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Vegetative Compatibility Groups in *Fusarium oxysporum* f.sp. *ciceris* and *F. oxysporum* Non-pathogenic to Chickpea

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Abstract

Forty-seven isolates of *Fusarium oxysporum* f.sp.*ciceris*, representative of the different pathogenic races and originating from California, India, Tunisia and Spain, and twenty-five non-pathogenic *F. oxysporum* isolates obtained from chickpea roots in Algeria, Italy, Morocco, Pakistan and Spain, were analyzed for vegetative compatibility. Only one vegetative compatibility group (VCG) was found among the 47 isolates of *F. oxysporum* f.sp. *ciceris*, although some isolates failed to complement with one or more isolates in this VCG. Among the twenty five non-pathogenic isolates, we found three multi-member VCG, each group containing two to five isolates. Pathogenic and non-pathogenic isolates complemented sporadically. Most of the non-pathogenic isolates were compatible with a yellowing-type low virulent *F. oxysporum* f.sp. *ciceris* isolate (Foc USA 3/1 JG), thus suggesting the possibility of the existence of transition isolates between pathogenic and non-pathogenic populations. Heterokaryon formation between compatible isolates was shown by both pathogenicity and molecular tests. The virulence pattern displayed by the heterokaryons indicated the presence of genetic factors controlling pathogenicity from both parental races. RAPD-PCR analysis of total DNA of the heterokaryon also showed the presence of the two genomes in it. The existence of a single VCG in the *F. oxysporum* f.sp. *ciceris* population support the hypothesis of its monophyletic origin.

Introduction

Fusarium oxysporum Schlechtend.: Fr. f.sp. *ciceris* (Padwick) Matuo & K. Sato (Foc) is the causal agent of Fusarium wilt in chickpea (*Cicer arietinum* L.). The most practical and economic disease control method is the use of resistant cultivars (Jalali and Chand, 1992), whose effectiveness is limited by the existence of pathogenic races. Therefore, the identification of pathogenic races in Foc is important for disease resistance breeding, and for the efficient use of available resistant cultivars. So far, eight pathogenic races have been identified in California (races 0, 1A, 1B/C, 5 and 6), India (races 1A, 2, 3 and 4), Morocco (races 1A and 6), Spain (races 0, 1A, 1B/C, 5 and 6), Tunisia, Syria, and Turkey (races 0 and 1B/C), Israel (races 0, 1A and 6) and Lebanon (race 0) (Haware and Nene, 1982; Jiménez-Díaz et al., 1989, 1993; Halila and Strange, 1996). Two pathotypes, causing yellowing and wilting, have been differentiated: races 1A, 2, 3, 4, 5 and 6 induce the wilt syndrome, and races 0 and 1 B/C induce the yellowing syndrome (Trapero Casas and Jiménez-Díaz, 1985).

Attempts to assess genetic variability in *Fusarium* species have been made using a number of techniques, including vegetative compatibility group (VCG) testing (Puhalla, 1985). Vegetative compatibility in fungi is a genetic trait controlled by *vic* or *het* loci (Anagnostakis, 1982) and identical alleles at each loci must be present in two compatible hyphae before anastomosis takes place. Vegetatively compatible isolates of a fungal species are placed in the same VCG. VCGs are identified using nitrate nonutilizing (*nit*) auxotrophic mutants that show thin but expansive growth on minimal medium with nitrate as sole nitrogen source. Isolates are considered vegetatively compatible when complementing *nit* mutants anastomose and produce wild type growth (Puhalla, 1985). In the absence of a sexual stage, it was proposed that exchange of genetic material would be limited to compatible isolates within a VCG and so each VCG represents a genetically isolated population. Vegetative compatibility has been used to classify isolates of *F. oxysporum* belonging to distinct formae speciales (Katan and Katan, 1988; Gordon and Okamoto, 1991; Katan et al., 1991; Klein et al., 2005), races within a special form (Katan et al., 1991; Marlatt et al., 1996; Katan and Di Primo, 1999; Elena and Pappas, 2006), special forms and/or races within or between different geographical origins

(Katan et al., 1996; Fernandez et al., 1997; Di Primo et al., 2002; Pasquali et al., 2005), and non-pathogenic isolates (Correll et al., 1986; Kalc Wright et al., 1996; Edel-Hermann et al., 2004).

Until now, 49 formae speciales of *F. oxysporum* have been subjected to vegetative compatibility grouping analysis (Katan, 1999; Katan and Di Primo, 1999), but compatibility was never explored in *F. oxysporum* f.sp. *ciceris* isolates. The objective of this work was to determine: (i) VCGs in a collection of isolates of *F. oxysporum* f.sp. *ciceris* of different pathogenic races and different geographical origins, and in a collection of non-pathogenic *F. oxysporum* isolates obtained from asymptomatic chickpea roots in different countries; (ii) compatibility between pathogenic and non-pathogenic isolates; and (iii) pathogenicity and RAPD-PCR pattern of heterokaryons in comparison with their parents.

