



# First report of vascular wilt caused by *Fusarium proliferatum* on chickpea in Cuba

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Chickpea (*Cicer arietinum*) is one of the most important legume crops, constituting more than 20% of grain production worldwide, and providing a significant carbohydrate and protein source for human and animal consumption (Arefian *et al.*, 2014). In Cuba, production still does not meet the demands of the population, making it necessary to import (Echevarria *et al.*, 2014). The diseases that cause the greatest loss in chickpea yields are root rots, mainly caused by different species of *Fusarium*, e.g. *F. oxysporum* f. sp. *ciceris*.

During the 2014-2015 harvest, field samples of wilting chickpea plants with chlorotic and necrotic roots were taken to the laboratory at the National Center for Animal and Plant Health (CENSA), Mayabeque Province, Cuba. Samples of necrotic roots were surface-sterilised with sodium hypochlorite solution (1%), and ethanol solution (70%), rinsed three times with sterile water, air dried, and cultured on potato dextrose agar (PDA, Biocen) plus chloramphenicol (0.01 g/l) at 25 ±2°C for one week. Several fungal colonies were isolated, which were transferred to fresh PDA plates to obtain pure cultures (French & Hebert, 1982). The aerial mycelium of all samples was initially white but became violet with age (Fig. 1). Macroconidia were slender and to almost straight, with thin walls, apical cell curved, basal cell poorly developed and 3 to 5-septate. Microconidia were pyriform, 0-septate, found in false heads, with visible monophialides and polyphialides as described by Leslie & Summerell (2006) (Fig. 2).

Total genomic DNA was extracted from seven-day-old cultures using the CTAB method. EF1/EF2 primers (O'Donnell *et al.*, 1998) were used in PCR amplification and sequencing of the translation elongation factor 1- $\alpha$  (*tef*) gene. The sequences were deposited in Genbank (Accession Nos. MT110193, MT110194, MT110195, MT163660 and MT185674). The aligned sequences were analysed for phylogeny (MEGA 7) and BLAST analysis revealed the highest identity (99%) with *Fusarium proliferatum* (JF740779). Based on both the culture morphology and DNA sequence analyses, the isolates were identified as *Fusarium proliferatum*.

To confirm pathogenicity, four seeds were sown in pots (500 ml) containing red ferralitic soil, previously sterilised on two consecutive days (121°C × 60 min). Plants were inoculated in vegetative stage V2 (Toledo, 2018) via root immersion. A 100 ml of conidial suspension (1 × 10<sup>5</sup> spores/ml) of each isolate was added to Petri dishes. Roots of the plants were immersed into this conidial suspension for 10 minutes. Sterile water was used for controls. The inoculated plants were subsequently replanted into their original pots and symptom development was checked daily. The

symptoms of root rot were observed visually 10 days post-inoculation. Pathogenicity tests were performed twice under the same conditions. Symptoms identical to those of natural infections were observed on all inoculated plants but not on the negative controls. The isolates of *F. proliferatum* were reisolated from the inoculated plants and they were morphologically identical to the original isolate, thus fulfilling Koch's postulates. To our knowledge, this is the first report of *Fusarium proliferatum* on *C. arietinum* in Cuba.

## Acknowledgements

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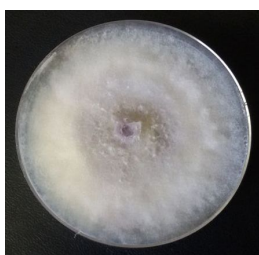


Figure 1

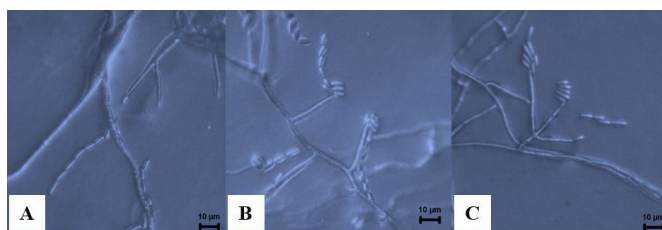


Figure 2

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