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Isolation and identification of *Fusarium* spp. associated with Fusarium wilt of chickpea (*Cicer arietinum* L.) in Algeria

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Summary. Chickpea (*Cicer arietinum* L.) is an important vegetable crop in many Mediterranean countries, and *Fusarium* is known to cause wilt in these crops. *Fusarium oxysporum* f. sp. *ciceris* and *Fusarium redolens* are the only species which have been reported as the causes of Fusarium wilt in Algeria. *Fusarium* isolates (74) were obtained from roots of chickpea plants showing symptoms of wilting and necroses in xylem tissues. The plants were from 11 eleven principal provinces for chickpea crops in Algeria. Laboratory identifications for 31 isolates were achieved by sequencing their transcription elongation Factor 1- α (*Ef-1 α*) gene regions. Four principal species were identified including *F. oxysporum* (20 isolates), *F. redolens* (ten isolates), *F. solani* (one isolate) based on PCR using species-specific primers, and three *formae speciales* were identified within *Fusarium oxysporum*. *Fusarium redolens* and *F. oxysporum* f. sp. *ciceris* were dominant in the sampling sites. *Fusarium redolens* was identified in the western region of Algeria, and *F. oxysporum* f. sp. *ciceris* was not isolated from this region. In contrast, from the eastern region, *F. redolens* was not isolated but *F. oxysporum* f. sp. *ciceris* was detected. Pathogenicity tests showed that chickpea was susceptible to various *Fusarium* species. Inoculated plants exhibited gradual wilting that eventually affected entire plants. This study provides new knowledge of the distribution of *Fusarium species* causing chickpea wilt in Algeria. Precise identification and the localization of pathogen species is important for developing Fusarium wilt management strategies, including development of wilt resistant chickpea cultivars.

Keyswords. Koch's postulates, transcription elongation factor 1-α gene.

Abbreviations: FR: *Fusarium redolens*; **FS:** *Fusarium solani*; **FO:** *Fusarium oxysporum*; **FOC:** *Fusarium oxysporum* f. sp. *ciceris*; **FOL:** *Fusarium oxysporum* f. sp. *lactuceae*. Transcription elongation Factor 1-α gene (EF-1α).

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop for human nutrition, and is a major component of food production systems that are resilient to climate change. Chickpea is a preferred food legumes (Siddique *et al*., 2000) in some regions because of its multiple uses. The high protein content of the seeds (approx. 40% of seed weight), has potential human health benefits, including reduced risk of diabetes, cardiovascular disease, and cancer (Merga and Haji, 2019). Chickpea also contributes to soil fertility by fixing atmospheric nitrogen in the soil through symbiosis with rhizobia, which is especially important in dry climate areas. However, chickpea crops are often affected by telluric diseases such as *Fusarium* wilt, *Verticillium* wilt and those caused by *Sclerotinia* sp. and *Rhizoctonia* sp. (Nene *et al*., 2012).

Fusarium wilt is now widespread in most chickpea production areas of Africa, southern Europe, the Americas, and Asia. This disease is caused by several *Fusarium* species, and accurate and rapid identification of the responsible pathogens is important for development of appropriate and efficient management of Fusarium wilt of chickpea (Tekeoğlu *et al*., 2017). In Algeria, Fusarium wilt of chickpea has been attributed to *Fusarium oxysporum* f. sp. *ciceris* (FOC), but in January 2022 Zaim and Bekkar (2022) reported a second pathogen, *F. redolens* (FR), causing this disease in the western region of the country.

Formal identification of the pathogen responsible for chickpea-wilt in Algeria was made using classical methods, including morphological characteristics and fulfilment of Koch's postulates. *Fusarium oxysporum* (FO) was defined by morphological criteria, including the shape of the microconidia, macroconidia and conidiophore structure (false head on short phialides formed on hyphae). This morphological identification is problematic, however, because of the diversity of non-pathogenic and saprophytic isolates in soil. *Fusarium* isolates are usually tested on the chickpea ILC-482 genotype, which is susceptible to all races of *F. oxysporum* f. sp. *ciceris* (Jimenez-Diaz *et al.,* 1989), and is supposed to confirm the identity of the pathogen.

Identification of *Fusarium* spp. based only on morphology is difficult, because informative morphological characteristics are limited, and microscopic traits can be influenced by environmental conditions, so that their plasticity and intergradation make them subject to misinterpretation (Leslie *et al*., 2001). For example, distinguishing *F. redolens* from *F. oxysporum* can be particularly challenging, because this relies on differences in size of their macroconidia (Gordon, 1952), and intermediate conidium forms may occur (Baayen and Gams, 1988). For these reasons, the taxonomic position of *F. redolens* has been problematic. Whereas Booth (1971) considered this fungus to be a variety of *F. oxysporum,* Nelson *et al*., (1983) considered *F. redolens* and *F. oxysporum* to be synonymous. The use of DNA-based methodologies now makes it possible to differentiate between different species of *Fusarium* (Jimenez-Gasco and Jimenez-Diaz., 2003; Jiménez-Fernandez *et al*., 2011).

During spring of 2020 and 2021, the present study investigated occurrence of *Fusarium* wilt of chickpea in the north of Algeria. Samples were collected from eleven provinces. Wilting and yellowing symptoms were observed on aerial parts of diseased chickpea plants, and 74 fungal isolates were obtained from roots. To identify these isolates, their translation elongation factor 1-alpha (EF-1α) gene regions were sequenced.

