 5-7 day (Nelson *et al*., 1983; Burgess *et al*., 1994).

Based on macro-and microscopic characteristics, colonies belonging to FOC were grown on PDA and monospore cultures were grown and then macroscopically characterized by color, appearance and growth rate after 7 day at 25°C; microscopically the size of their macroconidia, microconidia and chlamydospores was measured (Booth, 1975; Nelson *et al*., 1983). A control strain of FOC was used (ATCC 200784).

Abiotic factors: Using thematic and digital maps (INEGI, 2000), the climate, geology, edaphology, physiography and hydrology of the sampling sites were obtained. The physical and chemical properties of the soil were also analyzed for 36 samples made up of 10 subsamples per quadrat. Following the methodology of Castellanos *et al*. (2000), the following were determined: pH, electrical conductivity, nitrate, phosphate and potassium concentration, as well as organic matter content and texture.

Statistical analysis: ANOVAs with a 5% level of significance were used to analyze the physical and

Table 2: Primers used in the analysis of FOC strains

Primers	Primer Sequence (5´-3´)	Race	Fragment size (Kb)
FOC-f	GGCGTTTCGCAGCCTTACAATGAAG		
FOC-r	GACTCCTTTTTCCCGAGGTAGGTCAGAT	FOC	1.5
FOC-0f	GGAGAGCAGGACAGCAAAGACTA		
FOC-0r	GGAGAGCAGCTACCCTAGATACACC	R0	0.9
FOC-1B/Cf			
	GAGAGCAGGGTCAGCGTAGATAG		
FOC-1B/Cr			
GCAGCAGAAGAGGAAGAAAATGTA			
		R1B/C	0.5
FOC-5f	GGAAGCTTGGCATGACATAC		
FOC-5r	AAGCTTGGGCACCCTCTT	R5	0.9
FOC-6f	GAGCAGTCAATGGCAATGG		
FOC-6r	AGAGCAGGGTCAGCGTAGATA	R6	1.0

*Primers were designed by Jimenez-Gasco and Jimenez-Diaz (2003) and purchased from eurofins HW6 operon

chemical properties of the soil and the microscopic characteristics of the FOC strains. When there was a significant difference ($p \leq 0.05$) between fields, means were compared with a Tukey test in the SAS (1999) statistical computing software package. In order to group the fields sampled based on their soil properties and the phenotypes of the FOC strains, data were subjected to a multivariate analysis and a principal component analysis using the NCSS software package (Hintze, 2007).

Characterization by PCR: With the PCR of the FOC primers and amplifying a 1500 bp fragment the FOC positive strains were identified. This was followed by specific PCR for the races reported for the Americas: 0, 1B/C, 5 and 6, the first two yellowing races and the last two wilt races (Jimenez-Gasco and Jimenez-Diaz, 2003). To determine the pathotype to which each of the strains identified as FOC belong, genomic DNA, was extracted using the commercial kit (ZR-Fungal) followed by PCR. In Table 2 the primers used to detect the FOC and pathotypes 0, 1B/C, 5 and 6 are given.

PCR conditions were: 94°C 10 min^{-1}; 36 cycles of 94°C 1 min^{-1}; 58°C 1 min^{-1} and 72°C min^{-1}; a polymerization cycle at 72 °C 5 min^{-1} and 4°C storage temperature. Electrophoresis was done on 1.0% agarose gel in Tris-borate-EDTA buffer (TBE buffer) and the samples were dyed with ethidium bromide to visualize the amplified DNA using a UV transilluminator and a picture was taken with a Polaroid camera (Kodak).

RESULTS

Isolation of FOC: Based on the 360 diseased garbanzo plants (varieties Blanco Sinaloa 92, Costa 2004, Aura and Teki Blanco) with symptoms of yellowing and wilting, 355 strains belonging to *Fusarium* were isolated, 161 of which were positive for FOC. Per
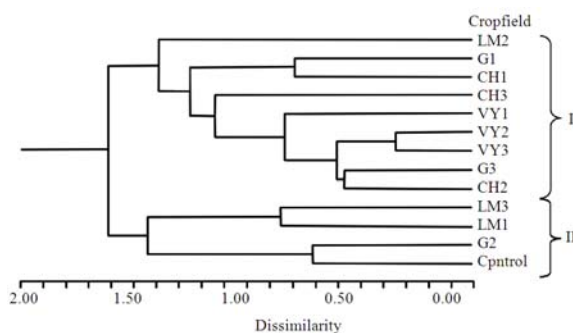

