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Identification and virulence of *Fusarium falciforme* and *Fusarium brachygibbosum* as causal agents of basal rot on onion in Mexico

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Abstract: *Fusarium* basal rot is a major disease of onion production in Mexico. Recently, *Fusarium falciforme* and *F. brachygibbosum* were reported as two new causal agents of basal rot of onion in Mexico. However, little is known about the pathogenicity and virulence of these pathogens on this crop globally. The virulence (severity of disease) of *F. falciforme* and *F. brachygibbosum* isolates recovered in Culiacan Valley (Sinaloa, North-west Mexico) was determined and compared with isolates of *F. oxysporum* collected from the same crop and region. Two pathogenicity tests were conducted on onion under controlled conditions, one on onion bulbs, and another on onion seedlings of the cv. 'Carta Blanca'. All of the isolates were pathogenic on onion, indicating that these three *Fusarium* species are a serious threat to production of this crop in the Culiacan Valley. The isolates showed significant differences in virulence, with *F. falciforme* the most aggressive at the bulb stage and *F. brachygibbosum* the most aggressive at the seedling stage, compared with *F. oxsyporum*. The isolates of these three species caused similar symptoms including yellowing and darkening of the vascular tissues on onion plants, highlighting the need for a specific diagnostic tool to discriminate among members of the species complex. This information is valuable for resistance breeding programmes, to reduce the devastating effects of *Fusarium* basal rot of onion in Mexico and worldwide.

Keywords: aggressiveness, basal rot, *Fusarium brachygibbosum*, *Fusarium falciforme*, onion

Résumé: Le pourridié fusarien est une grave maladie de l'oignon au Mexique. Récemment, on a rapporté que, dans ce pays, *Fusarium falciforme* et *F. brachygibbosum* étaient deux nouveaux agents du pourridié de l'oignon. Toutefois, nous en savons peu sur la pathogénicité et la virulence de ces agents pathogènes chez l'oignon à l'échelle mondiale. La virulence (gravité de la maladie) des isolats de *F. falciforme* et de *F. brachygibbosum* collectés dans la vallée de Culiacan, dans l'État du Sinaloa (nord-ouest du Mexique), a été établie et comparée avec celle d'isolats de *F. oxysporum* collectés sur la même culture et dans la même région. Deux tests de pathogénicité ont été menés sur l'oignon dans des conditions contrôlées, un sur les bulbes et l'autre sur les semis du cultivar 'Carta Blanca'. Tous les isolats étaient pathogènes de l'oignon, ce qui indique que ces trois espèces de *Fusarium* représentent une sérieuse menace pour la production de cette culture dans la vallée de Culiacan. Comparativement à *F. oxysporum*, les isolats ont affiché des différences significatives quant à la virulence, *F. falciforme* étant le plus agressif à l'égard des bulbes et *F. brachygibbosum*, à l'égard des semis. Les isolats de ces trois espèces ont causé des symptômes similaires, y compris le jaunissement et le noircissement des tissus vasculaires chez les plants d'oignons, accentuant le besoin de concevoir un outil de diagnostic permettant de distinguer les membres du complexe

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d'espèces. Cette information utile profitera aux programmes de sélection visant à accroître la résistance afin de réduire les effets dévastateurs du pourridié fusarien chez l'oignon au Mexique et partout dans le monde.

Mots clés: Agressivité, *Fusarium brachygibbosum*, *Fusarium falciforme*, oignon, pourridié fusarien

Introduction

Onion (*Allium cepa* L.) is an important vegetable crop in Mexico, with a cultivated area of 52 499 ha and an estimated production of 1.6 million tons in 2017 (SIAP 2017). However, one of the production limitations is basal rot disease caused by various pathogens including *Fusarium* spp. These pathogens lead to reductions in the quantity and quality of yield all over the world (Schwartz and Mohan 1995). Basal rot can reduce onion yield by interfering with the absorption and translocation of water and nutrients, causing the premature death of plants. The symptoms may include chlorosis starting at the leaf tip and developing progressively downwards, dwarf plants, soft consistency in the bulbs, necrosis in the basal part of the bulb and a decrease in the roots (Delgado-Ortiz et al. 2016).

In northwest Mexico, the disease occurs every year and reduces the yields of onion crops. There is limited information, however, on the diversity, abundance and geographical distribution of *Fusarium* spp. that cause root rot in these crops. Recently, *Fusarium falciforme* (FSSC 3 + 4) and *F. brachygibosum* were reported as causal agents of basal rot disease in onion cultivation in Mexico (Tirado-Ramírez et al. 2018, 2019). The *Fusarium* strains pathogenic on onion survive in the soil and are difficult to eradicate once they have infested a field. To make decisions on plant protection measures, it is important to identify the *Fusarium* species that cause basal rot of onion and to determine their virulence. To improve understanding of the populations of *Fusarium* species that cause basal rot in northwestern Mexico it is essential to study their aggressiveness at the onion seedling and bulb stages.

The objectives of this study were to determine the virulence of isolates of *Fusarium oxysporum* Schlechtend.:Fr f. sp. *cepae* (H. N. Hans.) W. C. Snyder & H. N. Hans., *F. falciforme* and *F. brachygibbosum* Padwick on onion bulbs and seedlings.

Materials and methods

Fungal isolation and purification

In October 2016, in the central-northern area of the state of Sinaloa (Mexico) (the main onion producing area in the state), 95 onion plant samples were collected which showed symptoms of yellowing of the leaves from the tips downwards, slight dwarfing of the plant, soft bulbs and roots with a reddish-brown colouration (Fig. 1). The bulbs were cut longitudinally and slices of diseased tissue of approximately 5 mm in length were obtained, surface-sterilized with sodium hypochlorite (2%) for 2 min followed by ethanol (70%) for 2 min, and rinsed with sterile distilled water for 5 min. Subsequently, the tissue samples were dried and incubated in Petri dishes containing Potato Dextrose Agar (PDA) at 25°C with a 12 h photoperiod. After five days, fragments from the periphery of typical *Fusarium* colonies were transferred to new Petri dishes with PDA to obtain pure cultures. To obtain monosporic cultures, the methodology described by Hansen and Smith

Fig. 1 (Colour online) Symptoms on diseased onion plants. **(a)** Chlorotic leaves. **(b-c)** Soft bulbs and roots of reddish-brown colouration. **(d)** Dead plant.