MATERIALS AND METHODS

Plant sampling, and isolation and maintenance of fungi

During the chickpea growing seasons of spring 2020 and 2021, chickpea plants with yellowing and wilting symptoms on their areal parts were collected from 23 farmer fields in different areas in north western and eastern Algeria. These areas were in to 11 provinces, five in the western region and six in the eastern region. The major chickpea sowing method in the west is strip sowing, whereas in the east it is broadcast sowing.

Tissue pieces (5 × 5 mm) were taken from diseased tap root tissues of wilted chickpea plants, and were surface-sterilized in 2% sodium hypochlorite for 30 sec, then rinsed three times in sterile distilled water, and dried on sterile paper. Samples were then plated on potato dextrose agar (PDA), and incubated at 27°C in the dark for 7 d. Growing tips of hyphae developing from in cultures were then transferred onto fresh PDA. Monoconidial isolates were prepared from cultures of fungi obtained from each region, and cultures were stored at 4°C.

A total of 74 *Fusarium* sp. isolates were obtained, of which 31 were sequenced using the EF-1α gene. The 31 isolates were chosen based on macroscopic characteristics (colony colour and appearance on PDA). Two to four representative isolates of the morphotypes from each province were considered.

DNA extractions

The selected fungal cultures were grown on PDA 25 ± 2°C in the dark for 7 d. For each extraction, 100 mg of mycelium was collected by scraping the colonies

from three plates per isolate. DNA of each isolate was extracted using a commercial NucleoSpin Plant II kit (Macherey-Nagel), following the supplier's instructions. The quality and concentration of extracted DNA were verified after 1% agarose gel electrophoresis, using a NanoDrop spectrophotometer (at 260/280 nm).

Molecular characterization of Fusarium *isolates*

The translation elongation factor-1alpha (EF-1α) gene region was amplified by PCR, using the universal primers EF-728F (CATYGAGAAGTTCGAGAAGG) and EF-2 (GGARGTACCAGTSATCATGTT) (Carbone and Kohn, 1999), in a 25 µL reaction volume containing 1.5 mM $MgCl_2$ (Promega), 0.2mM dNTPs (Invitrogen), 2 µg mL^{-1} fungal DNA, 0.5µM primers each, 1× Taq Buffer (Promega), and 1-unit Taq polymerase (Promega).

Amplifications were carried out in a thermal cycler (Icycler Bio-Rad), with an initial denaturation step for 5 min at 95°C, followed by 35 cycles each of 30 sec of denaturation at 95°C, 30 sec at annealing temperature (52°C), and 45 sec at 72°C, and an extension step of 7 min at 72°C. Amplification products were separated by electrophoresis on a 1.5% agarose gels. The gels were stained with MidoriGreen (Nippon), and were visualized under UV light by using the Gel doc system (Biorad). The size marker was a 100 bp DNA ladder (Invitrogen). The PCR products were purified with a NucleoSpin® Gel and a PCR Clean-up kit (Macherey-Nagel), following the manufacturer's instructions. PCR products of the EF-1α regions were sequenced by Sanger sequencing (Sanger *et al*., 1977) directly from the PCR products, without a cloning step, using Applied Biosystems BigDye Kit v3.1 and the primers used for PCR amplification of the fragments. Sequencing was performed in the direction (forward/reverse) for each amplicon. Sequencing was carried out by Gene Life Sciences.

The sequences obtained were analyzed and cleaned using the CHROMAS PRO software, and were then compared with those from the GenBank database using the BLAST Program (https://blast.ncbi.nlm.nih.gov/Blast.cgi Blast) to identify the isolates based on percentage homology with reference strains. A dendrogram was established using the UPGMA method algorithm to show the genetic diversity of the isolated fungi.

Assessment of Koch's postulates for representative fungi

To verify the pathogenicity of *Fusarium* spp. isolates, five species [*F. redolens* (FR), *F. solani* (FS), *F. oxysporum* (FO), *Fusarium oxysporum* f. sp. *ciceris* (FOC), and *F. oxysporum* f. sp. *lactuceae* (FOL)] were inoculated individually onto plants of cultivar ILC-482 from ICARDA. The seeds were surface sterilized, and then pre-germinated on moist cotton wool, which provided favorable conditions for seedling germination (ISTA, 2015; Korter *et al.,* 2023).

The pre-germinated seeds were then sown in plastic pots (30 × 20 cm) containing a sterile mixture of potting soil and sand (1:1 v:v). For each *Fusarium* sp., three pots each containing four plants were used. Seven d after sowing (3–4 leaf stage), the plants were removed from soil and their root systems were soaked for 30 min in a conidial suspension (10^6 conidia mL^{-1} (Gao *et al*., 1995; Ficcadenti *et al*., 2002). Five pots were used as controls, where the plants were soaked in sterile water without inoculum.

RESULTS AND DISCUSSION

In the assayed chickpea fields, diseased plants were spread throughout each field, with incidence up to 30% (Figure 1, a and b). In addition to wilting and yellowing symptoms, the stems and root xylem tissues of the diseased plants had black discolourations (Figure 2 a). Brown necrotic lesions in the tap roots and necroses of lateral roots (Figure 2 b) also occurred on most of the sampled plants from the west of Algeria. The same symptoms were observed by Jimenez-Fernandez *et al*. (2011).

