Short note [Nota corta]

FIRST REPORT OF BASAL ROT CAUSED BY *Fusarium equiseti* IN ONION CROPS FROM PUEBLA, MEXICO †

[PRIMER REPORTE DE PUDRICIÓN BASAL CAUSADA POR *Fusarium equiseti* EN CULTIVOS DE CEBOLLA DE PUEBLA, MÉXICO]

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SUMMARY

Background: Species of the *Fusarium* genus are considered as devastating phytopathogens of onion crops around the world. **Objective**: This work aimed to know the causal agent of basal rot in onion crops from Puebla-México recorded in 2019. **Methodology**: The causal agent was isolated from diseased samples by tissue incubation in Potato Dextrose Agar medium (PDA) and the pathogenicity tests were done with the causal agent to demonstrate its involvement in basal rot. Monosporic cultures of the causal agent were generated for further microscopic characterization and molecular identification by Internal Transcribed Spacers ITS1 and ITS2. **Results**: According to the pathogenicity tests, the causal agent produced apical constriction and necrosis in the radicle and leaves accompanied by brown spots surrounded by yellowing as those observed in natural conditions. A 533 bp amplicon of the causative agent was obtained by partial amplification of the 5.8S rDNA gene. The sequence of the amplicon was compared with the sequences deposited in the database of the National Center for Biotechnology Information (NCBI) showing 100% homology with *Fusarium equiseti*. **Implications**: Our investigation reveals *F. equiseti* as an emergent causal agent of onion basal rot in crops from the community of "La Soledad" Puebla, México. **Conclusion**: Herein we report for the first time *F. equiseti* as a new phytopathogen of onion and further strategies should be considered for its control.
Key words: *Fusarium equiseti*, pathogenicity, *Allium cepa*, basal rot.

RESUMEN

Antecedentes: Las especies del género *Fusarium* son consideradas como fitopatógenos devastadores de los cultivos de cebolla en todo el mundo. **Objetivo:** El objetivo de este trabajo fue conocer al agente causal de la pudrición basal en cultivos de cebolla en Puebla-México registrados en 2019. **Metodología:** El agente causal se aisló de muestras enfermas mediante incubación de tejidos en medio Agar Dextrosa y Papa (PDA) y las pruebas de patogenicidad se realizaron con el agente causal para demostrar su participación en la pudrición basal. Se generaron cultivos monospóricos del agente causal para su posterior caracterización e identificación

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microscópica y molecular mediante espaciadores transcritos internos ITS1 e ITS2. **Resultados:** De acuerdo con las pruebas de patogenicidad, el agente causal produjo constricción apical y necrosis en la radícula y hojas acompañada de manchas marrones rodeadas de amarillamiento tal y como fue observado en condiciones de campo. Se obtuvo un amplicón de 533 pb del agente causal por amplificación parcial del gen 5.8S rDNA. El amplicón previamente secuenciado se comparó con las secuencias depositadas en la base de datos del Centro Nacional de Información Biotecnológica (NCBI) mostrando un 100% de homología con *Fusarium equiseti*. **Implicaciones:** Nuestra investigación revela a *F. equiseti* como agente causal emergente de la pudrición basal de cebolla en cultivos de la comunidad de "La Soledad" Puebla, México. **Conclusión:** Reportamos por primera vez a *F. equiseti* como un nuevo fitopatógeno de la cebolla por lo cual se deben considerar nuevas estrategias para su control.
Palabras clave: *Fusarium equiseti*, patogenicidad, *Allium cepa*, pudrición basal.

INTRODUCTION

Onion (*Allium cepa* L.) is considered one of the most consumed vegetables worldwide whereas in Mexico it is considered an iconic condiment for Mexican cuisine (Joaheer *et al*., 2019). The production of this food in the country earns about 26,029,376 USD per year and the state of Puebla, México is regarded as the fifth producer, yielding 21,371 tons per hectare (Joaheer *et al*., 2019).