(1932) was used. Briefly, a small fragment of the mycelium growing on PDA medium was mixed with 1.0 mL of sterile distilled water, and then serially diluted on PDA. Two days later, individual germinated spores were transferred, with the aid of a microscope, to a new Petri dish with PDA supplemented with PCNB and chloramphenicol, then incubated at 25°C for five days.

DNA extraction and PCR amplification

The mycelium of each isolate (n = 18) was collected by scraping the surface of colonies growing on PDA, previously incubated for 1 week at 25 °C. After grinding 100 mg of fungal mycelia in liquid nitrogen, genomic DNA was extracted following Ausubel et al. (2003). The DNA concentration and quality were estimated using a Thermo Scientific NanoDrop 1000 Spectrophotometer (ThermoFisher Scientific, Watltham MA).

The DNA from the *Fusarium* isolates was analysed by PCR, with the sequences of all the primers and PCR conditions used provided in Table 1. The final reaction mixture (25 µL) contained 100 ng DNA template, an equimolar mixture of dNTPs, 25 mM $MgCl_2$, PCR buffer, 1 U Taq DNA polymerase and 40 pmoles of each oligonucleotide (Bioline).

The PCR products (ITS and TEF-1α DNA) were purified and sequenced by Macrogen Inc. The ITS and EF-1α DNA sequences were used to search for sequence similarity against the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov) using the BLASTN programme. The molecular identification was confirmed via BLAST on the FUSARIUM ID and Fusarium MLST databases.

Phylogenetic analysis

The ITS and the TEF-1α sequences were aligned with reference sequences obtained from GenBank (reported on onion and other crops in Mexico and other regions of the world) using the multiple alignment in ClustalW of the software Geneious R9, and phylogenetic relationships were inferred based on the nucleotide sequence alignment of the gene among the *Fusarium* isolates. Trees were constructed by the Neighbour-Joining method based on distances determined by the method of Jukes and Cantor using 1000 bootstrap replicates.

Morphological characterization

Morphological characterization was performed according to the criteria described by Leslie and Summerell (2006). The isolates (n = 18) were grown in Petri dishes containing PDA, incubated at 25°C in the dark for 4 days, and three replicates were used to evaluate the growth rate of the mycelium. The colour of the colony and the formation of aerial mycelium were observed after 14 days of incubation on PDA at 20°C. Microconidial and macroconidial shape and septation, the arrangement of conidia and the presence or absence of chlamydospores was observed from colonies grown on Carnation Leaf Agar medium (CLA) (Leslie and Summerell 2006) at 20° C. For each characterized structure, 40 measurements were recorded under a light microscope with 40× magnification.

Pathogenicity tests

The pathogenicity of the isolates on onion bulbs and seedlings was evaluated following Taylor et al. (2016). The methodology of Lomas-Cano et al. (2016) was used for the inoculation of the pathogen and for the evaluation of the disease severity index (DSI).

Pathogenicity test on onion bulbs. The pathogenicity tests were carried out on healthy onion bulbs (cv. 'Carta Blanca'). The external scales of the bulbs were removed, after which the basal plate of each onion was excised and the bulb surface was sterilized with 70% ethanol. A PDA plug (8 mm) taken from the edge of an actively growing colony of each isolate (grown for 7 days at 20°C) was then positioned on the basal

Table 1. Conditions of annealing temperatures and sequences of primers TEF-1α and ITS for PCR analysis of *Fusarium* spp.

Code primer	Sequence (5′ → 3′)	Expected fragment size (bp)	Species-specificity	PCR conditions
F: ITS 1	TCCGTAGGTGAACCTGCGG	≈600	Fungi[a]	30 cycles; 30 s at 94°C, 1 min at 59°C, 1 min at 72°C
R: ITS 4	TCCTCCGCTTATTGATATGC			
F: EF-1	ATGGGTAAGGARGACAAGAC	≈698	*Fusarium* spp.[bc]	30 cycles; 30 s at 94°C, 30 s at 59°C, 1 min at 72°C
R: EF-2	GGARGTACCAGTSATCATGTT			

F: primer forward.
R: primer reverse.
[a]White et al. (1990).
[b]Geiser et al. (2004).
[c]O'Donnell et al. (1998).

plate of each bulb. Control bulbs were inoculated with a sterile plug of PDA. Bulbs were placed on moist paper tissue in a plastic box (four bulbs per box for each isolate), sealed in a plastic bag to maintain high humidity, and incubated at 20°C in the dark. Boxes were randomized in trays following an alpha design. After 48 h, each bulb was wrapped with cling-film to ensure that the agar plug did not dry out. After 9 weeks, each bulb was bisected longitudinally and a digital image was taken (including a 10-cm scale bar). Images were then analysed using ImageJ software to quantify the area of infection as a percentage of the total bulb area. Three independent replicates (four onion bulbs per replicate) were set up for each isolate (n = 18).

Pathogenicity test on onion seedlings. The same *Fusarium* spp. isolates used in the onion bulb tests were assessed for pathogenicity on healthy onion seedlings of cv. 'Carta Blanca' (45 days-old) grown on autoclaved vermiculite. Ten plants per isolate were inoculated by drenching with 20 mL of a conidial suspension (1×10^5 CFU mL^{-1}) of each isolate per plant. The suspension was obtained by collecting the spores of each isolate grown on PDA, with 10 mL of an isotonic saline solution. Twenty non-inoculated plants drenched with diluted saline solution served as controls. Plants were maintained for 60 days in a growth chamber with a 12-h photoperiod at 23 to 26°C. The experiment was carried out in a completely randomized design, and the assay was conducted twice. The pathogen was re-isolated from the necrotic tissue from all inoculated plants and was identified by sequencing the partial EF1-α gene again.