Materials and Methods

Fungal strains

We have used 47 isolates of *Fusarium oxysporum* f.sp. *ciceris* (Foc) originating from California (10), India (8), Tunisia (1) and Spain (28), representing the different races (Table 1), and 25 non-pathogenic isolates of *F. oxysporum* (Fo) obtained from roots of asymptomatic chickpeas in Algeria (6), Italy (11), Morocco (1), Pakistan (1) and Spain (6) (Table 2). The isolates were maintained as monoconidial cultures in tubes with soil or Potato-Dextrose-Agar (PDA), at 4°C.

Table 1
Isolates of *Fusarium oxysporum* f.sp. *ciceris* used in this study, with indication of race and geographic origin

Isolate	Pathogenic race	Geographic origin
Foc 7802	0	Andalucía (Spain)
Foc 7952	0	Andalucía (Spain)
Foc 8291	0	Andalucía (Spain)
Foc 82108	0	Andalucía (Spain)
Foc 82113	0	Andalucía (Spain)
Foc 82115	0	Andalucía (Spain)
Foc 82128	0	Andalucía (Spain)
Foc 8503	0	Andalucía (Spain)
Foc 8717	0	Andalucía (Spain)
Foc 8733	0	Andalucía (Spain)
Foc 9018PV	0	Andalucía (Spain)
Foc 9018JG	0	Andalucía (Spain)
Foc 9032	0	Andalucía (Spain)
Foc 90111PV	0	Andalucía (Spain)
Foc 90111JG	0	Andalucía (Spain)
Foc Buetton	0	California (USA)
Foc Túnez 3	0	Tunisia
Foc 8720	1	Andalucía (Spain)
Foc 8726	1	Andalucía (Spain)
Foc 9027pv	1A	Andalucía (Spain)
FocUSA1633 – 2R1JG	1A	California (USA)
FocUSASD8415K1JG	1A	California (USA)
FocUSA1420 – 1R1JG	1A	California (USA)
Foc 7989	1A	Hyderabad (India)
Foc W-17	1B/C	California (USA)
Foc USA 3/1JG	1B/C	California (USA)
Foc R1N	1	India
Foc 8605	2	Kanpur (India)
Foc R2N	2	India
Foc 8606	3	Gurdaspur (India)
Foc R3N	3	India
Foc 8607	4	Jabalpur (India)
Foc R4N	4	India
Foc 8012	5	Andalucía (Spain)
Foc 8257	5	Andalucía (Spain)
Foc 8408	5	Andalucía (Spain)
Foc 8507	5	Andalucía (Spain)
Foc 8508	5	Andalucía (Spain)
Foc 9094JG	5	Andalucía (Spain)
Foc W6-I	5	California (USA)
Foc USA 1/1JG	5	California (USA)
Foc USA 6/1JG	5	California (USA)
Foc 9027JG	6	Andalucía (Spain)
Foc 9093PV	6	Andalucía (Spain)
Foc 9026JG	6	Andalucía (Spain)
Foc 90117JG	6	Andalucía (Spain)
Foc Tonini	6	California (USA)

Selection, characterization and storage of nit mutants

The *nit* mutants were selected by Puhalla's method (Puhalla, 1985). For this, 2 mm PDA blocks with fungal mycelium were transferred to 6 cm plates that contained PDA with 1.5% chlorate (PDC) or a salt Minimal Medium with 1.5% chlorate (MMC) (Puhalla, 1985), and were incubated at 25°C for 1 to 3 weeks. Fast growing sectors were transferred to Minimal Medium (MM) and those which grew as fine and expanding colonies, without aerial mycelium, were considered to be *nit* mutants. In those cases in which we were not able to select mutants in the conditions described above, we introduced some modifications, such as to increase the concentration of chlorate in MMC up to 6%, or to use a Czapek-Dox medium with 1.5% of chlorate (CDC), or two consecutive cultures on MMC (1.5% chlorate).

From each isolate, we obtained 2 to 10 *nit* mutants, which were further characterized on the basis of their capacity to utilize different sources of nitrogen (Correll et al., 1987). One or various *nit* 1 and *Nit* M mutants of each isolate were selected and stored at 4°C, both in tubes with sterile soil and on sterile Whatman paper. Small fragments of this paper were later used as inoculum for the complementation tests.

Vegetative compatibility tests

Vegetative Compatibility was determined by observing the formation of a heterokaryon between complementary *nit* mutants. For this, small fragments of Whatman paper containing the mycelium of the complementary mutants were placed, 2 cm each other, on MM agar plates and incubated at 25°C, 12 h photoperiod at 40 μEm-2 s-1, for 1 to 4 weeks.