The *Fusarium* genus is comprised of thousands of species with several clonal lineages and these species are mainly distributed in the soils, or they are associated with plants as endophytes or potential phytopathogens (Michielse and Rep, 2009; Summerell *et al*., 2010). Basal rot caused by the genus *Fusarium* spp. is widely distributed around the globe and has become a limitation in onion and garlic producing areas (Kiehr and Delhey, 2015). The main species within the genus *Fusarium* that harm the onion crop around the world are *F. proliferatum*, *F. solani* and *F. oxysporum*, reducing its yield up to 50% (Haapalainen *et al*., 2016). These species produce symptoms in the onion plant that include wilting, rotting of the roots and basal blade of the bulb (Sanogo and Zhang, 2015). In Mexico there are few studies related to the presence of some *Fusarium* species in onion crops. However, these have been associated with devastating loses for local producers (Montes-Belmont *et al.*, 2010). In the same context, Pulido-Herrera *et al.* (2008) reported serious root rot incidence caused by *Fusarium oxysporum*, *F. subglutinans* and *Pyrenochaeta terrestris* in onion crops at the Trinidad Valley, in Baja California-Mexico. Due to this fact, the present investigation focused on the identification of the causal agent of the basal rot of in onion crops that emerged in the community of "La Soledad" Puebla, México. The identification of the causal agent was obtained through pathogenicity tests and molecular techniques.

MATERIALS AND METHODS

In the summer of 2019, onion crops (var. 'Crystal white') grown in the community of "La Soledad" Puebla, México (18°27'39.3258"N; -98°37 '11.2614"W) experienced a devastating rot. The symptoms were basal rot, bulb rot, poor root development, leaf discoloration, chlorosis and necrosis in the central part of the leaf (Figure 1a). Approximately, 40% of the crops showed these symptoms.

Samples of diseased tissues (rot discs and bulbs) were collected in an onion plot (var. 'Crystal white') of 3,144.3 m^2 located in "La Soledad" Puebla, México. This geographical area has a warm-wet climate (cw), with an annual rainfall average of 1,500 mm and an altitude of 1,090 masl. The diseased crops showed loss of leaf turgor, weakness and wilting. The samples were kept at 4 °C and transported in plastic bags to the laboratory for immediate analysis. Thirty bulbs were cut into small pieces (~1.5 cm) and the surface was sterilized with 0.1% sodium hypochlorite for 1 min. The pieces were washed three times with sterile distilled water and dried with sterilized filter paper. Pieces of 0.5 cm^2 were placed in Petri dishes containing PDA medium and incubated for 10 days under 8 h natural light (day) and 16 h darkness at 28 °C). The colonies were purified using monosporic cultures which were maintained in 20% glycerol at -84 °C (Morales-Mora *et al*., 2020).

The characterization was carried out through fungal microcultures that were visualized in a Carl Zeiss®, (Jena, Germany) at 1000x. Anamorphic structures with morphological characteristics associated with the *Fusarium* genus were observed, measured and compared with dichotomous keys (Barnett and Hunter, 2006; Leslie and Summerell, 2006). Forty-five certified onion plants of the "Crystal white" var. with a germination percentage of 90% were used for pathogenicity tests. Plants of 30 days old (10 cm tall and 5 mm in diameter) were individually placed in a plastic pot (1 L) containing

a sterilized mixture of peatmoss and Agrellite (1:1 v/v) (Martínez- Salgado *et al*. 2021). The plants were kept under greenhouse conditions (70% RH, 28 °C) in two separate areas. The inoculation of the "CFbC" strain (*F. equiseti*) was done in these plants by spraying a suspension of 1×10^5 conidia/mL until dropping. The micro- and macroconidia were obtained from cultures developed in PDA and were recovered with 10 mL of sterile physiological saline solution in a laminar flow hood to be incubated for 7 days at 28 °C (Morales-Mora *et al.*, 2019). Fifteen seedlings were only sprayed with distilled water and kept under the same conditions.