Plants were examined after 60 days incubation and the symptoms were recorded. Disease severity was rated on a scale of 0–4, where: 0 = plants without symptoms; 1 = ≥10% secondary roots showing necrosis; 2 = ≥10% primary roots showing necrosis; 3 = ≥50% primary root and lower stem necrosis, ≥20% secondary root necrosis; and 4 = total primary root necrosis, necrosis of the lower and basal portions of the stem, ≥50% secondary root necrosis, irreversible wilting of the aerial part and dead plants. The disease severity index (DSI) was calculated by applying the formula of Parke and Grau (1993), with values adapted to a percentage scale (*i.e.* 0 = 0 and 4 = 100%).

Data analysis

The data from the pathogenicity test of onion seedlings was subjected to a nonparametric variance analysis with Kruskal-Wallis and a Dunn rank test ($p \leq 0.05$). The pathogenicity test data from the onion bulbs was subjected to an analysis of variance and comparison of means by Tukey's HSD ($p \leq 0.05$), in order to determine significant differences in virulence between the isolates, as well as a Pearson correlation analysis to determine if there is a relationship between severity in onion plants and bulbs. All statistical analyses were performed with the XLSTAT software.

Results

Morphological characterization

Eighteen monospore isolates were identified as *Fusarium* spp. based on the morphology of their colonies using the *Fusarium* synoptic keys of Leslie and Summerell (2006) for species identification. Within the 18 *Fusarium* isolates, 16 isolates showed typical morphological markers for *F. oxysporum*, one for *F. falciforme* and one for *F. brachygibbosum.*

Isolates of *F. oxysporum* on PDA had a white or creamy cottony mycelium with pigmentation at the bottom of the colony that varied from yellow to brown, purple and pink. On CLA medium, the microconidia were cylindrical to renal form, with 0–1 septa, 6.1–-14.8 × 2.6–4.5 µm, and the macroconidia were straight, thin-walled form, 21.9–30.2 × 4–6 µm, 3–4 septa. All of the isolates showed short phialides. Chlamydospores were not evident (Fig. 2a–c).

For *F. falciforme*, on PDA, the colonies had abundant white aerial mycelium, and light purple pigmentation was observed in the centre of old cultures. From 10-day-old cultures grown on CLA medium, macroconidia were falciform, hyaline, three to five septate, with well-developed foot cells and blunt apical cells, and measured 27.3 to 46.3 × 2.1 to 7.1 µm (n = 40). The microconidia (n = 40) were hyaline, one to two celled, oval or reniform, 8.2 to 12.3 × 1.6 to 4.8 µm, and borne in false heads that measured 7.5 to 20 (average 13.75) µm × 2.3 to 9.0 (average 5.3) µm (n = 40). Chlamydospores were not evident (Fig. 2d–f) (Tirado-Ramírez et al. 2018).

Fusarium brachygibbosum colonies were white with abundant aerial mycelium and produced red pigmentation on PDA. From 10-day-old cultures grown on CLA media, macroconidia were slightly curved, with five septa, wide central cells, slightly sharp apexes, basal cells with a foot-like shape, and measuring 20.3 ± 4.4 × 3.3 ± 0.4 µm (n = 50). Microconidia were rarely observed on either PDA or CLA. Spherical chlamydospores of 6.8 ± 0.4 × 5.5 ± 0.7 µm (n = 50) were produced from mycelium in all isolates (Fig. 2*g–i*) (Tirado-Ramírez et al. 2019).

Phylogenetic analysis

Partial sequences of the TEF1-α and ITS genes from two representative *F. oxysporum* isolates, one *F. falciforme*