Fig. 1: Dendrogram generated with UPGMA based on Euclidian distances for the macro- and microscopic characteristics of FOC
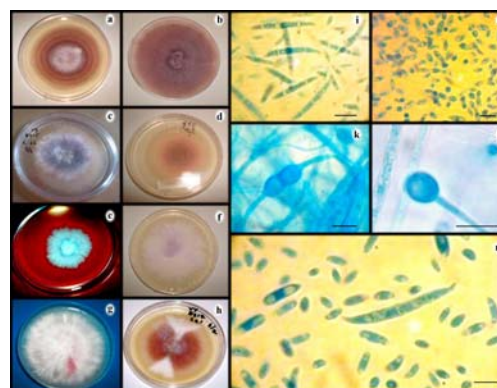

Fig. 2: Macro- and microscopic characteristics of FOC strains. (a) control strain, violet-velvety (b) group 2, whitish-violet-cottony (c) group 6, patchily violet (d) group 4, whitish-cottony (e) group 1, 3, 5 and 6, pinkish-whitish-cottony (f, g) group 1 and 4, violet-brown-whitish-feathery (h) groups 1 and 7, brown-violet-whitish-feathery (i) fusiform macroconidia, with 2-4 septa (j) oval, unicellular microconidia (k) intercalary chlamydospores in pairs (l) terminal chlamydospore (m) control strain, macro- and microconidia. Scale bar: 10 µm

field, these had the following proportion of the FOC strains: Valle del Yaqui (36.0%), La Costa de Hermosillo (24.8%), Guamúchil (20.5%) and Los Mochis (18.7%).

Morphological variability of FOC: The multivariate analysis of the macro- and microscopic characteristics of the 161 FOC strains returned two large clades with a dissimilarity of 1.52 (Fig. 1). All the cropfields studied in Sonora, two from Guamúchil (G1 and G3) and one from Los Mochis (LM2) formed one clade; the

remaining cropfields from Sinaloa were in the other. These two clades give rise to eight subgroups, with fields VY3 and VY1 the most similar (0.35), while the strains from G2 were most similar to the control strain.

For the macroscopic characteristics, the color of the strains went from whitish (groups 1, 3, 5 and 6), brownish-white (groups 2 and 4), pinkish-white (group 7), to purplish-brownish-white (group 8).

The appearance of the colony varied from cottony with an abundant mycelium (groups 1 and 2), patchily cottony (groups 3 and 6), cottony velvet (groups 4 and 5), cottony-feathery (groups 1 and 7) and patchily cottony velvet (group 8).

At the microscopic level, the mean length of the macroconidia was 16.5-34.1 μm for the eight groups (Table 3), with the two extremes recorded in the fields in Sinaloa, LM3 and LM2 respectively ($p \leq 0.05$); most had 2-4 septa. Mean length for the other fields were similar to that of the control ($p > 0.05$). Mean macroconidia width was the same as that of the control ($p > 0.05$), with the exception of the samples from cropfield VY2 (4.2 μm). In Fig. 2, the macro- and microscopic characteristics of the FOC strains isolated are given.

Microconidia were abundant, unicellular, oval and did not vary significantly in length ($p > 0.05$) (Table 3),although width was significantly different for the samples from cropfield CH3 (3.3 μm) ($p \leq 0.05$). Length and width means ranged from 7.2-9.8×2.5-3.3 μm. Chlamydospores had a thick wall, were spherical and had an intercalary or terminal location. Mean diameter was 4.8-8.1 μm, chlamydospore size for the eight groups was the same as that of the control ($p > 0.05$), with the exception of cropfield LM3 (4.8 μm). At day 7 the growth rate of the strains was between 6 and 7.5 cm ($p > 0.05$) (Table 3).

The principal component analysis revealed that macroconidia length is the variable that most influences differentiation between the fields studied for the morphological characteristics of FOC. The first principal component explains 92% of the total variation. This is confirmed in Fig. 1 which shows that the most dissimilar field was LM2 where the greatest mean macroconidia length was recorded (Table 3).