On PDA, colonies of the isolates had different morphotypes (Figure 3), with differences in colour, growth rates, and aspect of mycelium. All colonies initially had white to cream-coloured mycelium, which turned (after 3 to 7 d incubation) to purple and pink for isolates of *F. redolens*, purple tinged or slightly orange for *F. solani*, or cream or purple for *F. oxysporum* f. sp. *ciceris* and *F. oxysporum*. The mycelium texture was fluffy or appressed.

Microscopic observations of most of the isolates revealed septate mycelium which carried phialides bearing false-headed conidia (Figure 4).

The morphological and cultural characteristics on PDA identified the isolates as *Fusarium* sp., based on the descriptions of Booth (1977).

BLASTn sequence analyses with (https://blast.ncbi.nlm.nih.gov/Blast.cgi) identified the isolates as follows: ten isolates were *F. redolens*, six were *F. oxysporum* f. sp. *ciceris*, nine were *F. oxysporum, five were F. oxysporum* f. sp. *Lactuceae*, and one isolate was *F. solani* (*Ascomycota*, *Pezizomycotina*, *Sordariomycetes*, *Hypocreomycetidae*, *Hypocreales*, *Nectriaceae*) with 94.85 to 100% sequence similarity to the sequences of type isolates from Genbank.

A dendrogram was constructed based on the EF-1α sequences, using the UPGMA method (Sneath and

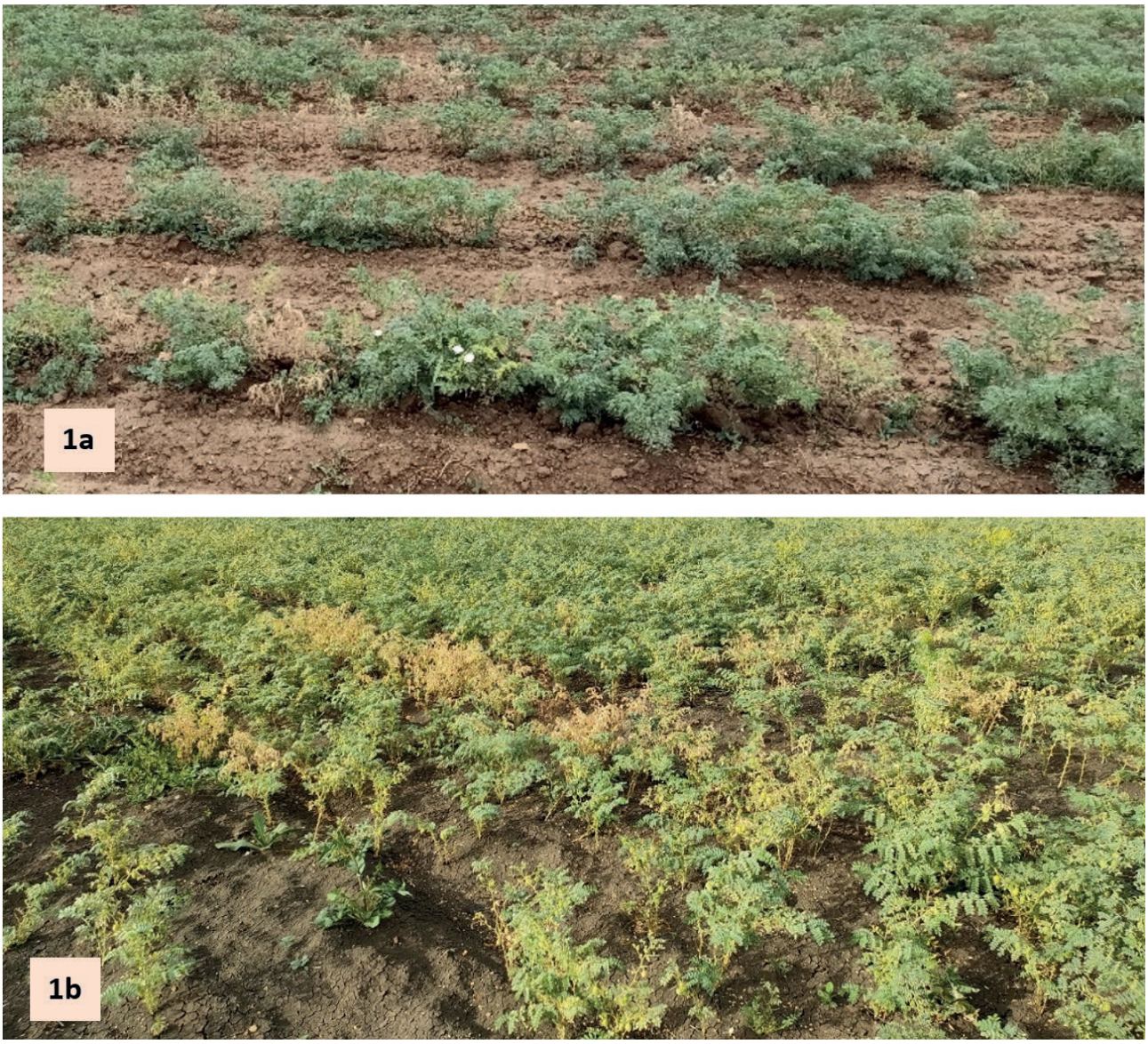

Figure 1. (**1a**) Typical symptoms of Fusarium wilt caused by *Fusarium redolens* on chickpea plants in the Ain Temouchent region (Western Algeria). (**1b**) Typical symptoms of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* in chickpea plants in Mila (Eastern region).