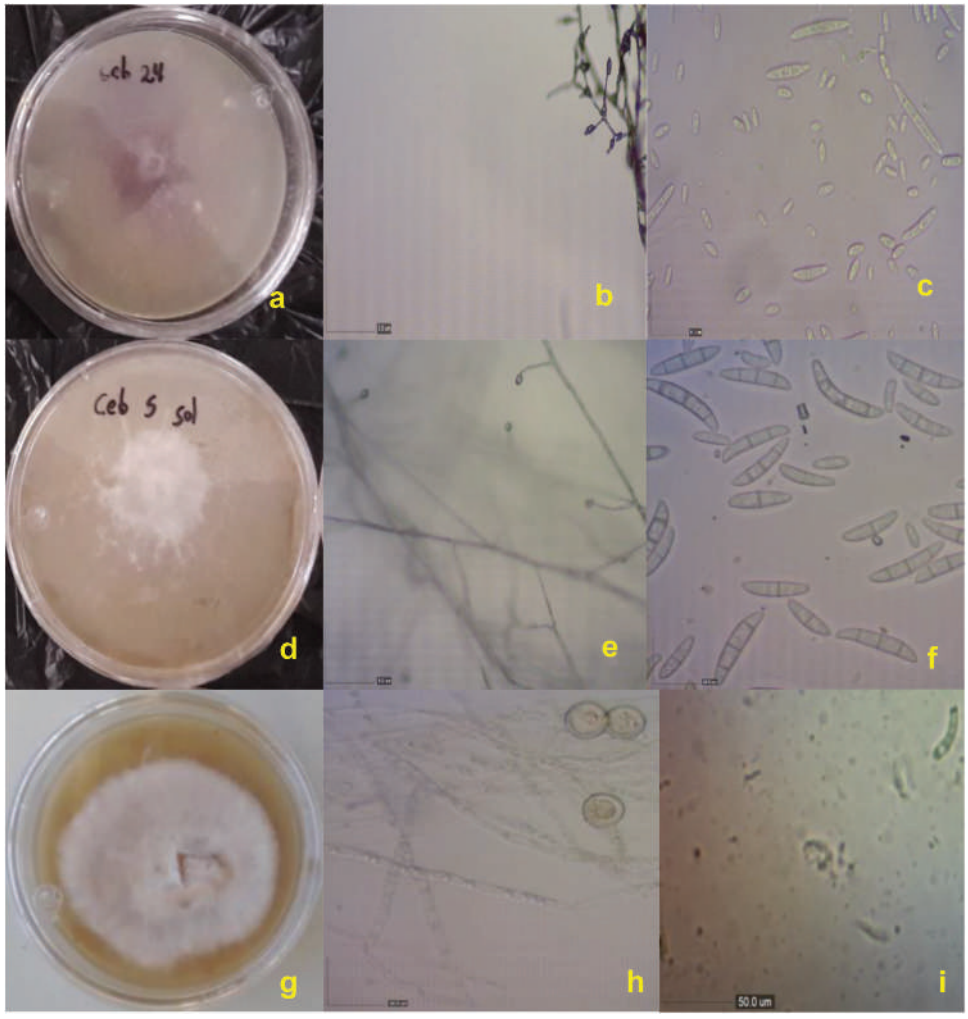

Fig. 2 (Colour online) Fungal isolates from diseased onion plants. (**a)** Top view of a *Fusarium oxysporum* colony growing on PDA. (**b)** Phialides of *F. oxysporum*. (**c)** Microconidia and macroconidia of *F. oxysporum*. (**d**) Top view of a *Fusarium falciforme* colony growing on PDA. (**e**) Phialides of *F. falciforme*. (**f**) Microconidia and macroconidia of *F. falciforme*. (**g**) Top view of a *Fusarium brachygibbosum* colony growing on PDA. (**h**) Chlamydospores of *F. brachygibbosum*. (**i**) Macroconidia of *F. brachygibbosum.*

isolate and one *F. brachygibbosum*, were generated and submitted to NCBI (Figs 3, 4). The analysis of ITS and TEF-1α sequences of these isolates by BLAST in the GenBank, Fusarium ID and Fusarium MLST databases showed that the TEF1-α sequence MH041264 (isolate Ff05) was affiliated to the species *Fusarium falciforme* (FSSC 3 + 4) with a 98% coverage and 99.85% identity with the TEF1-α sequence KY514181 (*F. falciforme*). The TEF1-α sequence MH041261 (isolate Fb20) had a 96.60% identity with the *F. brachygibbosum* sequence KR108742. The ITS sequence MH038176 (isolate Fb20) had a 98% coverage and 100% identity with the *F. brachygibbosum* ITS sequence JX162372. The ITS sequence MH038177 (isolate Ff05) had a 100% coverage and 100% identity with ITS sequences of the species *F. falciforme* (FSSC 3 + 4). Neighbour-Joining (NJ) trees were produced from the sequence data. In addition to isolates that were sequenced during the current study, other sequences were obtained from GenBank as reference strains and used for analysing phylogenetic relatedness.

In the phylogenetic tree resulting from the TEF-1α neighbour joining analysis (Fig. 3) three groups with high bootstrap values were recognized. Clade 1 included *F. falciforme* isolates from different geographical regions and different hosts. Clade 2 was composed of *F. oxysporum* isolates. Clade 3 consisted of *F. brachygibbosum* isolates. The phylogenetic analysis, based on partial sequences of the TEF-1α gene,

showed that the *F. falciforme* isolates, *F. oxysporum*, as well as the *F. brachygibbosum* isolates showed sequence variation among the isolates used as references, suggesting that these isolates represent relatively divergent populations.

The phylogenetic analysis of the ITS sequences helped to delineate the recovered isolates into the respective species. All strains formed distinct clades with the reference strain sequences used for each species. The two *F. oxysporum* isolates (MH038178 and MH038179) clustered into the clade of *F. oxysporum* reference sequences (Fig. 4), which included isolates from Mexico, Brazil, China and Egypt. Another clade was formed by the *F. falciforme* (FSSC 3 + 4) isolates, which

