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Research article

Pathogenic and mating type characterization of an uncommon wilt disease of citrus in Tunisia

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Abstract

In Tunisia, wilt disease of citrus trees has increased in significance in the north and center of the country. Trunks and secondary branches of wilted trees were collected and used to isolate the main pathogen and assess pathogenicity on sour orange rootstock. In addition, mating type (MAT) specific primers were used to assess the mating type of *Fusarium oxysporum* Schlecht isolates. The distribution of MAT idiomorph may suggest that *MAT1-1* is a predominant mating type in the *F. oxysporum* population. Wilt disease symptoms, recorded at the end of the pathogenicity tests, were associated with reduced plant growth and vigour, with shorter and thinner shoots, and limited number of roots. Principal component analysis (PCA) based on the host specificity of isolates (sweet orange and tangerine), geographical origin (CapBon area and Kairouan) and severity index of the isolates showed three groups related to the specific host and origin. The present study has proved essential information on the occurrence of mating types of *F. oxysporum* isolated from citrus.

Key words: *Citrus aurantium*, Fusarium wilt, Principal Component Analysis, Mating types.

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Introduction

Citriculture represents a strategic sector in Tunisia. Citrus is a common practice in littoral and sub-humid areas in Tunisia. This crop appeared to be introduced during the 10th century. Citrus orchards occupy approximately 20,400 ha, with a production oscillating between 210,000 and 300,000 tons during the last decade (FAO, 2013). In addition, sour orange is still the most widely used traditional rootstock of Tunisian citriculture (Snoussi et al., 2012). *Fusarium* species are commonly encountered in soils of citrus orchards (Le Roux 1985; Sherbakoff 1953) and are associated with different citrus diseases, such as dry root rot, root rot, feeder root rot, wilt, twig dieback, and citrus decline. *F. oxysporum* was isolated from the roots and rhizosphere less frequent than *F. solani*. Several researchers had also isolated *F. oxysporum* from citrus orchards in South Africa (Labuschagne et al., 1987) and California (Nemec et al., 1989). Otherwise, a serious wilt and dieback of Mexican lime (*Citrus aurantifolia*) caused by *F. oxysporum* f. sp. *citri* was described earlier in Florida (Timmer, 1982). During 2010, an unusual cause citrus tree death of was detected in the Cap Bon area which represents 80% of the total surface of citrus. This devastating decline has been observed in some citrus groves grafted on sour orange rootstock (*Citrus aurantium*) and was comparable to the wilt in Mexican limes (Hannachi et al. 2014; Timmer et al. 1979). Therefore, the development of wilt disease is quite uncommon because sour orange is generally tolerant to this disease. Recently, the replacement of this rootstock should be considered to prevent the possible spread of Citrus tristeza virus (CTV). Thus, a survey should be anyway carried out in nurseries and fields where the fungus can induce symptomless infections. However, *F. oxysporum* has a high level of host specificity with over 120 described formae specials and related races which capable of causing wilt disease on several crop species and varieties (Armstrong & Armstrong, 1981). Furthermore, *F. oxysporum* has been associated with vegetative compatibility (Gordon & Martyn, 1997), with isolates belonging to a vegetative compatibility group (VCG) showing high genetic similarity. *F. oxysporum* formae specials differ in symptomatology, epidemiology and cultivar susceptibility, and can be distinguished by pathogenicity tests with appropriate hosts (Vakalounakis & Fragkiadakis, 1999). The aim of this study was to characterize the mating type of *F. oxysporum* isolated from wilted trees of citrus and to assess the aggressiveness of its isolates on sour orange cv ‘bigaradier Gou-tou”.

Materials and methods

Occurrence of Fusarium wilt on citrus plants: Wilt disease monitoring, including spatial distribution, severity and frequency was carried out in several private orchards in the Cap Bon area, Bizerte, Kairouan, Jandouba and Ben Arous. Data was compiled using observations provided by citrus growers, extension agents and plant health authorities from wider ranges of growing conditions. Collected samples were

placed in paper bags and labeled with required data such as date, place, name of farmer, area of orchard, tree condition, namely the age and the variety.