Sokal, 1973) to show phylogenetic relationships among the *Fusarium* isolates obtained from different regions of Algeria (Figure 5). The branches of the dendrogram were supported by bootstrap values, demonstrating the robustness of the phylogenetic relationships among these isolates. For 15 isolates from the western region, ten isolates (67%) were FR, four (27%) were FO of one *forma specialis* (*F. oxysporum* f. sp. *lactuceae*), and one isolate (6.7%) was FS. In contrast, among the 16 isolates from the eastern region of Algeria, all were FO, in which two *formae speciales* were identified, six of FOC, and three of FOL (Table 1).

These results indicate that *F. redolens* is dominant in the western region of Algeria, while *F. oxysporum* prevails in the eastern region, where *F. oxysporum* f. sp. *ciceris* was the most frequently identified *forma specialis* among the isolates collected from the east. *Fusarium oxysporum* f. sp. *ciceris* was not isolated from the western region, and *Fusarium redolens* was not isolated from the eastern region of the country (Figure 6).

Koch's postulates were confirmed by inoculating chickpea cultivar ILC-482 and re-isolating the fungi from the inoculated chickpea roots, which indicates that all the *Fusarium* isolates were infectious agents of

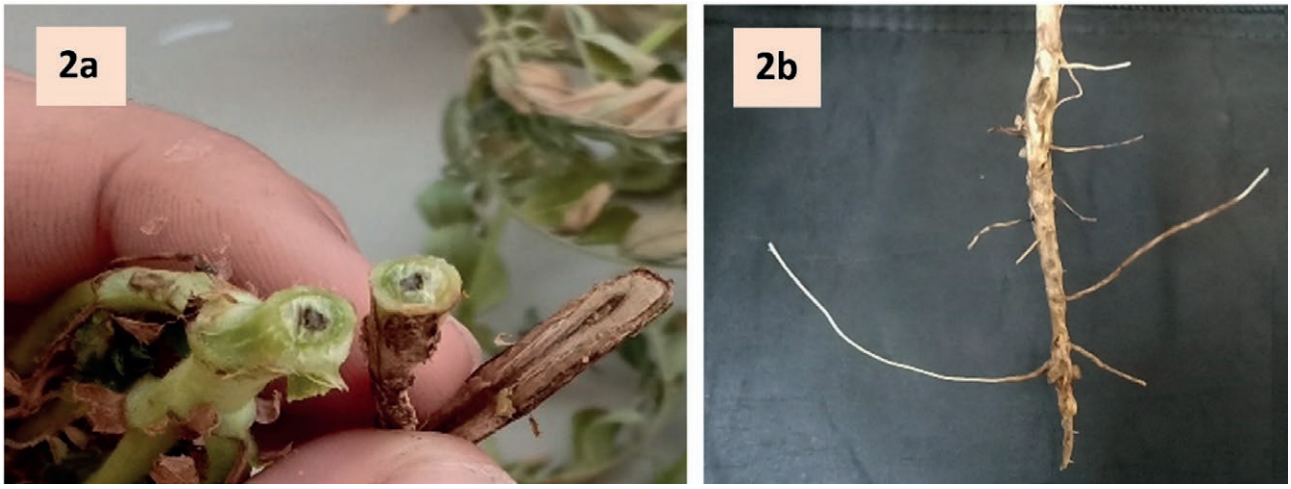

Figure 2. (2a) Cross section of a chickpea stem showing discoloration (black and brown necrosis) of the xylem and pith tissue caused by *Fusarium redolens*. **(2b)** Brown necrotic lesions in the tap root and necrosis of lateral roots showing on chickpea infected with *Fusarium.*