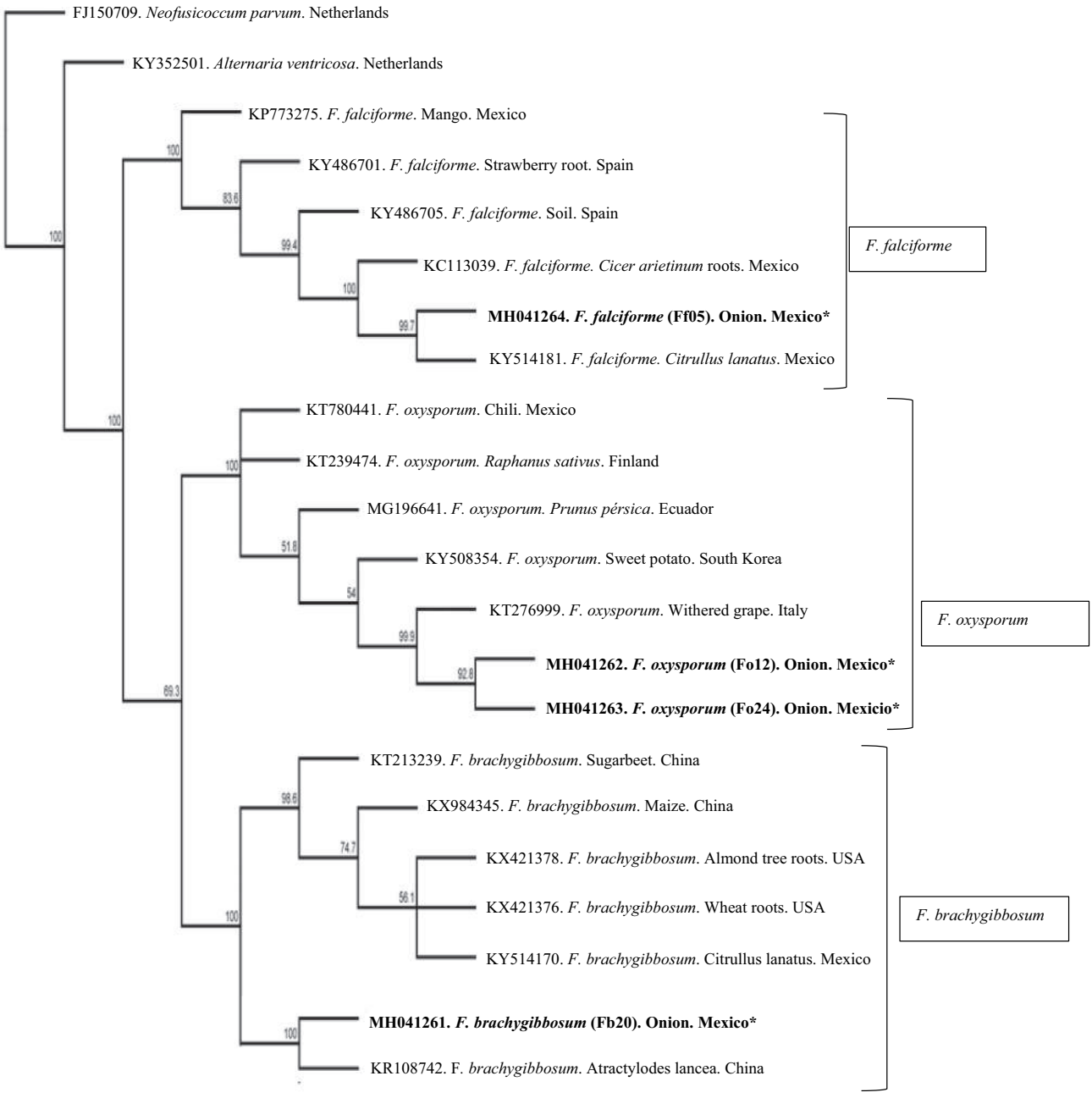

Fig. 3 Phylogram of Neighbour Joining for *TEF-1α* gene from *F. oxysporum, F. falciforme* and *F. brachygibbosum* species. Values at the nodes represent the percentage bootstrap scores (1000 replicates). The tree has *Neofusicoccum parvum* (FJ150709) and *Alternaria ventricosa* (KY352501) as a outgroup.

included isolate Ff05 from this study (MH038177). Another distinct clade included the *F. brachygibbosum* isolates, which included isolate Fb20 from this study (MH038176).

Pathogenicity tests

A significant difference was observed in the analysis of variance of the onion bulb pathogenicity data (Fig. 5), where the numerically highest percentage of damage (66.6%) was caused by isolate Ff05 (*F. falciforme*), but was statistically equal to the damage caused by isolates Fb20 (*F. brachygibbosum*, 59.9%), Fo24 (*F. oxysporum*, 57.4%) and Fo12 (*F. oxysporum*, 56.4%). Ff05, Fb20, Fo24, Fo12, Fo22, F028 and Fo03 were statistically higher then Fo26, Fo30, Fo23, Fo08, Fo10, Fo11, Fo29, Fo02, Fo09, Fo25 and Fo33. The damage caused by the isolates of *Fusarium* spp. consisted of a soft, light brown rot, the same as observed on onion bulbs obtained from the field. Symptoms were not observed on the non-inoculated bulbs (Fig. 6).

In the case of the analysis of variance of pathogenicity on onion seedlings, isolate Fb20 (*F. brachygibbosum*) did not show a significant difference compared with the other isolates (Fig. 7), but was significantly different from the control, unlike the rest of the isolates. The highest disease incidence was observed in plants inoculated with isolate Fb20. Fungi recovered from symptomatic plants showed the same morphological characteristics as the isolates used for inoculation,