Initially, a *nit* 1 and a Nit M mutant from each isolate were paired to determine whether the isolate was self-compatible or self-incompatible. After that, complementary *nit* mutants of all isolates were paired in all possible combinations to establish which of the isolates were vegetatively compatible. The complementation test was considered: (i) negative, when there was no prototrophic growth in the mycelial line of contact; (ii) weak, when interaction became evident by the appearance of a thin, sometimes non-continuous, zone of prototrophic growth with very little aerial mycelium and (iii) strong, when a dense line of prototrophic growth with abun-

Table 2
Isolates of *Fusarium oxysporum* non-pathogenic to chickpea used in this study

Isolate	Geographic origin
Fo 9009	Andalucía (Spain)
Fo 9014	Andalucía (Spain)
Fo 9033	Andalucía (Spain)
Fo 9081I	Andalucía (Spain)
Fo 90101	Andalucía (Spain)
Fo 90105	Andalucía (Spain)
Fo 9169	Tánger (Morocco)
Fo 91117	Castilla-León (Spain)
Fo Pakistán	Pakistán
F sp 2	Algeria
F sp 3	Algeria
F sp 4	Algeria
F sp 5	Algeria
F sp 7	Algeria
F sp 9	Algeria
Fo 420	Oristano (Italy)
Fo 425	Bellizi (Italy)
Fo 442	Italy
Fo 457	Racale (Italy)
Fo 506I	Tarquinia (Italy)
Fo 511I	Italy
Fo 526I	Leonessa (Italy)
Ceralicultura 3p	Italy
Ceralicultura 4p	Italy

dant aerial mycelium was obtained. Weak and strong reactions were taken as evidence of compatibility.

Microscopical observations

Hyphal fusion between different *nit* mutants was examined microscopically. For this purpose, a *nit 1* and a *nit* M mutant were grown 4 cm apart over a thin layer of 2% water-agar on a slide. Observations were made with a Nikon Optiphot® (Nippon kogaku K. K., Shijuku-ku, Tokio, Japan) and optics of Nomarsky.

Pathogenicity tests

Pathogenicity of the heterokaryons formed between complementary *nit* mutants from races 0 and 5, and 1 and 5 respectively, was determined by their differential virulence reactions on chickpea cultivars 12071/10054, P2245, JG62 and ICCV2. Inoculum production and inoculation method have been previously described (Trapero Casas and Jiménez-Díaz, 1985). Inoculum was increased in a cornmeal-sand (CMS) mixture and mixed with an autoclaved soil mixture (clay loam/sand/peat, 1 : 1 : 1, v/v). Germinated chickpea seeds were sown in 15-cm-diameter clay pots (three seeds per plot) filled with the infested soil mixture. Non-inoculated plants, as well as plants inoculated with wild-type isolates and their associated *nit* mutants, were used as controls. Plants were grown in a growth chamber and observed at 2-day intervals for symptom development (Trapero Casas and Jiménez-Díaz, 1985).

RAPD-PCR analyses of the DNA

Total genomic DNA of the heterokaryon, as well as those from the wild-type parentals and the corresponding *nit* mutants, were analyzed by RAPD-PCR amplification. Mycelia were obtained from culture plates with PDA (wild-type parents) or MM (*nit* mutants and heterokaryons). DNA were extracted from the lyophilized mycelia according to Raeder and Broda (1985), as previously described (Pérez-Artés et al., 1995).

Primers used for amplification were Ks, P2 and P6, that generate fragments of DNA specifically associated with races 0 and 5 of Foc (Kelly et al., 1994). Reaction mixtures and conditions of amplification were the same as those described by Kelly et al. (1994). Products of amplification were separated in 1.5% agarose gels, stained with ethidium bromide and observed under ultraviolet light.

Results

Isolation, characterization and storage of nit mutants

Isolation of chlorate-resistant mutants and phenotypic diversity of *nit* mutants recovered was compared between MMC and PDC media. The frequency of appearance of rapid growth sectors was similar in both media, but the proportion of those sectors that were chlorate-resistant but able to utilize nitrate (*crn* mutants, of no use for VCG testing) was lower with MMC.

Whenever possible, at least two different *nit* mutants, preferably a *nit* 1 and a Nit M, were selected and stored as indicated in Materials and Methods.

Vegetative compatibility tests

Initially, two different *nit* phenotypes of each isolate were paired on MM to check for self-compatibility. Out of the 47 Foc isolates tested, 16 (34%) were self-incompatible. In contrast, non-pathogenic Fo isolates showed a much lower percentage of self-incompatibility: out of 14 isolates tested, two were self-incompatible. Nevertheless, this proportion could be actually higher, because self-compatibility could not be checked for the 10 Fo isolates from which we obtained only a single *nit* phenotype.

When possible, a *nit* 1 and a Nit M mutant from each isolate were selected and paired in all possible combinations. The pattern of heterokaryon formation between the mutants of the different isolates showed the existence of a single VCG, although the extension of the compatibility between isolates was variable (Fig. 1).

Among the 25 non-pathogenic Fo isolates, a much lower proportion of compatible pairings was found. Three VCG were established, two of them with only two isolates (Fig. 2). One VCG comprised five isolates representing three geographic origins: Fo 90105, Fsp2, Fo 511I, Fo 90101 and Fsp9. The two others VCGs included respectively, isolates Fsp3 and Fsp4 from Algeria and isolates Fo 457 and Fo 442 from Italy.