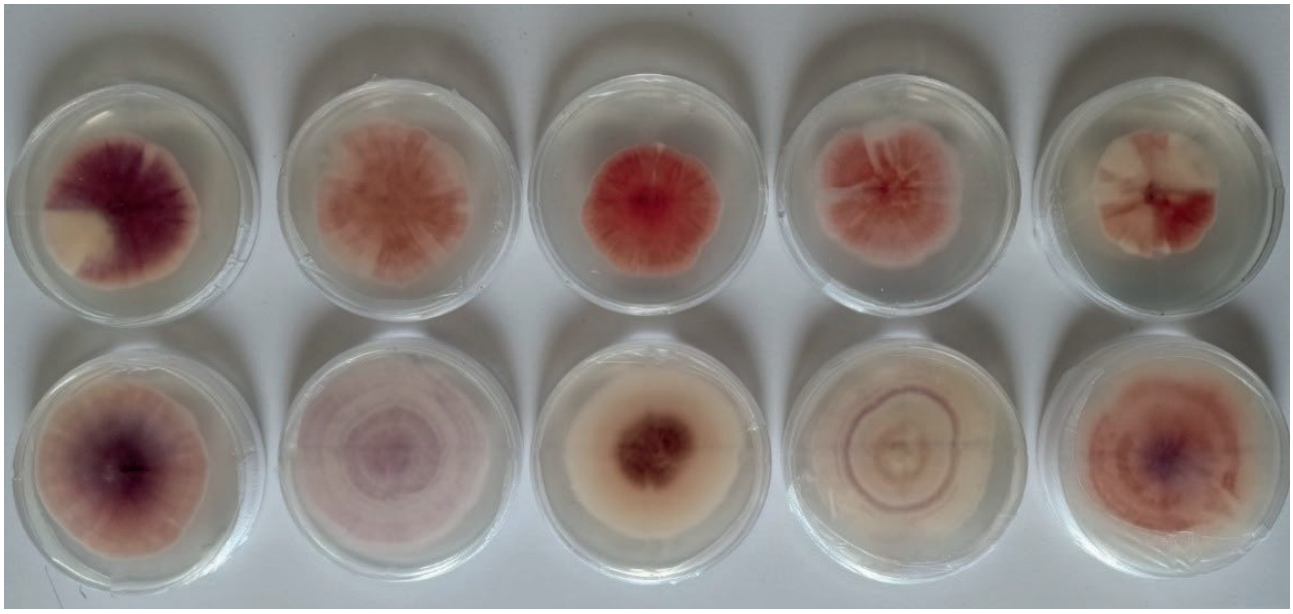

Figure 3. Different morphotypes of *Fusarium* spp. of a colony isolated from chickpea, on PDA medium, after 8 days of incubation.

diseased plants sampled from the chickpea fields. These results show that chickpea culture can be susceptible to different *Fusarium* species, and even to FOL that has been previously shown to be a pathogen of lettuce. All the inoculated plants developed progressive wilting. Although *formae speciales* of *F. oxysporum* are mainly described as highly specific, their host ranges have been found to increase. For example, only 53 of the 106 *formae speciales* listed by Edel-Hermann and Lecomte (2019) remain associated with a unique plant species. Similarly, Ramirez-Suero *et al*. (2010) showed susceptibility of *Medicago truncatula* to several *F. oxysporum formae speciales*, even from non-legume hosts.

In the present study, wilting symptoms appeared on inoculated plants at 21 d after inoculation for all of the *Fusarium* isolates. Leaves at the base of plants initially showed marginal yellowing before wilting (Figure 7). The symptoms then developed progressively towards the upper leaves, and most rapidly in plants inoculated with *F. oxysporum* f. sp. *ciceris* and *F. redolens*. Roots of inoc-

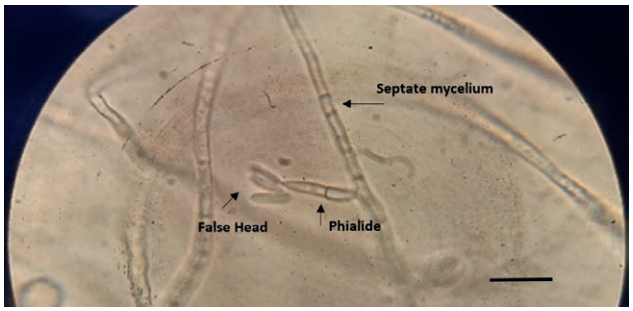

Figure 4. Microscopic features of *Fusarium redolens* (isolate FR1). Note the phialide bearing false-headed conidia on a septate hypha (x 100). Bar = 20 µm.

ulated plants had reduced root hairs and brown/black discolouration, which extended to the plant collars and sometimes caused collar narrowing (Figure 7b).

Longitudinal sections of primary roots of the diseased plants had dark vascular necroses, which were more or less dark according to the inoculated *Fusarium* isolates. Root xylem necroses were sometimes accompanied by wet rots, where plants were inoculated with *F. solani* or *F. redolens* (Figure 8). All non-inoculated (control) plants remained healthy. Symptoms on roots and leaves were more severe on plants inoculated with isolates of FOC or FR than on those inoculated with the other isolates (Figure 7, a and b).

To date, chickpea wilt in the western region of Algeria has been attributed to *Fusarium oxysporum* f. sp. *ciceris,* using morphological characteristics of the pathogenic fungi (Benfreha *et al*., 2014) or Koch's postulates for the chickpea ILC-482 genotype (Tlemsani *et al*., 2015; Zaim, 2016). The present study indicates that FOC does not exist in the western region of Algeria. Using ITS and EF-1α, Zaim and Bekkar (2022) identified only *Fusarium redolens* of 20 *Fusarium* isolates from chickpea in the western region of this country. The present study further confirms the absence of FOC, since among the 15 *Fusarium* isolates that were obtained from the western region, ten were *F. redolens*, and the remaining five were FS (one isolate), FO (two isolate), and FOL (two isolates). Although only 31 of 74 isolates were sequenced in the present study, these results and those of Zaim and Bekkar (2022) confirm that FOC is absent from western Algeria.