Isolation and identification of the causal pathogen: Samples collected from trunks and secondary branches of wilt-diseased trees were used to isolate potential causal pathogens, as described by Ricker and Ricker (1936). Sterilized samples of plant pieces were plated in Petri dishes containing potato dextrose agar medium (PDA, Difco, USA) amended with with 0.5 mg/ml Sterptomycin sulphate, and incubated at 27 ± 2°C with 12 h periods of light for 3 to 4 days. The growing colonies of isolated fungi were purified using hyphal tip and single spore culture techniques. Table 1 provides details of the isolates, including codes, year of isolation, geographical origin and host source. All isolated fungi were identified on the basis of their spore morphology and colony growth characters (Leslie & Summerell 2006; Nelson et al., 1983; Booth 1971). To stimulate spore production especially, chlamydospores, each isolate was cultured on carnation leaf-piece agar (CLA) (Fisher et al., 1982) and were incubated at 27 ± 2°C for two weeks. Pure cultures of all isolated fungi were given code numbers and maintained at 5°C in PDA slant for further studies.

Pathogen inoculums: For pathogenicity tests, each *F. oxysporum* strain was grown on casein hydrolysate liquid medium for 12 days at 27±2°C in a rotatory shaker at 90 rpm and 12 h of fluorescent light per day. Suspensions were filtered through a layer of cheesecloth and the concentration of conidia and mycelial fragments was evaluated with a Bürker's hemocytometer (Morris & Nicholls, 1978). Original suspensions were adjusted with deionised water to a final concentration of 10^6conidia/ml.

Experimental design: Two tests were conducted on *Citrus aurantium* plants (cv 'Gou-Tou') approximately 7 months old in greenhouse conditions (24 ± 2°C and a 12-h day/night photoperiod with high relative humidity 90 - 98%). The assay was performed on ten plants per isolate in separate pots containing 2 kilograms of sterile soil. Before inoculation, roots were scraped with a sterile scalpel.

Pathogenicity assay: Pathogenicity tests of 18 *F. oxysporum* isolates were conducted on 7-month-old seedlings of under greenhouse conditions. Inoculation was achieved by dipping roots in a conidial suspension of *F. oxysporum* (10^6 conidia mL^{-1}) for 20 min. Inoculated plants were transplanted into pots filled with steamed mix (sphagnum peat/perlite/pine bark/clay; 50:20:20:10) and maintained in a plant growth chamber at 24±2°C under a regimen of 12 h per day of fluorescent light. Non-inoculated plants with wounded roots dipped in sterile distilled water served as controls. Three months after root inoculation, the criteria for plant growth as length of the longest root (cm), root dry weight (g), shoot length (cm) and shoot dry weight (g) were determined.

Re-isolation and identification of the pathogen: The causal pathogen was re-isolated from diseased stems and leaf midribs of inoculated plant to prove and

confirm Koch's postulates. Fragments of the lesions were disinfected in 3% NaOCl for 1 minutes, washed in sterile distilled water twice, and then kept on PDA at 27± 2°C for 7 days. Re-isolated fungi were identified based on conidial morphology and the sequence of the rDNA ITS region as described previously.

DNA extraction: Mycelial plugs (10 mm in diameter) were transferred to 50 mL of potato dextrose broth (PDB) (Difco). Cultures were grown at 25 ± 2°C on a rotary shaker (100 r.p.m.) for 5 days (8 h light/16 h dark per day). Total genomic DNA was extracted according to Möller et al., (1992). Proteinase K (Invitrogen, Life Technologies, Carlsbad, USA) was used for protein removal. DNA was extracted in chloroform: isoamylalcohol (24:1), precipitated with ethanol, and washed twice with 70% ethanol. Extracted DNA was resuspended in TE buffer (10 mM Tris-HCl pH 8.0) and stored at -20 °C. DNA was quantified by comparing the band intensity of samples on 0.8% agarose gels to a known amount of Lambda DNA standard (QiaGen, Düsseldorf, Germany).