Vegetative compatibility between pathogenic and non-pathogenic isolates was also tested. For that, two complementary *nit* mutants (*nit* 1 and Nit M) from nine Foc isolates representative of the seven different races were selected by its ability to form strong heterokaryons with many others mutants. The selected Foc

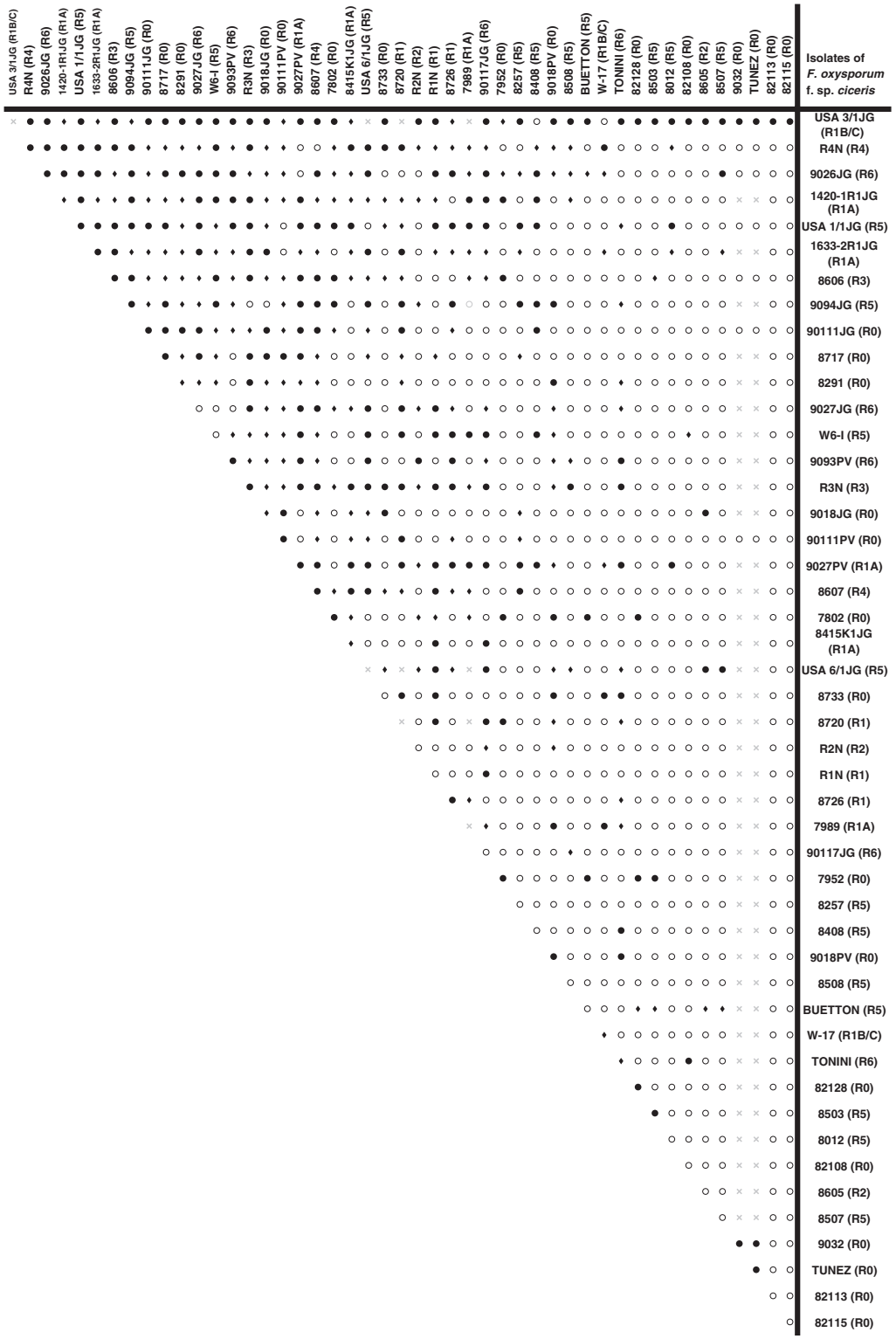

Fig. 1 Heterokaryon formation between complementary *nit* mutants of *Fusarium oxysporum* f.sp. *ciceris* isolates. ●: strong complementation; ♦: weak complementation; ○: no complementation;×: complementation not tested

nit mutants were paired with complementary mutants from the 25 non-pathogenic Fo isolates. Results showed that most of the non-pathogenic isolates complemented with the Foc isolate USA 3/1 JG, a race 1B/C isolate from California (USA). Isolates Fo 91117 and Fo 9014, both, from Spain, were also compatible with the race 0 isolates Foc 9032 (Spain) and Foc Tunez (Algeria) respectively (Fig. 3).