Fusarium redolens has been reported to cause wilt of tomato (Edel-Hermann *et al*., 2012), and damping-off of Aleppo pine (*Pinus halepensis*) (Lazreg *et al*., 2013) in the North west of Algeria. Association of *F. redolens* with wilt symptoms of chickpea has been reported in Morocco, Spain, Lebanon, and Pakistan (Jimenez-Fernandez *et al*., 2011), Saskatchewan (Taheri, 2011), Tunisia (Bou-

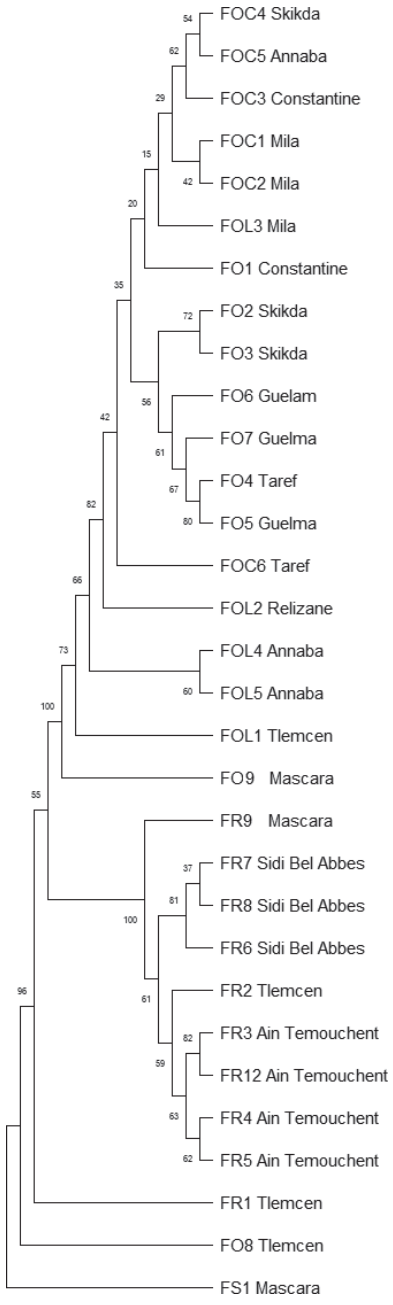

Figure 5. Dendrogram of *Fusarium* wilt species, obtained using the UPGMA method. The bootstrap consensus tree inferred from 1000 replicates (Tamura K. *et al*., 2004) is taken to represent the evolutionary history of the taxa analyzed (Tamura *et al*., 2004). The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Tamura K. *et al*., 2004). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura K. *et al*., 2021), and are the numbers of base substitutions per site. This analysis involved 31 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were 1042 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Felsenstein J., 1985).

Table 1. Identities of *Fusarium* isolates, their sampling site fields, and their EF-1α sequence similarities with reference strains, for isolates from different regions and provinces in Algeria.

Region	Province	Code	Identification	Field coordinates	Percentage similarity	Blast Best Hit ID	Accession number NCBI
Western region	Tlemcen	FOL1	*Fusarium oxysporum* f. sp. *lactuceae*	35°09'54.3"N+1°26'50.7"W	98.64	MK801786.1	PQ899234
		FR1	*Fusarium redolens*	34°56'42.5"N+1°14'59.6"W	94.85	*HQ731060.1*	PV388859
		FR2	*Fusarium redolens*		99.75	*HQ731060.1*	PV388860
		FO8	*Fusarium oxysporum*	35°09'54.3"N+1°26'50.7"W	100.00	*ON703236.1*	PQ899228
	Ain Temouchent	FR12	*Fusarium redolens*	35°13'56.7"N+1°20'50.5"W	99.16	*HQ731063.1*	PV388869
		FR3	*Fusarium redolens*	35°13'56.7"N+1°20'50.5"W	99.58	*MT305223.1*	PV388861
		FR4	*Fusarium redolens*	35°16'36.2"N+1°13'43.1"W	99.58	*OR270920.1*	PV388862
		FR5	*Fusarium redolens*	35°17'43.2"N+1°05'10.6"W	99.57	*OR105855.1*	PV388863
	Sidi Bel Abbes	FR6	*Fusarium redolens*	35°14'18.9"N+0°43'51.5"W	99.79	*OR270920.1*	PV388864
		FR7	*Fusarium redolens*	35°16'15.8"N+0°35'04.5"W	99.79	*OR270920.1*	PV388865
		FR8	*Fusarium redolens*	35°23'25.0"N+0°29'03.4"W	99.85	*MK172061.1*	PV388866
	Mascara	FR9	*Fusarium redolens*	35°26'56.5"N+0°08'48.9"E	99.57	*HQ731060.1*	PV388867
		FS1	*Fusarium solani*	35°24'23.9"N+0°18'27.0"E	99.57	*HE647956.1*	OR234389
		FO9	*Fusarium oxysporum*		97.53	*MW361989.1*	-
	Relizane	FOL2	*Fusarium oxysporum* f. sp. *lactuceae*	35°59'58.4"N+0°52'33.9"E	99.35	*MH412703.1*	PQ899235
Eastern region	Mila	FOC1	*Fusarium oxysporum* f. sp. *ciceris*	36°30'22.9"N+6°16'35.1"E	99.55	FJ538240.1	PQ899229
		FOC2	*Fusarium oxysporum* f. sp. *ciceris*	36°27'54.3"N+6°13'21.8"E	100.00	FJ538240.1	PQ899230
		FOL3	*Fusarium oxysporum* f. sp. *lactuceae*	36°27'21.7"N+6°12'51.0"E	99.85	OP918954.1	PQ899236
	Constantine	FOC3	*Fusarium oxysporum* f. sp. *ciceris*	36°17'58.0"N+6°38'41.8"E	100.00	FJ538241.1	PQ899231
		FO1	*Fusarium oxysporum*	36.294952, 6.641571	98.46	*PP795998.1*	PQ613583
	Skikda	FOC4	*Fusarium oxysporum* f. sp. *ciceris*	36°43'22.0"N+6°51'29.3"E	100.00	FJ538241.1	PQ899232
		FO2	*Fusarium oxysporum*	36°42'43.2"N+6°47'47.8"E	100.00	*OQ511027.1*	PQ613583
		FO3	*Fusarium oxysporum*		99.85	*OQ511027.1*	PQ899231
	Annaba	FOC5	*Fusarium oxysporum* f. sp. *ciceris*		98.85	FJ538241.1	PQ899233
		FOL4	*Fusarium oxysporum* f. sp. *lactuceae*	36°46'38.1"N+7°44'18.4"E	99.57	*MW316853.1*	PQ899237
		FOL5	*Fusarium oxysporum* f. sp. *lactuceae*		99.78	*MW316854.1*	-
	El Taref	FOC6	*Fusarium oxysporum* f. sp. *ciceris*	36°42'28.4"N+7°44'26.5"E	98.81	FJ538240.1	-
		FO4	*Fusarium oxysporum*		99.85	*OQ511027.1*	PQ613586
	Guelma	FO5	*Fusarium oxysporum*	36°39'03.1"N+7°42'35.2"E	99.85	*KF537337.1*	PQ613587
		FO6	*Fusarium oxysporum*		100.00	*OQ511027.1*	PQ613588
		FO7	*Fusarium oxysporum*		99.85	*OQ511027.1*	PQ899227