Abiotic factors: The physical and chemical properties of the soil all differed significantly among the cropfields ($p < 0.05$; Table 3). pH was 6.9 (LM1 and G1) to 7.9 (VY2 and VY3). Electrical conductivity (EC) was 1.6 (LM3 and VY2) to 6.3 dS m^{-1} (G3). Na$^+$ concentration was below 5 mg L^{-1} in all cropfields, except in G3 (11.8) and CH2 (6.5). Cl$^-$ was lower than 5 meq L^{-1} in all cropfields, except G1 (8.4) (data not
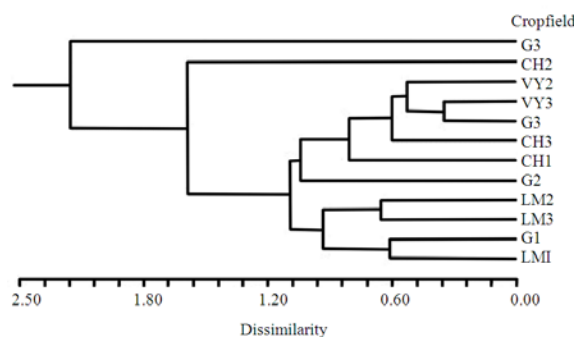

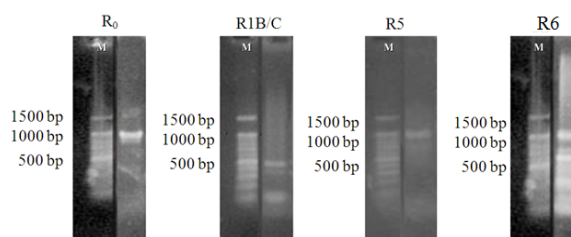Fig. 3: Dendrogram of the physical and chemical soil properties

Fig. 4: Agarose gels showing amplification products from Polymerase Chain Reaction (PCR) using genomic DNA from isolates of *Fusarium oxysporum* f.sp. *ciceris*. Size of amplified bands was: R0, 0.9 Kbp; R1B/C, 0.5 Kbp; R5, 0.9 Kbp and R6, 1.0 Kbp. M: 100 bp Ladder DNA marker

shown). The lowest nitrate concentration was recorded for field G1 (20 mg kg^{-1}) and the values for all the other fields were higher than 30 mg kg^{-1}. In G1, G2 and G3 phosphate concentration was less than 30 mg kg^{-1} (the minimum acceptable for agricultural crops) and in the other fields it was higher. In all of the cropfields, potassium concentration was greater than 150 cmol kg^{-1}, the minimum recommended for agricultural land. Organic matter content was low in all of the cropfields from 1.1 (LM2 and CH1) to 1.8% (G1 and VY1). Soil texture ranged from clayey to loam, through silty clay and silty clay loam (Table 4).

The multivariate analysis of the physical and chemical properties of the soil revealed that field G3 in Sinaloa was the most dissimilar (2.22), followed by CH2 in Sonora. The remaining fields fell into two clades: one comprised of the Sonora fields and cropfield G2 in Guamúchil, Sinaloa and the other comprised only of the remaining fields in Sinaloa (Fig. 3).

The principal components analysis applied to the soil properties returned salinity (45.8%) and texture (24.4%) as the variables with the greatest influence. In

Table 3: Microscopic characteristics and growth rate of FOC strains

Group	Cropfield	Macroconidia (µm)		Microconidia (µm)		Chlamydospores (µm)	GR
		L	W	L	W	D	cm 7 day
1	VY1	22.4bcde	3.4ab	8.4a	2.8ab	6.9abc	6.5a
	VY3	24.9cde	3.6ab	7.8a	3.0ab	8.2ab	7.0a
2	G3	18.4ed	3.2ab	7.6a	3.0ab	7.4ab	6.0a
	CH2	27.2ab	3.7ab	8.9a	2.9ab	6.9abc	7.5a
3	G2	19.5cde	2.8ab	7.2a	2.6ab	8.1ab	7.0a
	Control	21.9bcde	2.6b	7.8a	2.5b	7.4ab	7.0a
4	G1	21.2bcde	3.7ab	7.9a	2.9ab	8.1a	7.0a
	CH1	20.3bcde	3.7ab	7.5a	3.2ab	8.0ab	7.0a
5	LM3	16.5e	3.1ab	7.6a	2.9ab	4.8c	7.5a
	LM1	17.4ed	3.1ab	7.9a	2.9ab	6.5abc	7.0a
6	VY2	26.6abc	4.2a	8.8a	2.7ab	5.8bc	7.5a
7	CH3	23.0bcde	3.9ab	9.1a	3.3a	6.2ab	7.5a
87	LM2	34.1a	3.6ab	9.8a	3.0ab	7.2abc	7.0a