DNA amplifications: The ITS1-5.8S rDNA-ITS2 region was amplified by PCR using ITS1 (*5'*-CTTGGTCATTTAGAGGAAGTAA-*3'*) and ITS4 (*5'*-TCCTCCGCTTATTGATATGC-*3'*) primers (White et al. 1990). PCR was performed in a 25 µl reaction containing 30 ng of template DNA, 2 µl of 10 x PCR buffer containing 25 mM $MgCl_2$ (Qiagen, Düsseldorf, Germany), 2.5 mM dNTPs (Qiagen), 10 µM of each primer and 2 units of Taq DNA polymerase (Qiagen). Amplification was performed under the following conditions: 4 minutes at 94°C; 35 cycles of 40 seconds at 94°C, 45 seconds at 55°C, 2 minutes at 72°C, and a final extension at 72°C for 7 minutes. The mating type of each isolate of *F. oxysporum* was determined by MAT 1-1 primer sets Falpha1 (*5'*-CGGTCAYGAGTATCTTCCTG-*3'*) and Falpha2 (*5'*-GATGTAGATGGAGGGTTCAA-*3'*), (Arie et al. 2000). PCR was performed in 30 µl of reaction which contained 30 ng of genomic DNA, 3µl of 10 x PCR buffer 25 mM $MgCl_2$, 2.5 mM dNTPs, 10 µM of each primer and 2 Unit of Taq DNA polymerase. DNA amplification was performed in thermocycler (Gene Amp 9700, Applied Biosystems) using the following program: 94°C for 4 minutes, 38 cycles with denaturation at 94°C for 45 seconds, annealing at 55°C for 30 s, extension at 72°C for 2 minutes and final extension at 72°C for 5 minutes (Ortu et al. 2013). PCR products were separated on 1.5% agarose gel, stained with ethidium bromide. The PCR products were purified using QIAquick PCR purification Kit (Quiagen Inc.) according to the manufacturer's instructions. The ITS regions and mating type gene were sequenced at BMR Genomics, (Padova, Italy). Orthologous sequence of the ITS and Mating type of *F. oxysporum* isolates entries to the GenBank data-base were identified using both the BlastN and tBlastX algorithms Table (1).

Table 1: List of *F. oxysporum* strains used in this work. Code, Origin and Year of isolation, Specific host, and accession number for ITS and Mating type genes.

Code	Origin	Year of isolation	Host	ITS	Mating type (1-1)
F12/BKR	CapBon	2011	*C. tangerina*	KC304817	KJ477026
F18/E2r4	CapBon	2010	*C. sinensis*	KC304813	KJ477025
F21/E2r7	CapBon	2010	*C. sinensis*	KC304805	nd
F32/MB5C	CapBon	2010	*C. sinensis*	KC282839	KJ477035
F37/SMVDA2t1	Kairouan	2011	*C. sinensis*	KC304797	nd
F38/SMVDA2t2	CapBon	2011	*C.tangerina*	nd	KJ477027
F4/TK4	CapBon	2010	*C. sinensis*	KC304799	KJ477033
F44/E2P2b	CapBon	2011	*C. tangerina*	KC304820	KJ477029
F30/MB1C	CapBon	2010	*C. sinensis*	KC282839	KJ477028
F29/MB14	CapBon	2011	*C.sinensis*	KC304821	KJ477032
F58/SBatP1FB	CapBon	2011	*C. sinensis*	KC304801	KJ477036
F60/SBatP1Fc1	CapBon	2010	*C. sinensis*	KC304796	KJ477030
F7/BKR1	CapBon	2011	*C. tangerina*	KC304800	KJ477034
F98/BKR5	CapBon	2010	*C. tangerina*	KC304824	KJ477031
F1/TK1	Kairouan	2011	*C. sinensis*	KC304813	nd
F87/Kr1t9	Kairouan	2010	*C. sinensis*	KC304806	KJ477037
F90/Kr1t1	Kairouan	2010	*C. sinensis*	KC304815	nd

n.d. = not determined.