Pathogenicity test

Chickpea plants from cultivars P2245 (susceptible to races 0, 1 and 5), JG 62 (susceptible to races 1 and 5

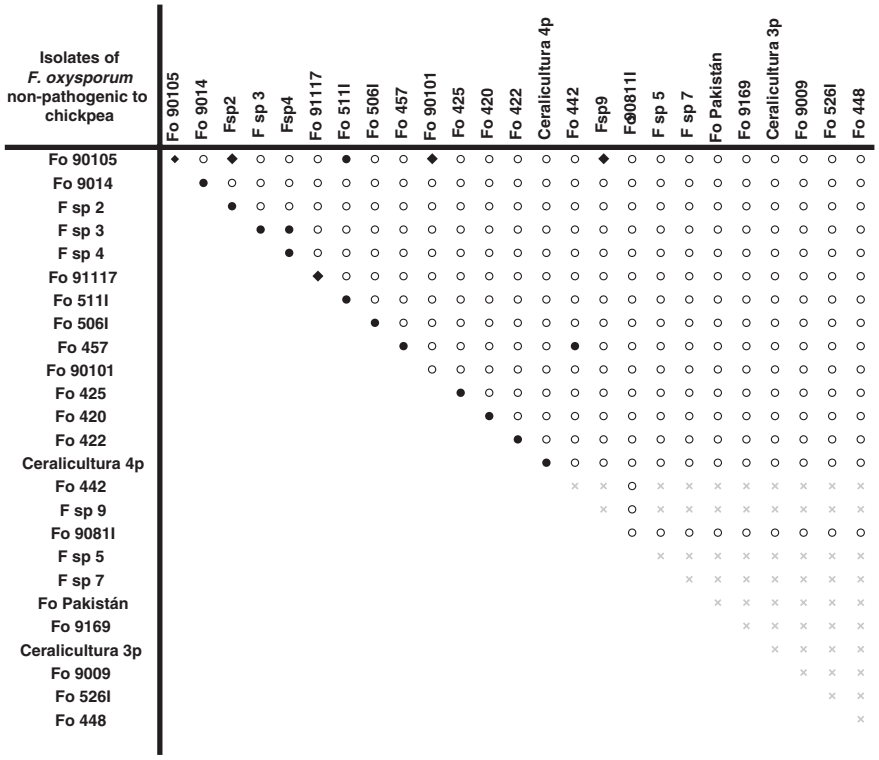

Fig. 2 Heterokaryon formation between complementary *nit* mutants of *Fusarium oxysporum* non-pathogenic to chickpea. ●: strong complementation; ♦: weak complementation; ○: no complementation; ×: complementation not tested

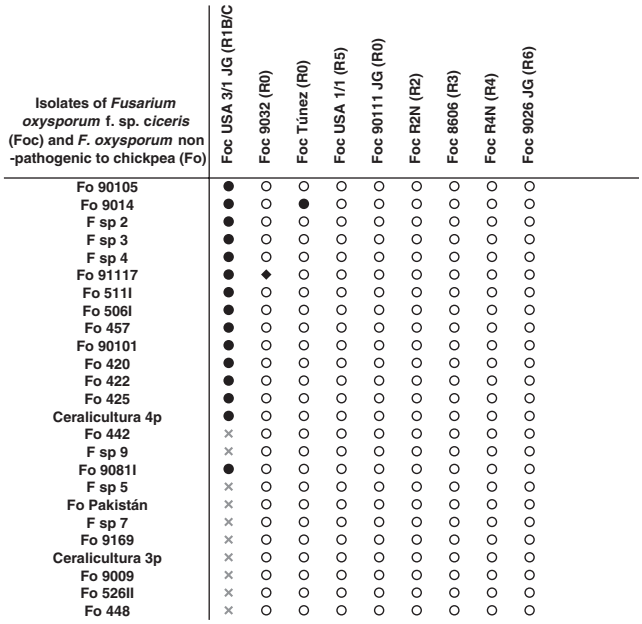

Fig. 3 Heterokaryon formation between complementary *nit* mutants of a selection of *Fusarium oxysporum* f.sp. *ciceris* representing the different races, and the collection of *Fusarium oxysporum* isolates non-pathogenic to chickpea. ●: strong complementation; ♦: weak complementation; ○: no complementation; ×: complementation not tested

and resistant to race 0), 12071/10054 (susceptible to races 0 and 1 and resistant to race 5), and ICCV2 (susceptible to race 5, and resistant to races 0 and 1), were inoculated with the heterokaryons formed between each pair of isolates Foc 8291 (race 0) and Foc W6-I (race 5), and Foc 3/1 JG (race 1B/C) and Foc W6-I (race 5) (Table 3). Non-inoculated plants as well as plants inoculated with the parentals and with their *nit* mutants were used as controls in each case.

Table 3
Differential virulence reactions on chickpea cultivars of the heterokaryons formed between complementary *nit* mutants from races 0 and 5 (0/5), and 1B/C and 5 (1/5) respectively

	Race				
Cultivar	0	1	5	0/5	1/5
P 2245	Y	Y	W	Y	Y
JG 62	R	Y	W	(W)	–
12071/10054	Y	Y	R	Y	Y
ICC V2	R	R	W	(W)	(W)

Y, yellowing symptoms; W, wilting symptoms; (W), slight wilting.