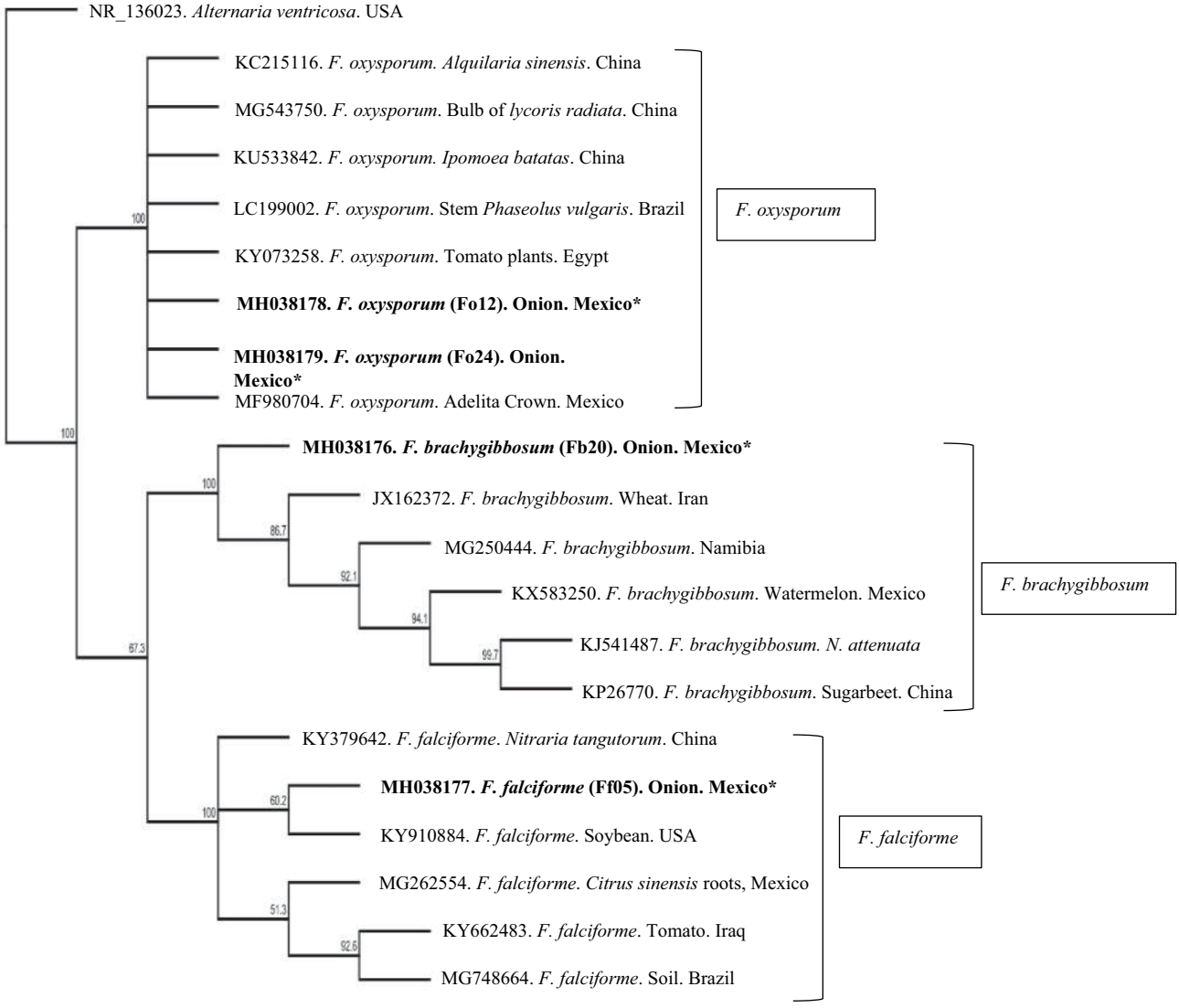

Fig. 4 Phylogram of Neighbour Joining for ITS gene from *F. oxysporum, F. falciforme* and *F. brachygibbosum* species. Values at the nodes represent the percentage bootstrap scores (1000 replicates). The tree has *Alternaria ventricosa* (NR_136023) as a outgroup.

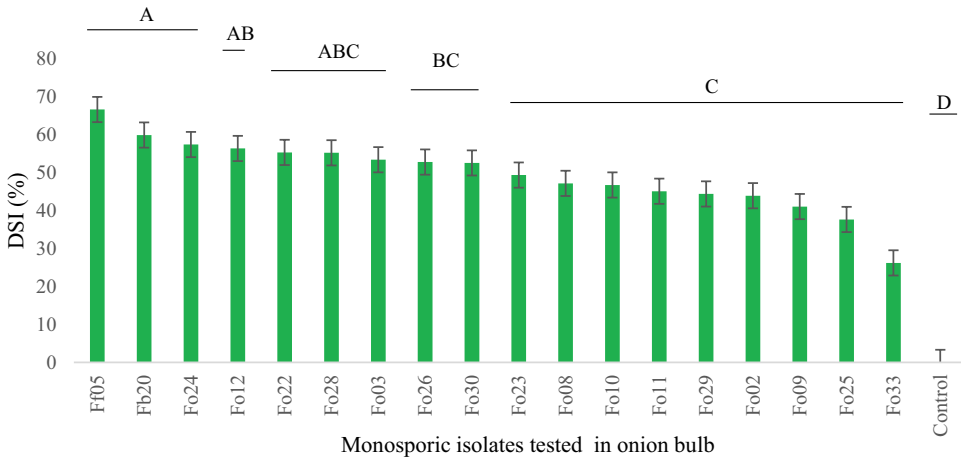

Fig. 5 (Colour online) Disease severity index (DSI) and the result of analysis of variance for the onion bulbs. The DSI is expressed as a percentage, and the comparison of means was performed by Tukey with $p \leq 0.05$.

thus confirming their pathogenicity. Control plants showed no symptoms at all (Fig. 8). Inoculations were performed twice, showing similar results. Also, a high correlation ($r = 0.82$, $P < 0.0001$) was observed between isolate pathogenicity on the bulbs and seedlings.

Discussion

This study confirmed that *F. oxysporum, F. falciforme* (FSSC 3 + 4) and *F. brachygibbosum* are associated with basal rot in onion in northwest Mexico. *Fusarium oxysporum* was the most commonly isolated species from onion crops, whereas *F. falciforme* and *F. brachygibbosum* were found less frequently. These results provide the basis for the development and implementation of appropriate disease management practices for these pathogens in Mexico.

Dissanayake et al. (2009) and Rabiei et al. (2010) reported a higher incidence of *F. oxysporum* on onions in Iran and Japan, and found *F. solani, F. proliferatum* and *F. verticillioides* less frequently. Stankovic et al. (2007) reported a high incidence of *F. proliferatum* in garlic and onion in Serbia, while *F. oxysporum, F. solani, F. acuminatum* and *F. equiseti* occurred less frequently. In Morelos, Mexico, Mejia (2015) identified three *Fusarium*