Statistical analysis: Analysis of variance (ANOVA) was conducted using SAS software version 8 (SAS Institute, Cary, NC). Means between treatments in each test were compared using the least significant difference (LSD) at 0.05 and 0.01 levels of probability (Fleming et al., 1972). Indirect gradient ordination method, principal component analysis (PCA) (Jolliffe, 2002) and cluster analysis using Ward's minimum variance method (Ward, 1963) was used and with Dunnett's one-tailed *t*-test (Dunnett, 1955), to determine if there were significant differences among isolates in each experiment.

Results

Occurrence of *Fusarium* wilt in Tunisian orchards: A severe wilt attributed to *Fusarium* was observed in cultivars included *C. sinensis* (L) cv. orange 'Washington Navel' and on *Citrus clementina* hort cv. 'Cassar', cv. 'Hernandina' and cv. 'MA3' from commercial orchards in CapBon area. In the field, the highest percentage of infection was noted on rootstock of sour orange seedlings with discoloration of vascular tissue. Root rot was observed on these seedlings. The disease was only observed on young trees (10 to 25 years-old) grafted on sour orange. The early symptoms included sectored wilt,

chlorosis and epinasty of the young leaves. Late symptoms included brown discoloration of vascular tissue, wilting, and dying plants.

Identification of the causal pathogen: All isolates had typical *F. oxysporum* growth and hyphal characteristics on PDA and CLA. On PDA or CLA, by all isolates of *F. oxysporum* were white, with aerial mycelium. However, the lower surface of the colonies was pink or light to dark violet. Canoe-shaped macroconidia with a long apical cell and a foot-shaped basal cell formed with 3 to 5 septa. Uni or bicellular, ovoid to ellipsoid microconidia were abundant. Microconidia formed in groups on CLA medium from short or sometimes branched monophialides. Chlamydospores were mostly single on PDA or CLA in two-week old cultures (Fisher et al., 1982).

ITS amplifications and Mating-type assessment: The identification was further confirmed by molecular study (Figure 1). Nuclear rDNA internal transcribed spacer (ITS) regions, amplified by PCR using the universal primers ITS1 and ITS4, yielded products of approximately 600 pb (Figure 1B). All isolates were identified as *MAT 1-1* based on the presence of 370 bp (Figure 1A). as estimated by agarose gel. Each strain had one or the other of the MAT genes. We could not find any relationship between the pathotypes and mating types. All the sequences had 99 to 100% similarity to reference sequences collected from the GenBank. All sequences were deposited in GenBank (Table 1).

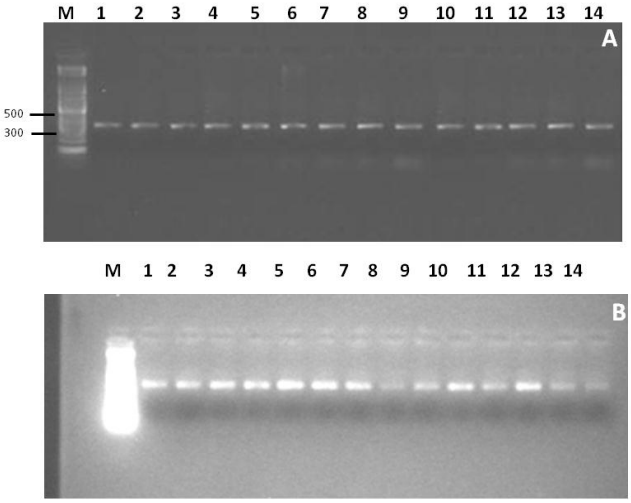

Fig. 1. Polymerase chain reaction (PCR) amplification of mating type specific sequences and ITS region from the selected *Fusarium oxysporum* isolates, (A): amplicon of *MAT1-1* specific ALPHA box (370 bp). M: a Gene Ruler™ 100 bp DNA Ladder Plus (Fermentas). (B) ITS PCR products amplified from different *Fusarium oxysporum* with ITS1/ITS4 primers. M: a Gene Ruler™ 100 bp DNA Ladder Plus (Fermentas).