Genomic DNA was extracted from mycelium of a monosporic culture grown for 7 days in PDA by the CTAB method (Rivera-Jiménez *et al.*, 2018). The genetic material was resuspended in 100 µL HPLC water and immediately quantified by spectrophotometry (Nanodrop 2,000 C, Thermo Scientific®) at 260/280 and 230/260 nm. Afterwards, the genetic material was diluted to a final concentration of 20 ng mL^{-1} and used as a template for PCR reactions. The amplicons were obtained using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) reported by White *et al*. (1990) to amplify a partial fragment of the ITS region. PCR mixtures (50 µL) consisted of 20 ng/µL template, 20 µM primers, 500 mM KCL_2, 100 mM Tris HCl (pH 9), 50 µM $MgCl_2$, 100 µM dNTPs and 2.5 U/µL Taq DNA polymerase (Promega®). The amplification protocol was performed in accordance with Salazar-González *et al*. (2016). The amplicons were purified using the kit ExoSAP-IT (Affymetrix®, Santa Clara, CA), following the manufacturer's instructions. The "CFbC" strain were sequenced using the kit BigDye Terminator v3.1 (Applied Biosystems®, Carlsbad, CA) in an Applied Biosystems 3130 sequencer (Carlsbad®, CA) (Juárez-Vázquez *et al*. 2019). Both complementary chains were assembled and edited using the software BioEdit v7.0.5. As a result, a consensus sequence was obtained for the strain CFbC. The phylogenetic analysis of the strain CFbC was performed by the neighbor-joining method using the MEGA X (Kumar *et al*., 2018) and the results were compared with five records of the nucleotide database of the National Center for Biotechnology Information.

RESULTS AND DISCUSSION

Ten representative fungi were isolated from 50 diseased onion bulbs. These isolates showed typical morphological features of *F. equiseti* including white mycelium with radial growth (Figure 1e). Nevertheless, the isolate named "CFbC" was the most abundant in all samples analyzed. After 11 days, the fungus turned the PDA medium to brown-orange color, which was observed at the bottom of the Petri dish (Figure 1f). The microscopic features of *F. equiseti* CFbC showed septate hyaline hyphae, septate macroconidia (with five septa) (Figure 1c-d), falcate shaped conidia with a curvature of 60-120 µm (n = 80); the curvature was arcuate in its ventral zone and the dorsal arcs show a prominent basal cell with foot shape and a filamentous apical termination. Microconidia were unicellular, no septate, hyaline and ellipsoid that measured 4.6-17.2 × 1.4-4.1 µm (n = 80). The morphological characteristics coincided with that described in previous studies for *F. equiseti* (Barnett and Hunter, 2006; Leslie and Summerell, 2006; Summerell *et al*., 2010).

After 20 days post-inoculation, infected plants exhibited brown spots surrounded by yellowing, necrosis of the root, constriction of apical shoot and wilt of young leaves (Figure 1b). Plants treated with distilled water remained asymptomatic. The fungal pathogen was re-isolated from the lesions and after examination, it exhibited the same morphological characteristics as those of the original isolate. These procedures fulfilled the criteria of Koch's postulates an endorsed *F. equiseti* as a causal agent of onion basal rot.

The corresponding ITS region was deposited in the nucleotide database of the NCBI with the accession code MN612793. After comparing the sequences of ITS region, the analysis revealed that this sequence had 100% homology with those of the accessions KX375792.1, HM999942.1, KM246255.1, KY554857.1, MG734215.1 and MH860607.1 of *F. equiseti* (Figure 2). To the best of our knowledge, this is the first report of *F. equiseti* as an emergent phytopathogen of onion var. 'Crystal white' detected in crops from the community of "La Soledad" Puebla, México. Nevertheless, *F. equiseti* has been reported as a common causal agent of watermelon rot (Li and Ji *et al*., 2015). Delgado-Ortiz *et al*. (2016), isolated and identified several phytopathogenic species, such as *F. oxysporum, F. proliferatum, F. verticillioides, F. solani* and *F. acuminatum* in onion crops from the states of Zacatecas and Aguascalientes, Mexico. Also, *Fusarium oxysporum*, *F. proliferatum* and *F. redolens* have been reported as phytopathogens in onion crops from Finland (Haapalainen *et al*., 2016). Dauda *et al*. (2018) reported *F. equiseti* as the causal agent of the dieback of onion crops from Nigeria and Bayraktar *et al*. (2010) confirmed 40% mortality in onion crops from Turkey caused by the same

phytopathogen. On the other hand, Ignjatov *et al*. (2015) reported the strain FIESC-3 of *Fusarium* sp. as part of the *F. incarnatum-equiseti* complex and its involvement in onion seed rot in crops from Serbia.