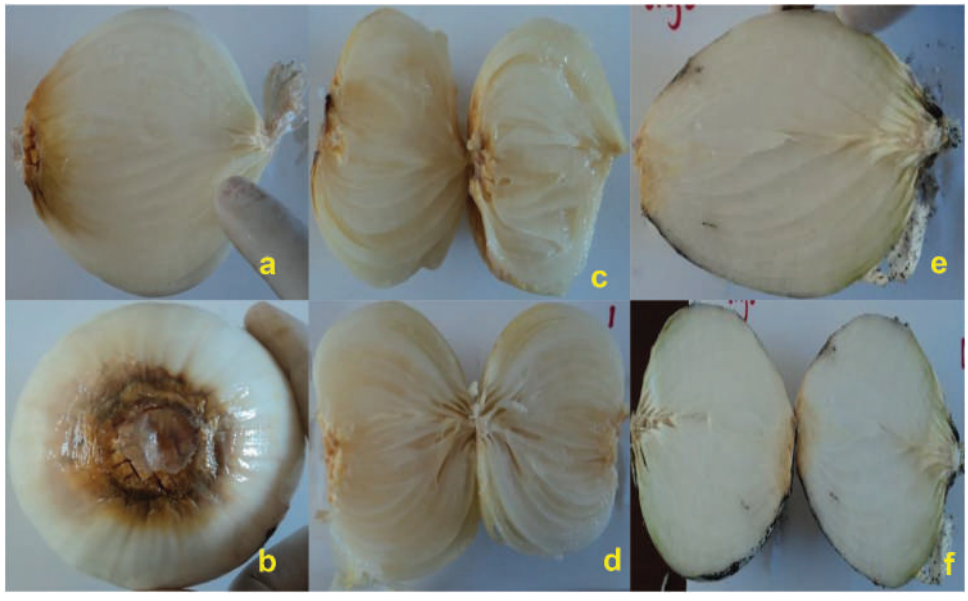

Fig. 6 (Colour online) Symptoms on diseased onion plants. (**a–d**), Basal rot caused by *Fusarium* spp.; (**e–f**), Healthy bulb (control).

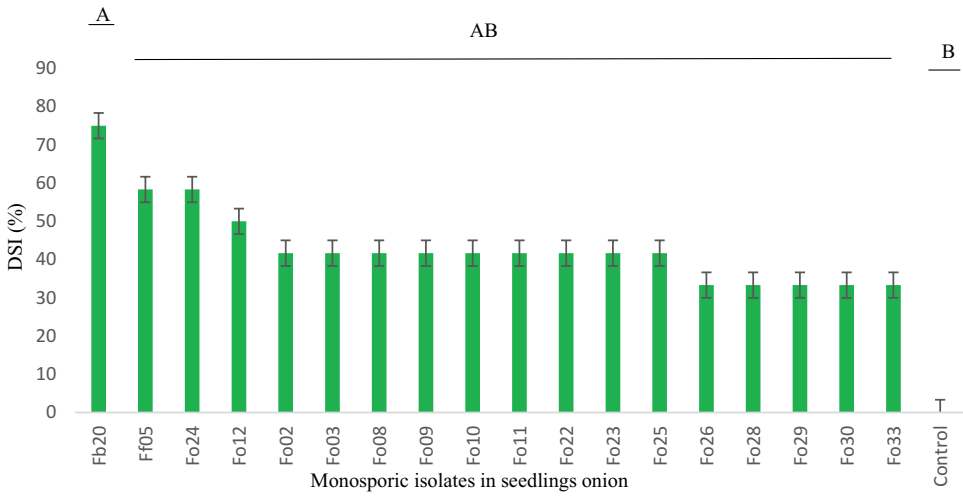

Fig. 7 (Colour online) Disease severity index (DSI) and the result of Kruskal-Wallis test for the onion seedlings 60 days after inoculation. The DSI is expressed as a percentage, and the range comparison was performed by Dunn with $p \leq 0.05$.

species causing disease on onion, *F. proliferatum, F. verticillioides* and *F. oxysporum*. Ochoa et al. (2012) identified four *Fusarium* species, *F. verticillioides, F. acuminatum, F. solani* and *F. oxysporum*, in garlic seeds in Aguascalientes, Mexico. Montes et al. (2003) reported the presence of *F. culmorum* causing disease on onion cultivars in the state of Morelos, Mexico. In Iran, Ghanbarzadeh et al. (2014) reported that *F. redolens* was the most virulent isolate on onion seedlings, but was less virulent on onion bulbs. In Finland, *F. redolens* isolates were highly virulent, killing onion seedlings (Haapalainena et al. 2016).

The phylogenetic trees based on the ITS and TEF-1α sequences helped to separate recovered isolates into distinct clades, with each clade containing reference strains of different species (Figs 3, 4). The phylogenetic analysis suggested that at least three phylogenetically distinct species of *Fusarium* are associated with basal rot in onion in northwest Mexico. This analysis confirmed that the three clades represent distinct phylogenetic lineages. These observations demonstrate the importance of using the phylogenetic concept to identify fungi from the genus *Fusarium*.

Fusarium falciforme was the predominant species associated with root rot in watermelon cultivation in Sonora, Mexico (Rentería-Martínez et al. 2018). It was also reported previously on papaya and tomato in Mexico (Vega-Gutiérrez et al. 2019a, 2019b), as well as on lima, bean and chickpea in Brazil (Cabral et al. 2016; Sousa et al. 2017). This fungus is one of the most prevalent clinically relevant species of the genus, capable of inducing disease in humans and other animals (O'Donnell et al. 2008; Sandoval-Denis and Crous 2019), with some strains also found as plant pathogens (Scheel et al. 2013; Short et al. 2013).

Fusarium brachygibbosum belongs to the *Fusarium sambucinum* Species Complex (FSSC) (Rentería-Martínez et al. 2018); Likewise, *F. brachygibbosum* has been reported from oleander in Iran (Mirhosseini et al. 2014), corn stem rot in China (Shan et al. 2017), watermelon wilt in Sonora, Mexico (Renteria-Martinez et al. 2015), and in olive trees causing an intense darkening of vascular tissues (Trabelsi et al. 2017)

The *Fusarium oxysporum* Species Complex (FOSC) causes vascular wilt and root rot in more than 100 different plant species, and based on its specificity with the host, more than 80 *formae speciales* have been reported (Rentería-Martínez et al. 2018; DeIulio et al. 2018), including *F. oxysporum* f. sp. *cepae*, the causal agent of basal rot in onion cultivation (Schwartz and Mohan 1995; Pulido-Herrera et al. 2008; Taylor et al. 2016; Delgado-Ortiz et al. 2016). Nonetheless, Azevedo et al. (2017) pointed out that the *formae speciales* naming system for *F. oxysporum* is obsolete, because the core genome phylogeny and the pathogenicity on a certain host plant do not always match.