Symptoms induced in the susceptible cultivars by isolates of races 0 and 1B/C, as well as by their *nit* mutants, were those associated with the yellowing syndrome. On the other hand, symptoms induced by isolate from race 5, as well as the induced by its *nit* mutant, were those of the wilting syndrome. Moreover, there were no differences in virulence between parents and *nit* mutants isolates. Isolation from vascular tissues of inoculated plants showed that both phenotypes, parental and *nit* mutant, were obtained and they corresponded with isolates used for inoculation.

Symptoms displayed by the different cultivars inoculated with each of the two heterokaryons were in good agreement with the 'heterokaryotic nature' of

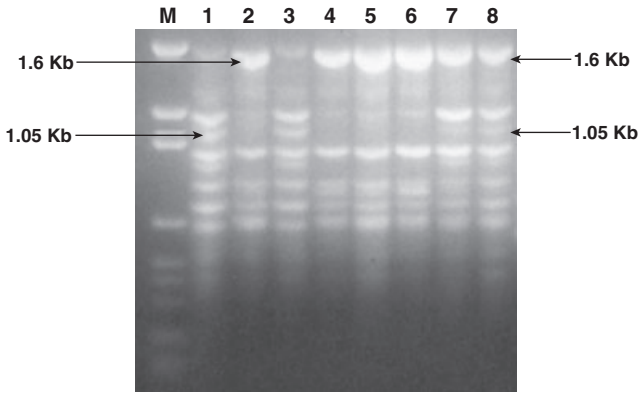

Fig. 4 RAPD-PCR amplification with primer Ks of total DNA from a *Fusarium oxysporum* f.sp. *ciceris* isolate of race 0 (lane 1), and of race 5 (lane 2); from their associated *nit* mutants (lane 3: *nit* 1 mutant of Foc race 0; lanes 4–5: Nit M mutant of Foc race 5); and from the heterokaryon obtained after complementation of the *nit* mutants (lanes 6–8). Numbers on the left and right side are the sizes of the marker bands that identify races 0 (1.05 kb) and 5 (1.6 kb). Lane M: molecular weight marker

the inocula (Table 3). Inoculation with the heterokaryon 0/5 produced symptoms in all the chickpea genotypes after 25 days. Symptoms in cvs P2245 (susceptible to both races) and 12071/10054 (resistant to race 5) consisted of yellowing; on the other hand, cvs JG 62 and ICCV2 (both resistant to race 0) showed a slight wilting. In inoculations with the heterokaryon 1/5, plants from cvs P2245 (susceptible to both races) and 12071/10054 (resistant to race 5) showed the yellowing syndrome and were dead at 35 days after inoculation; plants from cv ICCV2 (resistant to race 1) showed slight wilting at 32 days after inoculation (Table 3).

RAPD-PCR analysis of the heterokaryons

Results obtained with primers Ks, P2 and P6 constituted direct evidence of the presence of genetic material from both races in the heterokaryon. Results obtained with primer Ks and the heterokaryon 0/5 are shown in Fig. 4. Pimer Ks amplifies fragments of DNA of 1.05 kb and 1.6 kb that are specific for isolates of race 0 and race 5, respectively. The correct fragment was amplified from DNA of both (race 0 and 5) parentals, as well as from their associated *nit* mutants (Fig. 4). As expected, DNA from the heterokaryon 0/5 amplified the two fragments, the one specific of race 0 (1.05 kb) and the other one specific of race 5 (1.6 kb), thus demonstrating the presence of the two genomes in the heterokaryon (Fig. 4).

Discussion

Vegetative compatibility has been analyzed in a collection of *Fusarium oxysporum* f.sp. *ciceris* isolates and of *Fusarium oxysporum* isolates obtained from chickpea roots of asymptomatic plants. All these non-pathogenic isolates caused mild cortical necrosis in artificial inoculations of chickpeas, except the isolates Fo 9009 and Fo 9014, which induced asymptomatic vascular infections (Jiménez-Díaz et al., unpublished).

One VCG was found among 47 isolates of *Fusarium oxysporum* f. sp.*ciceris* of races 0, 1A, 1B/C, 3, 4, 5 and 6, representing the two pathotypes and the geographical range of the pathogen. This suggests that asexual genetic exchange may occur between isolates of different races. According with the systematic numbering proposed by Katan (1999), the code number for this VCG should be 0280.

Some isolate combinations produced weak or discontinuous reactions. These differences in the intensity of cultural interactions have previously been reported in *F. oxysporum* and could reflect the number of allelic differences at loci affecting vegetative compatibility (Gordon and Okamoto, 1991). Moreover, some isolates in the VCG were incompatible; that is, two isolates might be vegetatively incompatible even though each is compatible with a third isolate. The occurrence of these bridging strains is well known in *F. oxysporum* (Katan et al., 1991; Katan and Katan, 1999; Vakalounakis and Fragkiadakis, 1999).