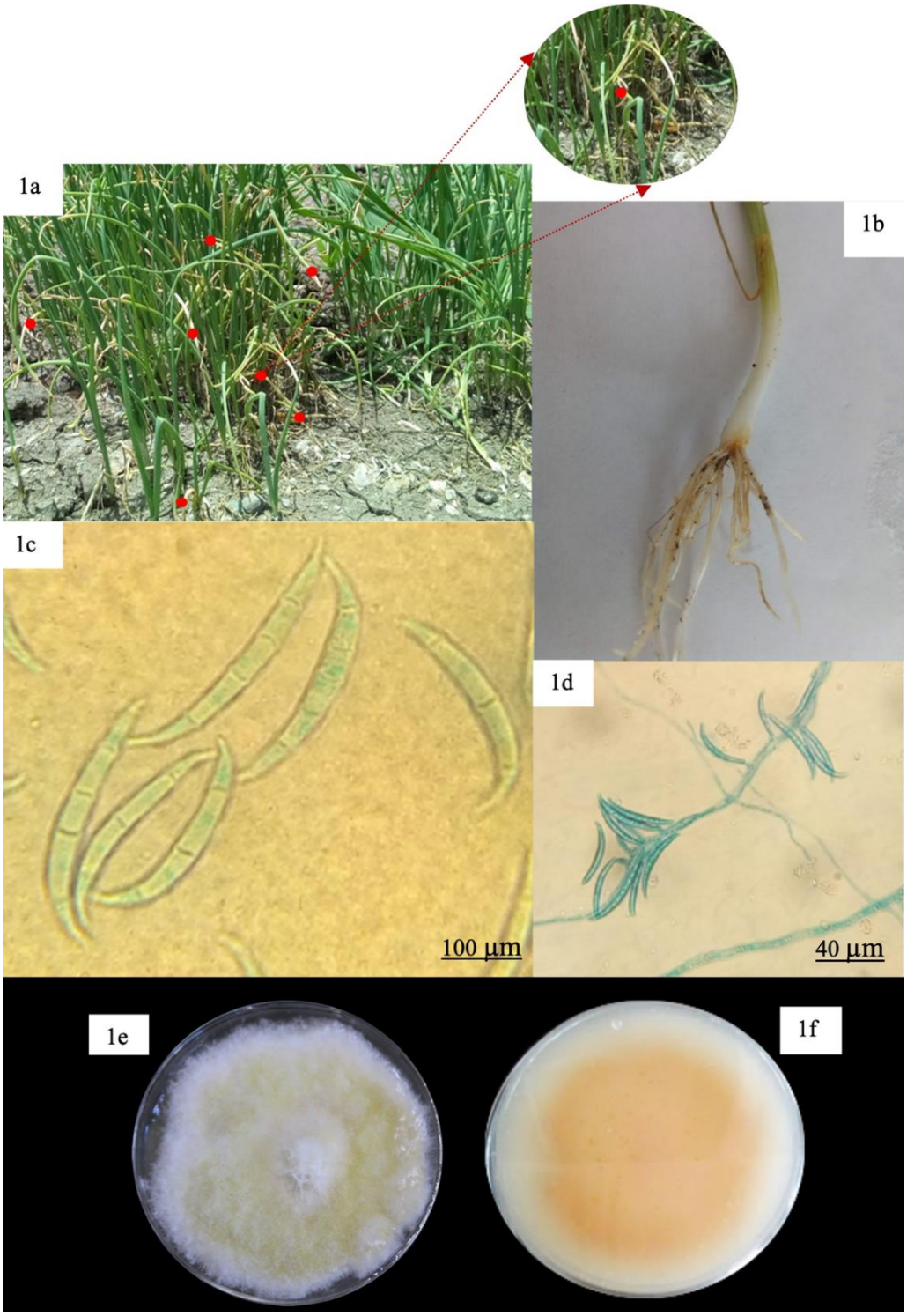

Figure 1. Pathogenicity tests of *F. equiseti* in onion crops from Puebla Mexico; red dots indicate characteristic symptoms of the reported disease. 1a) There was a loss of leaf turgor in infected plants; the plants show weakness and wilt whereas discoloration was observed in the severely affected leaves; curly wilted leaves displaying yellowing were evident. 1b) Necrosis in root and herbaceous shoots with orange-pink coloration. 1c-d) Lunate macroconidia stained with methylene blue at 100 and 40 X. 1e) Fungal colony showing white mycelium and radial growth. 1f) Bottom of Petri dish showing a brown-orange color.