In this study, pathogenicity tests were performed to identify the species that present a potential risk of causing *Fusarium* bulb rot disease on onion in northwest Mexico. Although *F. oxysporum* was more frequently isolated (89% of all isolates collected) than *F. brachygibbosum* (5.5%) and *F. falciforme* (5.5%) (Fig. 3), the tests indicated that *F. brachygibbosum* presents a potential risk to onion crop health at the seedling stage and *F. falciforme* at the bulb stage. These risks may be actualized if these pathogens become more frequent.

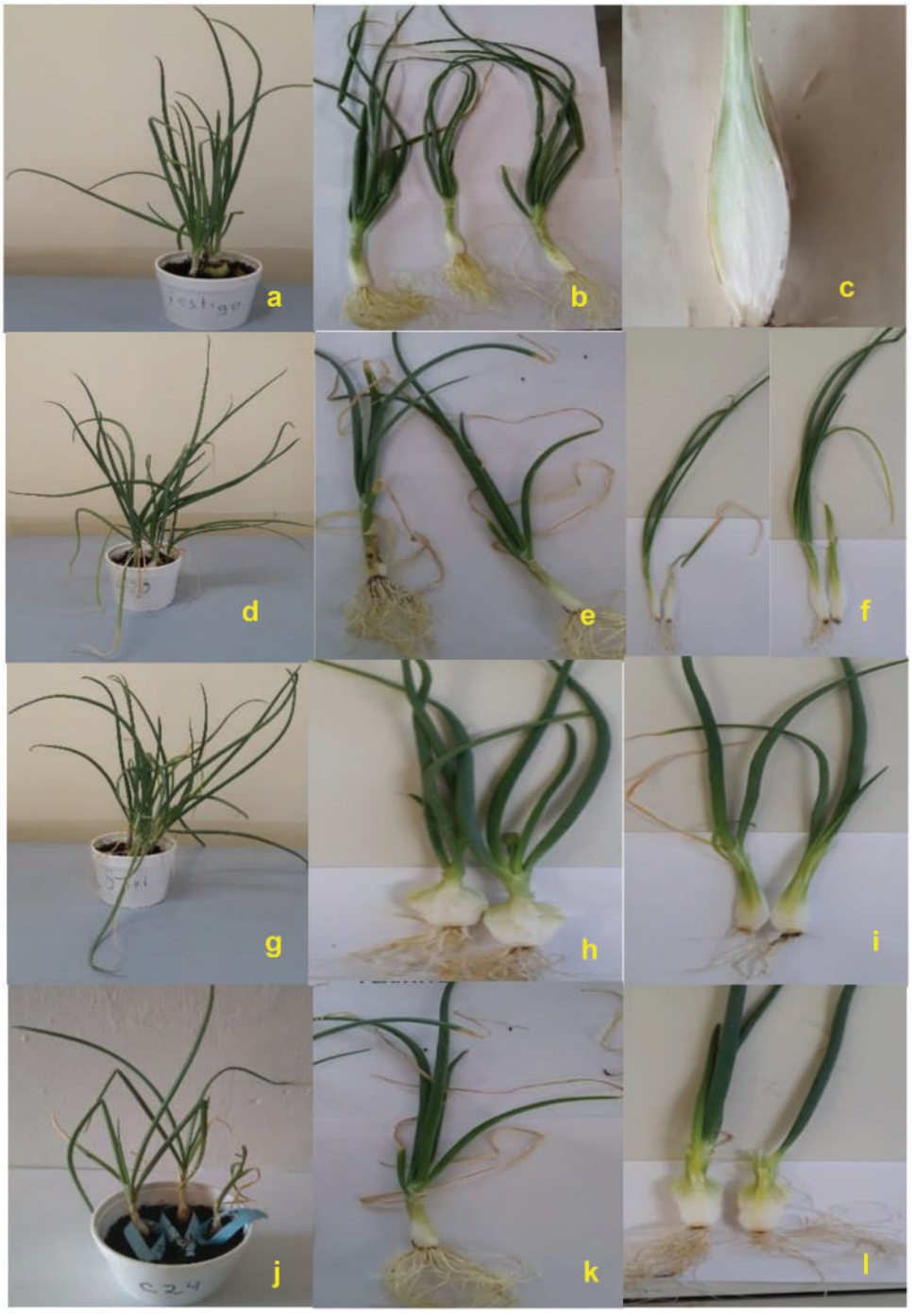

Fig. 8 (Colour online) Pathogenicity test on onion seedlings. (**a**–**c**) negative control. (**d**–**f**) Disease symptoms caused by *Fusarium brachyqibbosum*. (**g**–**i**) Disease symptoms caused by *Fusarium falciforme*. (**j**–**l**) Disease symptoms caused by *Fusarium oxysporum.*

Therefore, the situation needs to be monitored by analysing samples collected from onion crops. The analysis of variance of the pathogenicity data showed significant differences among the isolates with respect to the damage they caused on onion bulbs. Taylor et al. (2016) found highly significant correlations between pathogenicity tests on seedlings and onion bulbs. Isolates of the three species caused similar symptoms including leaf yellowing and darkening of the vascular tissues on onion plants, highlighting the need for specific

diagnostic tools to discriminate within the species complexes. Information on the different pathogens causing disease in onion is valuable for the development of breeding programs to select for resistant cultivars that can help to reduce the devastating effects of Fusarium basal rot of onion worldwide.

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