Pathogenicity test: *F. oxysporum* isolates were pathogenic, and field symptoms of the disease were reproduced consistently in the greenhouse test. The results of the analysis of variance (ANOVA) revealed the presence of significant differences ($P < 0.05$) among the different isolates of *F. oxysporum* for the root dry weight (RDW) shoot length (SL) and shoot dry weight (SDW) and not significant for the longest root (LR) (Table 2). Wilt symptoms were visible in inoculated plants. Symptoms included leaf chlorosis, epinasty of the young leaves, vascular discoloration, whereas a lack of root rot was observed on these plants (Figure 2).

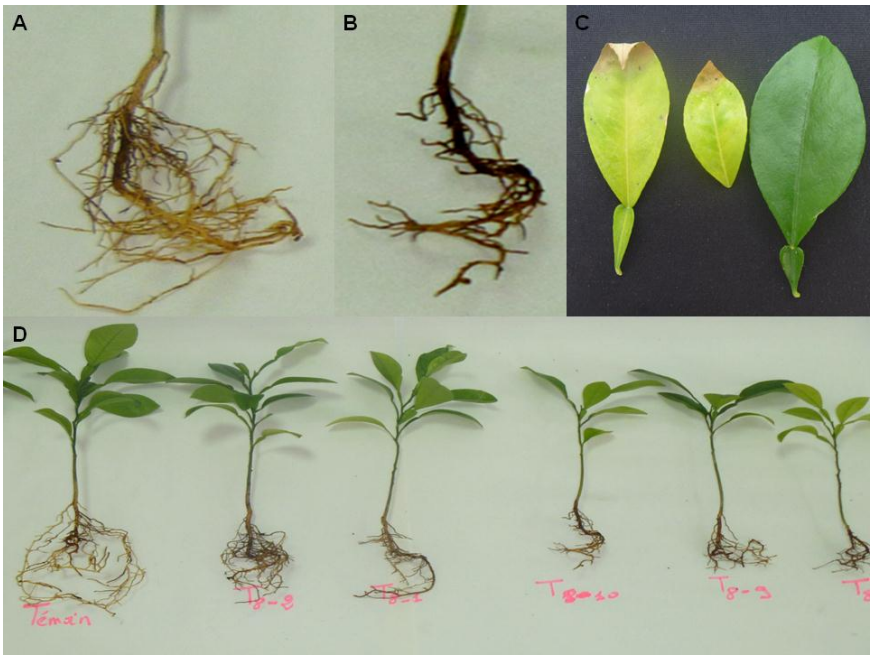

Fig. 2. *Fusarium* wilt symptoms in sour orange plant after inoculation with F21/E2r7. (A-B) Necrosis of rootlets, large roots and crowns. Control on the left side (A). (C) Wilting of sections of the canopy, chlorosis and epinasty of young leaves induced by *F. oxysporum* inoculation. Control on the right end side. (D) Symptoms of celery yellows. Wilt severity index: left = 1 to right = 5. Control on the left side.

Table 2: Analysis of variance for root dry weight (RDW), shoot length (SL), the longest root (LR) and shoot dry weight (SDW) after inoculation with *F. oxysporum* isolates.