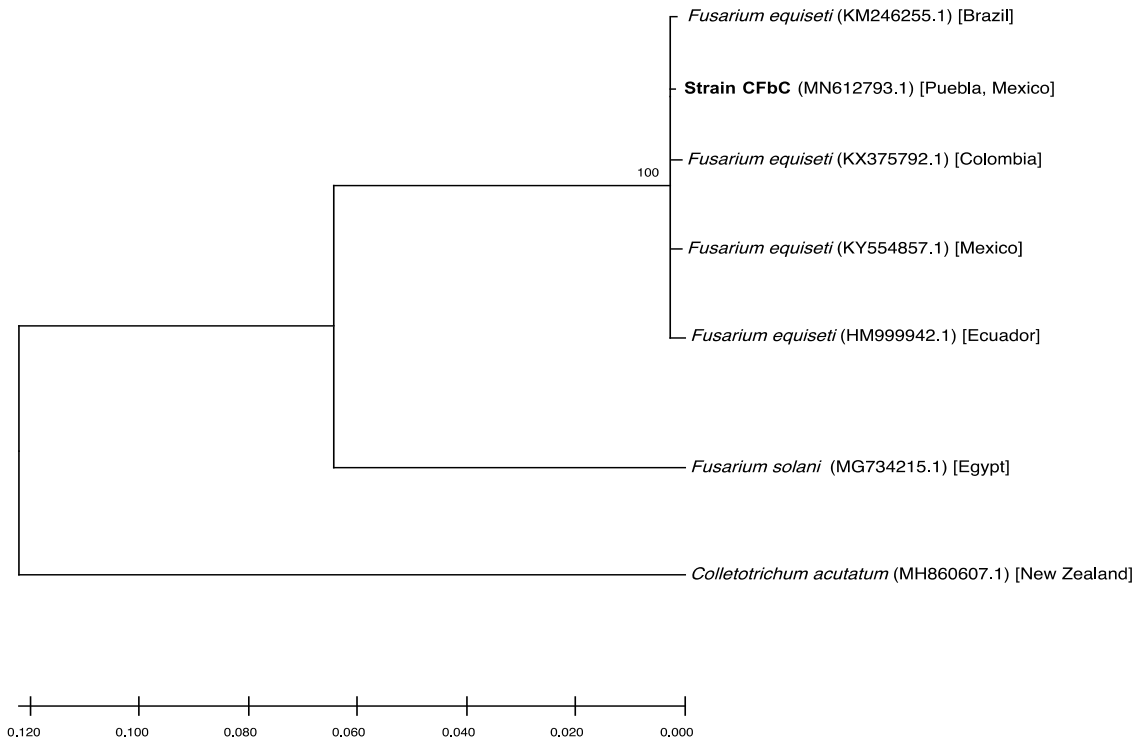

Figure 2. Phylogenetic analysis by neighbor-joining method generated in the Mega X program from ITS1-4 sequences of the 5.8S rRNA partial gene. *Colletotrichum acutatum* and *Fusarium solani* were used as outgroup. The CFbC strains obtained in this study are shown in black.

This research is the first evidence on the presence of *F. equiseti* as a causal agent of basal rot in onion var. 'Crystal white' from cultures developed in Puebla, Mexico.

CONCLUSION

The morphological and molecular data presented in this work, revealed *F. equiseti* as a new phytopathogen of onion crops from Puebla, México. Further strategies should be considered for its control.

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Compliance with ethical standards. The authors confirm that this investigation was conducted under the current ethical procedures.

Data availability. Data are available with Dr. Nemesio Villa-Ruano (necho82@yahoo.com.mx) upon reasonable request.

Author contribution statement (CRediT). O. Romero-Arenas – Conceptualization, Funding acquisition, Methodology, Validation, Supervision, Writing. **S.J. Martínez-Salgado** – Methodology, Validation, Writing. **A. Rivera** – Funding acquisition, Methodology. **M. Huerta Lara** – Funding acquisition, Methodology. **Beatriz Laug Garcia** – Funding acquisition, Methodology. **Nemesio Villa-Ruano** – Conceptualization, Validation, Data curation, Writing.

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