L = Length; W= Width; D = Diameter; GR = Growth Rate. Means in the same column but with different letters are statistically different (p≤0.05)

Table 4: Physical and chemical soil analysis for the fields sampled

Cropfield	pH	EC (dS m^{-1})	N-NO$_3$(mg kg^{-1})	P-PO$_4^=$(mg kg^{-1})	K(cmol kg^{-1})	OM(%)	Texture
Sinaloa							
LM1	6.9bc	1.9c	46cde	150b	242bcd	1.7a	C
LM2	7.5ab	1.8c	39ed	109bcd	272bcd	1.1b	CL
LM3	7.5ab	1.6c	79bc	127bc	283abcd	1.4ab	L
G1	6.9cb	1.9c	20d	14e	354a	1.8a	L
G2	7.7a	1.8c	37ed	18e	302ab	1.6ab	SL
G3	7.7a	6.3a	80bc	30e	230cd	1.5ab	SL
Sonora							
CH1	7.7a	2.0c	55bcde	52de	310ab	1.1b	SC
CH2	7.7a	3.8b	141a	66cde	242bcd	1.6ab	SC
CH3	7.7a	2.0c	75bcd	46de	353a	1.3ab	C
VY1	7.8a	2.1bc	75bcd	178ab	225d	1.8a	SCL
VY2	7.9a	1.6c	39ed	176ab	300abc	1.7a	SCL
VY3	7.9a	1.9c	89b	240a	270bcd	1.6ab	SL

EC= Electrical Conductivity; OM= Organic Matter; Texture: C= Clayey, CL= Clay Loam, L= loam, SL= Silty Loam, SC = Silty Clay, SCL= Silty Clay Loam. Means in the same column but with different letters are statistically different (p≤0.05)

fact, the highest EC was observed for G3 (6.3 dS m^{-1}) followed by CH2 (3.8 dS m^{-1}) (Table 4), the two least similar fields among all those evaluated (Fig. 3).

On analyzing the dendrograms of the physical and chemical properties of the soil (Fig. 3) and the microscopic characteristics of FOC (Fig. 1), we can see that fields VY1, VY2, VY3, CH3 and CH1 tend to group into the same clade, suggesting that edaphological conditions and geographic origin have some kind of influence on the microscopic characteristics of this fungus.

Pathotypes detection by PCR: Of the 161 strains positive for FOC and based on the races reported for America (0, 1B/C, 1A, 5 and 6), the first two yellowing and the last three wilting with the exception of 1A, the four remaining pathotypes were identified by specific PCR (Fig. 4). Race was identified for 91 of the 161 strains as follows: 0 (41%), 1B/C (15%), 5 (14%) and 6 (28%).

Thus, this is the first report of races of FOC in Mexico. The strains that did not fall into one of those previously mentioned have been stored for future study. These 70 remaining strains may belong to races previously not reported for the Americas, to new pathotypes or they may be nonpathogenic strains.

DISCUSSION

Isolation of FOC : The symptoms of the disease caused by FOC and even though they did not isolate the pathogen, they estimated that 60% of the area sown with garbanzo in La Costa de Hermosillo was affected by it. However, Fusarium wilt and root rot can be confused with diseases caused by other pathogens in the diagnosis if one bases the latter solely on the symptoms of the plant.

Morphological variability of FOC: According to morphological variability of FOC, our results coincide with those obtained by Trujillo *et al.* (2005), who also detected a high degree of phenotypic and genomic variability in the strains of *Fusarium* spp. responsible for wilting in carnations. Morphological and genetic

variability was also high in *Thanatephorus cucumeris* (A.B. Frank) Donk in vineyards of Sonora, Mexico (Meza-Moller *et al.*, 2007; Molina-Freaner *et al.*, 2010).

Fusarium oxysporum has been reported to vary in color on the PDA growth medium (Groenewald *et al.*, 2006). The aerial mycelium is white and can change to a variety of colors -from violet to dark purple- depending on the strain of *F. oxysporum*. Environmental conditions such as growth medium, light and temperature can cause pigment production in *F. oxysporum* (Rodrigues and Menezes, 2005).

The macro- and microscopic characteristics of the FOC strains this phenotypic variation coincides with that reported in other studies which identify *F. oxysporum* as one of the most variable species in its genus (Domsch *et al.*, 1993; Dueñas *et al.*, 2003; Groenewald *et al.*, 2006).