hadida *et al*., 2017), Turkey (Tekeoğlu *et al*., 2017), and Iran (Chehriand Sattar, 2018).

The present study isolated *F. solani* from the western region of Algeria. This fungus was previously reported to infect chickpea in China, India, Spain, Pakistan, Iran, and other countries (Zhuang *et al*., 2005; Jiménez-Fernández *et al*.,2011). In Algeria, *F. solani* has been reported to cause wilt of potato (*Solanum tuberosum*) (Azil *et al*., 2021) and tomato (*Solanum lycopersicum*) (Abdesselem *et al*., 2016), as well as damping-off of Aleppo pine (*Pinus halepensis*) (Lazreg *et al*., 2014). Therefore, the present study is the first to show that *F. solani* is a chickpea pathogen in Algeria.

While *F. redolens* and *F. oxysporum* f. sp. *ciceris* are known to cause yellowing and wilt of chickpea, the reasons for their distinct geographic distributions in Algeria remains unclear. Several factors could explain this separation of the pathogens, including variations in climate,

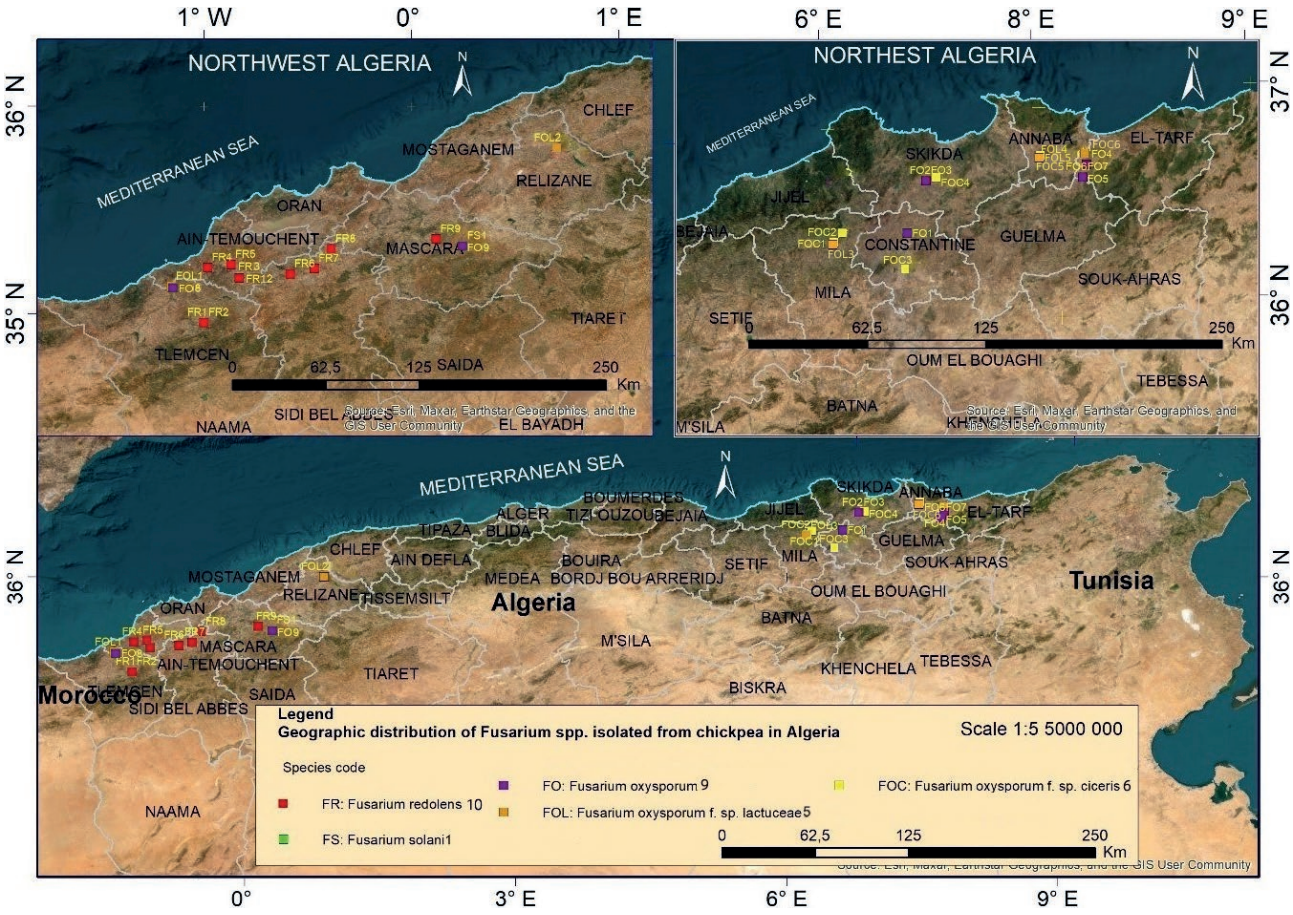

Figure 6. Geographic distribution of *Fusarium* spp. isolated from chickpea in Algeria, recorded with ArcGis software. The numbers after the names of the *Fusarium* species correspond to the numbers of obtained isolates of that species.

soil structure, and cropping systems. Further research is required to confirm these potential influences.

It is well-documented that plant pathogen prevalence can be linked to climatic conditions, such as temperature and rainfall, which differ across geographic regions. For example, *F. graminearum* tends to dominate in warm areas such as parts of the United States of America, while *F. culmorum* is common in cool, maritime regions such as the United Kingdom (Parry *et al*., 1995; Doohan *et al*., 2003). Similarly, optimal temperatures and moisture levels are critical for *Fusarium* sporulation and inoculum dispersal (Rossi *et al*., 2001), suggesting that local environmental factors may play a role in the distributions of FR and FOC.

Soil characteristics, including moisture and pH, also affect *Fusarium* spp. growth and resulting host disease development. Previous studies have shown that soil pH can influence *Fusarium* growth rates and expression of wilt symptoms (Chen *et al*., 2013; Gatch and du Toit, 2017). Additionally, resistance to *Fusarium* wilt has been observed in particular soils, with microbiological interactions such as competition for iron and/or carbon playing important roles (Elad and Baker, 1985b; Couteaudier and Alabouvette, 1990).