Complementation between pathogenic Foc isolates and non-pathogenic Fo isolates was also tested. Three out of the nine Foc isolates selected complemented with some non-pathogenic Fo isolates. These corresponded to races 0 (Foc 9032, Foc Túnez), and 1 B/C (Foc USA 3/1 JG). Isolate Foc USA 3/1 JG was compatible with all the non-pathogenic isolates tested (15 out of 26). In fact, this isolate shows a high level of compatibility with pathogenic (Foc) and non-pathogenic (Fo) isolates, in comparison to the other Foc isolates tested. Pathogenicity tests carried out with this Foc isolate showed that it was a yellowing type low virulent isolate, morphologically more similar to non-pathogenic isolates (Alcalá-Jiménez, 1995). This seems to indicate that it could be a transition isolate between pathogenic and non-pathogenic populations. In any case, more work would be necessary to study the possibility that mechanisms other than heterokaryosis could be involved in the particular behaviour of this isolate. On the other hand, race 0 isolates Foc 9032, and Foc Túnez were each one compatible with only one non-pathogenic Fo isolate. These sporadic complementations between pathogenic and non-pathogenic populations has also been described for other *F. oysporum* formae speciales (Elmer and Stephens, 1989).

Hyphal fusions were evidenced microscopically (result not shown), and the exchange of genetic material and formation of a heterokaryon between compatible isolates was shown by both pathogenic and molecular tests. For both tests, the heterokaryons used were those formed between isolates Foc USA 3/1 JG (race 1B/C) and Foc W6-I (race 5), and isolates Foc 8291 (race 0) and Foc W6-I (race 5).

Symptoms showed by the different cultivars inoculated with each of the two heterokaryons were in good agreement with the 'heterokaryotic nature' of these isolates, and constituted evidence of the presence of genetic material from both races in each heterokaryon.

Similarly, products amplified by the three different primers evidenced the presence of the two genomes in the heterokaryon.

Clonality in *F. oxysporum* has been associated with vegetative compatibility (Gordon and Martyn, 1997; Kistler, 1997), with isolates belonging to the same vegetative compatibility group showing higher genetic similarity. In the case of *Fusarium oxysporum* f.sp. *ciceris*, previous studies (Pérez-Artés et al., 1995, 1996; Jiménez-Gasco et al., 2002) showed that all Foc races had the same mtDNA RFLP pattern and showed identical sequences in introns of the translation elongation factor 1α (EF1α), β-tubuline, histone 3, actin and calmodulin genes, thus suggesting that *F. oxysporum* f.sp. *ciceris* has a monophyletic origin. The existence of a single VCG also support this hypothesis.

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References

Alcalá-Jiménez AR. *Aspectos epidemiológicos en el Patosistema Fusarium oxysporum Schlechtend. Fr. F.sp. ciceris (Padwick) Matuo & K. Sato/Cicer arietinum L.*, PhD Thesis. Córdoba, Spain, University of Córdoba, 1995.

Anagnostakis SL. (1982) Genetic analysis of *Endothia parasitica*: linkage data for four single genes and three vegetative compatibility types. *Genetics* **102**:25–28.

Correll JC, Puhalla JE, Schneider RW. (1986) Vegetative compatibility groups among nonpathogenic root-colonizing strains of *Fusarium oxysporum*. *Can J Bot* **64**:2358–2361.

Correll JC, Klittich CJR, Leslie JF. (1987) Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* **77**:1640–1646.

Di Primo P, Cappelli C, Katan T. (2002) Vegetative compatibility grouping of *Fusarium oxysporum* f.sp. *gladioli* from saffron. *Eur J Plant Pathol* **108**:869–875.

Edel-Hermann V, Gautheron N, Alabouvette C. (2004) Genetic diversity of pathogenic and non-pathogenic populations of *Fusarium oxysporum* isolated from carnation fields in Argentina. *Phytopathology* **94**:661–668.

Elena K, Pappas AC. (2006) Race distribution, vegetative compatibility and pathogenicity of *Fusarium oxysporum* f.sp. *melonis* isolates in Greece. *J Phytopathol* **154**:250–255.

Elmer WH, Stephens CT. (1989) Classification of *Fusarium oxysporum* f.sp. *asparagi* into vegetatively compatible groups. *Phytopathology* **79**:88–93.

Fernandez D, Ouinten M, Tantaoui A, Geiger JP. (1997) Molecular records of microevolution within the Algerian population of *Fusarium oxysporum* f.sp. *albedinis* during its spread to new oases. *Eur J Plant Pathol* **103**:485–490.

Gordon TR, Martyn RD. (1997) The evolutionary biology of *Fusarium oxysporum*. *Annu Rev Phytopathol* **35**:111–128.

Gordon TR, Okamoto D. (1991) Vegetative compatibility groupings in a local population of *Fusarium oxysporum*. *Can J Bot* **69**:168–172.

Halila MH, Strange RN. (1996) Identification of the causal agent of wilt of chickpea in Tunisia as *Fusarium oxysporum* f.sp. *ciceri* race 0. *Phytopathol Mediterr* **35**:67–74.

Haware MP, Nene YL. (1982) Races of *Fusarium oxysporum* f.sp. *ciceris*. *Plant Dis* **66**:809–810.