Microconidia size did not varied significantly, being similar to that recorded by Domsch *et al.* (1993). Chlamydospores size, for the eight groups was the same as that of the control, Martinez *et al.* (1996) observed that the number of *F. oxysporum* chlamydospores increases as the culture dehydrates, which points to their function as a resting spore.

Abiotic factors: The altitude of the chickpea growing region in northwestern Mexico is 12-75 m a.s.l. and the abiotic conditions that predominate are a dry to very dry climate, mean annual precipitation of 200 to 600 mm and a mean annual temperature of 23-25°C (INEGI, 2000). Ninety-eight percent of the area under cultivation and 92% of the world's chickpea production are in the semiarid tropics. Vujanovic *et al.* (2006) recorded *F. oxysporum* in Canada at temperatures between -5.7 and -17°C and temperatures between 24 and 27°C, along with high soil and air humidity are ideal conditions for FOC. In contrast, in northwestern Mexico under conditions of extreme aridity, FOC are widely distributed.

Recently, Karami *et al.* (2009) reported the morphological characteristics and pathogenicity of *Synchytrium psophocarpi* (Rac.) Baumann associated with false rust on winged bean, but further investigations to elucidate the influence of environmental factors on the disease and to determine host range is required.

The occurrence of FOC increases in acid pH and it is suppressed by alkalinity. Our results suggest the adaptation of FOC to neutral to slightly alkaline pH. Although the crop grows best at a pH of 6, it can grow when pH is 2 to 12. The optimal pH for FOC is lower than 4.5 (Martinez *et al.*, 1996), though it has been isolated from soils at pH 5.3-6.3 (Vujanovic *et al.*,

2006). The occurrence of FOC increases in acid pH and it is suppressed by alkalinity. Our results suggest the adaptation of FOC to neutral to slightly alkaline pH. Anuar *et al.* (2008) reported long-term applications of N decreased the soil pH from 4.2 to 3.7 and caused leaching of K to the lower depth.

Electrical conductivity (EC), crops grow quite well when it is 1.5-2 dS m^{-1}, growth is average between 2.1 and 4 and poor when EC is above 4 dS m^{-1}. Chickpea's water requirement is low (40-45 cm surface water), with an EC lower than 1 dS m^{-1} and a sodium absorption ratio of less than 5. Knowing the spatial losses of water beyond the root zone is important for precise management of the irrigation water (Aimrun *et al.*, 2010).

Vujanovic *et al.* (2006), observed a higher number of colony forming units (CFU g^{-1} soil) of *F. oxysporum* colonies in sandy soils. Chickpea crops need deep soils with a sandy loam to clay loam texture and with no salinity problems (Kukreja *et al.*, 2005).

Pathotypes detection by PCR: Of the four FOC races, two yellowing and two wilting pathotypes were identified. Races of FOC are geographically circumscribed: 2, 3 and 4 have only been reported for India, while 0, 1B/C, 5 and 6, are known for the Mediterranean region and the United States of America (California). In contrast, race 1A is the most widely distributed and has been observed in all three of the aforementioned regions (Jimenez-Gasco and Jimenez-Diaz, 2003).

In Sonora and Sinaloa, though these states are significant producers and exporters of chickpea, the diagnosis of the disease is based on the symptomology of the plants and on occasion plant yellowing and wilting is mistaken for root rot because the causal agent has not been isolated and identified. This means that studies are required to identify and understand the pathotypes, in order to contribute to the search for ways to control the disease.

In the Bajio region of Mexico, Luna-Paez *et al.* (2005) studied the distribution and genetic variability of FOC using genomic DNA polymorphisms and PCR-RAPD. However, they did not report the identity of the pathogenic races of FOC, just that there were two groups of isolates for yellowing and two for wilting, suggesting the possible presence of some of the races described for other countries.

The distribution and adaptation of the species in *Fusarium* are reported to be delimited by climate, geographic region, soil type, variety and cultivar (Vujanovic *et al.*, 2006).

CONCLUSION

Morphological variability in *Fusarium oxysporum* f.sp. *ciceris* was high in the main chickpea producing

regions in northwestern Mexico and was not a function of the physical and chemical properties of the soil, nor of the geographic location of the cropfields. Yellowing (0 and 1B/C) and wilting (5 and 6) races were found among the strains isolated, reflecting the symptoms observed in the areas sampled. This is the first report of FOC races in Mexico. There may be other races or nonpathogenic strains of FOC in the study area, which would require specific primers to carry out PCR on the strains yet to be identified.

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