Source of Variation	DF	LR	RDW	SL	SDW
Isolates	20	63.334 ^{NS}	0.181 **	24.615 **	0.337 **
Errors	189	62.424	0.032	3.343	0.096

NS = Non –significant, ** = Significant at 1% level of probability

Means of *Fusarium* wilt root dry weight (RDW), shoot length (SL), longest root (LR) and shoot dry weight (SDW) after inoculation with 18 isolates of *F. oxysporum* for two months in two replicated trials under greenhouse conditions presented in Table (3). Data showed that inoculated plants had shorter shoots as well as fewer and shorter roots, while no infection were observed on mock-inoculated plants. The average of (LR) for the control plants was 25.30 cm, which did not differ significantly from the maximum value recorded from inoculated plants (25.70 cm for the isolate F48/E4P1a). The minimum value for LR was 14.81 cm for isolate F57/MB2P2C. Reduced root dry weight (RDW) ranged from 0.32 g to 0.77 g for isolates F60/SBatP1FC1 and F21/E2r7 respectively, compared to 0.95 g measured in the mock inoculated plants. These reductions could be attributed to a limited translocation efficiency that was probably disrupted by the *F. oxysporum* mycelium in the vascular system of the

plant. A more pronounced reduction of the aerial parts of the trees and particularly the shoot growth were observed. The average (SL) was 35.10 cm in the control plants, while only shoot length of 18.20 cm and 13.95 cm were observed for F38/SMVDA2t2 and F60/SBatP1FC1 *Fusarium* isolates respectively. In addition, the (SDW) reduction ranged from 1.36 g to 0.82 g for F21/E2r7 and F60/SBatP1FC1 isolates, respectively, as compared to 1.99 g observed in mock inoculated plants. Most of the results suggested that the F60/SBatP1FC1 isolate was slightly more virulent than the other isolates, particularly in parameters concerning the aerial plant parts. *F. oxysporum* was re-isolated from the roots, stems, and leaf midribs of wilted plants. All the obtained isolates were identified as *F. oxysporum*, fulfilling Koch's postulates.

Table 3: Means of Fusarium wilt root dry Weight (RDW), shoot length (SL), longest root (LR) and shoot dry weight (SDW) after inoculation with 18 *F. oxysporum* isolates for three months in two replicated trials under greenhouse conditions.

Species	Strain code	LR[a] (cm)	RDW (g)	SL (cm)	SDW (g)
	Control	25.3 a-b	0.9 g	35.1 g	1.9 f
F. oxysporum	F12/BKR6	21.5 a-b	0.5 c-e	16.9 d-g	1.1 b-f
F. oxysporum	F18/E2r4	17.9 a-b	0.4 a-e	15.2 a-e	1 a-d
F. oxysporum	F21/E2r7	18.6 a-b	0.8 f	17.7 f-g	1.4 e-f
F. oxysporum	F5/TK5	21.4 a-b	0.6 d-f	16.3 b-f	1 a-d
F. oxysporum	F32/MB5C	16.5 a	0.5 a-e	14.4 a-b	0.9 a-d
F. oxysporum	F37/Kr2t2	18.7 a-b	0.5 b-e	16.2 b-f	0.9 a-d
F. oxysporum	F38/SMVDA2t2	20.1 a-b	0.6 e-f	18.2 f	1.2 d-f
F. oxysporum	F4/TK4	17.2 a-b	0.3 a-b	14.9 a-c	0.8 a-b
F. oxysporum	F44/E2P2b	25.7 b	0.4 a-c	14.0 a	0.8 a-b
F. oxysporum	F30/MB1C	15.9 a	0.5 a-e	14.8 a-c	0.9 a-d
F. oxysporum	F29/MB1A	16.9 a	0.4 a-e	15.4 a-e	1.2 c-f
F. oxysporum	F58/SBatP1Fb	18.5 a-b	0.3 a	13.9 a	0.8 a
F. oxysporum	F60/SBatP1FC1	20.5 a-b	0.6 c-e	17 e-g	1.1 b-f
F. oxysporum	F7/BKR1	17.3 a-b	0.4 a-c	15.1 a-d	0.8 a
F. oxysporum	F98/BKR5	18.7 a-b	0.5 a-e	15.5 a-e	1.0 a-d
F. oxysporum	F1/TK1	21.0 a-b	0.4 a-d	16.0 b-f	0.9 a-d
F. oxysporum	F87/Kr1t9	17.2 a-b	0.5 c-e	15.3 a-e	0.8 a
F. oxysporum	F90/Kr1t1	20.0 a-b	0.5 b-e	16.7 c-g	1.0 a-e