Jalali BL, Chand H. Chickpea wilt. In: Sing US, Mukhopadhayay AN, Kumar J, Chaube HS (eds), *Plant Diseases of International Importance.Vol I. Diseases of Cereals and Pulses*, Englewood Cliffs, NY, Prentice Hall, 1992, pp. 429–444.

Jiménez-Díaz RM, Trapero-Casas A, Cabrera de la Colina J. Races of *Fusarium oxysporum* f.sp. *ciceri* infecting chickpeas in southern Spain. In: Tjamos EC, Beckman CH (eds), *Vascular Wilt Diseases of Plants*, Vol. H28. Berlin, Springer, 1989, pp. 515–520.

Jiménez-Díaz RM, Alcalá-Jiménez AR, Hervás A, Trapero Casas JL. Pathogenic variability and host resistance in the *Fusarium oxysporum* f.sp. *ciceris/Cicer arietinum* pathosystem. In: Arseniuk E, Goral T (eds), *Proc Eur Semin Fusarium Mycotoxins, Taxonomy, Pathogenicity and Host Resistance*. Radzików, Plant Breeding and Acclimatization Institute, Hodowsla Ròslin Aklimatyazacja I Nasiennictwo, 1993, pp. 87–94.

Jiménez-Gasco MM, Milgroom MG, Jiménez-Díaz RM. (2002) Gene genealogies support *Fusarium oxysporum* f.sp. *ciceris* as a monophyletic group. *Plant Pathol* **51**:72–77.

Kalc Wright GF, Guest DI, Wimalajeewa DLS, van Heeswijck R. (1996) Characterisation of *Fusarium oxysporum* isolated from carnation in Australia based on pathogenicity, vegetative compatibility and random amplified polymorphic DNA (RAPD) assay. *Eur J Plant Pathol* **102**:451–457.

Katan T. (1999) Current status of vegetative compatibility groups in *Fusarium oxysporum*. *Phytoparasitica* **27**:51–64.

Katan T, Di Primo P. (1999) Current status of vegetative compatibility in *Fusarium oxysporum*: supplement. *Phytoparasitica* **27**:273–277.

Katan T, Katan J. (1988) Vegetative compatibility grouping of *Fusarium oxysporum* f.sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. *Phytopathology* **78**:852–855.

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

Katan T, Zamir D, Sarfatti M, Katan J. (1991) Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f.sp. *radicis-lycopersici*. *Phytopathology* **81**:255–262.

Katan T, Gamliel A, Katan J. (1996) Vegetative compatibility of *Fusarium oxysporum* from sweet basil in Israel. *Plant Pathol* **45**:656–661.

Kelly AG, Alcalá-Jimenénez AR, Bainbridge BW, Heale JB, Pérez-Artés E, Jiménez-Díaz RM. (1994) Use of genetic fingerprinting and random amplified polymorphic DNA to characterize pathotypes of *Fusarium oxysporum* f.sp. *ciceris* infecting chickpea. *Phytopathology* **84**:1293–1298.

Kistler HC. (1997) Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. *Phytopathology* **87**:474–479.

Klein KK, Edel-Hermann V, Gautheron N, Traore D, Steinberg C. (2005) High genetic diversity among strains of *Fusarium oxysporum* f.sp. *vasinfectum* from cotton in Ivory Coast. *Phytopathology* **95**:1391–1396.

Marlatt ML, Correll JC, Kaufmann P, Cooper PE. (1996) Two genetically distinct populations of *Fusarium oxysporum* f.sp. *lycopersici* race 3 in the United States. *Plant Dis* **80**:1336–1342.

Pasquali M, Dematheis F, Gilardi G, Gullino ML, Garibaldi A. (2005) Vegetative compatibility groups of *Fusarium oxysporum* f.sp. *lactucae* from lettuce. *Plant Dis* **89**:237–240.

Pérez-Artés E, González-Roncero MI, Jiménez-Díaz RM. (1995) Restriction fragment lenght polymorphism analysis of the mitochondrial DNA of *Fusarium oxysporum* f.sp. *ciceris*. *J Phytopathol* **143**:105–109.

Pérez-Artés E, Rodriguez-Jurado D, Jiménez-Díaz RM. Caracterizacion de aislados de *Fusarium oxysporum* no patogénicos de garbanzo mediante análisis de polimorfismos de ADN. *Libro de Resúmenes, Congreso Sociedad Española de Fitopatología, VIII*. Córdoba, Spain, 1996, p. 78.

Puhalla JE. (1985) Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Can J Bot* **63**:179–183.

Raeder U, Broda P. (1985) Rapid DNA preparation from filamentous fungi. *Lett Appl Microbiol* **1**:17–20.

Trapero Casas A, Jiménez-Díaz RM. (1985) Fungal wilt and root rot diseases of chickpea in southern Spain. *Phytopathology* **75**:1146–1151.

Vakalounakis DJ, Fragkiadakis GA. (1999) Genetic diversity of *Fusarium oxysporum isolates* from cucumber: differentiation by pathogenicity, vegetative compatibility and RAPD fingerprinting. *Phytopathology* **89**:161–168.