To fully understand the geographic distributions of FOC and FR, further research is required on effects on the fungi of soil physicochemical properties, microbial communities, and agricultural practices. It remains that FS and FOC could be serious future threats to chickpea cultivation in Algeria. It would also be worthwhile to test the aggressiveness of *Fusarium* spp. on different chickpea cultivars to understand whether those isolates have cultivar specificities.

The use of morphological traits to differentiate morphologically similar *Fusarium* spp., including *F. oxysporum* and *F. redolens*, may lead to incorrect pathogen identifications. However, accurate identification of pathogenic *Fusarium* spp. and *F. oxysporum formae speciales* is important for efficient management of diseases caused by these fungi, particularly when resistant cultivars are

Figure 7. (7a) Wilt symptoms, on chickpea cultivar ILC482 30 d after inoculation with *Fusarium isolates.* **a,** *F. oxysporum* f. sp. *ciceris;* **b,** *F. redolens;* **c,** *F. solani*; **d,** *F. oxysporum*; **e,** *F. oxysporum* f. sp. *lactucae*; **f,** Inoculation Control. **(7b)** Symptoms on chickpea cultivar ILC482 inoculated with *Fusarium isolates,* 30 days after inoculation: **a,** *F. redolens*; **b,** *F. oxysporum* f. sp. *ciceris;* **c,** *F. oxysporum;* **d,** *F. oxysporum* f. sp. *lactucae*; **e,** *F. solani*; **f,** Inoculation Control.

Figure 8. Symptoms in xylem tissues of chickpea cultivar ILC-482 inoculated with *Fusarium* isolates. **a,** Inoculation Control; **b.** inoculation with *F. oxysporum;* **c,** with *F. oxysporum* f. sp. *lactucae*; **d,** with *F. oxysporum* f. sp. *ciceris*; **e,** with *F. redolens*; **f**, inoculation with *F. solani.*

one of the few and most effective control measures for these diseases, as is the case for *Fusarium* wilt of chickpea (Nene and Reddy, 1987).

Identification of new pathogen species and races would help plant breeders to select appropriate germplasm for development of resistant crop cultivars. Identification of disease-causing *Fusarium* spp. may also help development of cultural practices for management of *Fusarium* wilt, which can be achieved with use of resistant cultivars and adjustment of sowing dates (Jiménez *et al*., 1991; Jalali and Chand, 1992; Jiménez *et al*., 1998; Landa *et al*., 2004; Navas-Cortés *et al*., 1998; Navas-Cortés *et al*., 2000), and appropriate crop rotations (Landa *et al*., 2006).

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