[a] In each column, values followed by the same letter do not differ statistically according to Tuey's test (P=0.05)

In the principal component analysis (PCA) performed on disease severity, the first two principal components (PCs) explained 95.11% of the variation for disease severity. PC1 (62.78) was mainly associated with the effect of host, while PC2 (32.33%) with the effect of origin. Cluster analysis grouped isolates into three clusters (Figure 3). Cluster I included isolates (F12/BKR6, F7/BKR1, F38/SMVDA2t2, F98/BKR5 and F44/E2P2b) which caused the most

severe symptoms as accounted for PC1. Cluster II included isolates (F87/Kr1t9, F90/Kr2t1 and F37/Kr2t2) and Cluster III included (F4/Tk4, F5/Tk5, F29/MB1A, F32/MB5c, F21/E2r7, F30/MB1c, F18/E2r4, F58/SBatP1FB, and F60/SBatP1Fc1). Isolates from Cluster II originated from Kairouan while isolates from Cluster III originated from CapBon. Isolates belonging to Cluster II and III caused milder symptoms, compared to isolates of Cluster I.

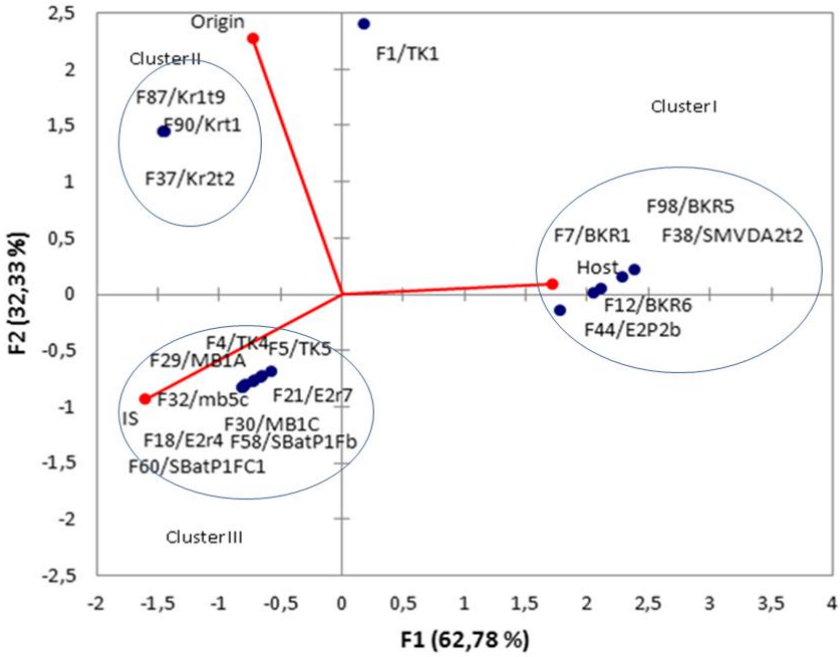

Fig. 3. Bi-plot of principal components computed from disease severity means of 18 isolates belonging to *F. oxysporum* from citrus, based on (CapBon and Kairouan) and Specific Host (*Citrus sinensis* or *C. tangerina*).

Discussion

Field observation, isolation trail from the crown of diseased mature trees, the pathogenicity test and molecular identification indicate that *F. oxysporum* is the causal agent of wilt disease of *Citrus* sp. in Tunisia. The disease was detected in the CapBon area (Takilsa, Bni Khaled, and Manzel Bouzalfa) and in the central area of Tunisia (Kairouan). Similar symptoms were described by Timmer (1982) on Mexican lime nurseries plants and other species of *Citrus* sp under greenhouses conditions (Timmer, 1982). This work revealed the morphological and pathogenicity of isolates of *F. oxysporum* on the major rootstock used in Tunisia. Isolates were successfully identified, based on morphological and cultural characteristics, such as pigment production, colour of mycelium, phialides, micro- and macroconidia shape and size, sporodochia and chlamydospores (Leslie & Summerell, 2006). Our results definitely confirmed that the causal pathogen of wilting on sour orange is *F. oxysporum* (Hannachi et al., 2014; Yaseen & D'Onghia 2010). As the first report described in one field in North east of Tunisia, the disease was wide speared in the major production regions: CapBon and Kairouan Pathogenicity and virulence could be

extremely useful for differentiating strains of *F. oxysporum* (Correll, 1991). Symptoms of *Fusarium* wilt disease recorded at the end of the experiment were associated with a reduction in plant growth and vigour and in the number of roots, and with shorter and thinner shoots. These reductions could be attributed to a limited translocation efficiency that was probably disrupted by the *F. oxysporum* mycelium developed in the vascular system of the plant. In fact, traits related to the aerial plant part were more informative than those related to the roots. However, the effect of these pathogens in the aerial parts should be interpreted bearing in mind that only ungrafted rootstocks (*C. aurantium*) were considered in this work. Further experiments using grafted plants of 'Clementine' and 'Washington Navel' would be necessary to reach conclusions on the effect of *F. oxysporum* on aerial parts of citrus plants. In spite of this, the results obtained revealed a different degree of severity on the aerial plant part. Isolates F60/SBatP1FC1 and F21/E2r7 were the most virulent, while the less virulent isolate was F38/SMVDA2t2. Analysis of variance established significant differences for various traits among the *F. oxysporum* isolates from Tunisia. In fact, maybe they could belong to different races within the new forma specials. Principal component analysis (PCA) employed in this study permitted to provide a realistic picture on the relationship between different cluster of *F. oxysporum* and their effect on sour orange. In addition, PCA based on host specificity of isolates (sweet orange and tangerine), geographical origin (CapBon area or Kairouan) and severity index of the isolates showed three groups related to the specific host and origin. However, sex plays important roles in pathogen evolution. The development of a new pathogenic race compatible to disease-resistant cultivar and of a new strain resistant to a fungicide was reported (Arie et al., 2000). *F. oxysporum* is known as asexual fungi. In the present study, only *MAT1-1*, alpha box *MAT-1* individuals were detected in a single closely related group of isolates which is similar to results of Arie et al., (2000). There could be two possible reasons for asexuality in Tunisian *F. oxysporum* strains: the fungus is a female sterile mutant from the original heterothallic fungus or has become a collection of asexual clones and, although the fungus still has the ability to reproduce sexually, no mating partner could be found in the population at this moment. Further studies on the evolution of fungal pathogenicity will reveal the mechanism by which they are asexual and, moreover, whether they are truly asexual or not. *Fusarium* wilt disease on citrus is a new disease syndrome was reproduced in Tunisia. Similar results were presented by Yaseen and D'Onghia, (2010), who confirmed the aggressiveness of *F. oxysporum* isolates on citrus 'Carrizo' citrange seedlings. Rarely *F. oxysporum* attack fruits trees. In addition these observations confirm our hypothesis that the pathogen may represent a new forma specials referred as *F. oxysporum* f sp. *citri*, causing wilt disease in *Citrus* sp. Consequently, the geographical area of origin might have an impact on the genomic differentiation within the *F. oxysporum* species complex (Hill et al., 2011). Additional investigations focusing on other loci (polygalacturonases genes, *β*-tubuline, calmodulin, etc) could be considered to confirm these results. SNP analysis could be used to develop markers for identifying pathogenic *F. oxysporum* isolates and possibly to predict the degree of pathogenicity of *F. oxysporum* isolates from citrus trees. The possible

effect of agronomic and environmental factors in the emergence of this disease as well as the role of infected planting stock, need to be further investigated**.**

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