Diseases Caused by Fungi and Fungus-Like Organisms

Occurrence of *Fusarium proliferatum* Causing Vascular Wilt on Cowpea (*Vigna unguiculata*) in Brazil

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Cowpea (*Vigna unguiculata* L. Walp.) is widely cultivated throughout the North and Northeastern regions of Brazil. Despite its adaptability to local environmental conditions, some diseases may limit its cultivation. In September 2017, cowpea cultivar BR-17 Gurguéia plants exhibiting root rot, vascular bundle darkening, and wilt symptoms were collected from a field located in Areia county, Paraíba state, Brazil (6°58'12" S, 35°42'15" W). About 14% of plants in the field were symptomatic. Infected roots and vascular bundle fragments from diseased samples were surface sterilized with a sodium hypochlorite (1%) solution for 2 min, rinsed multiple times with sterile water, plated on potato dextrose agar (PDA) medium, and incubated at $25 \pm 2^{\circ}$ C for 7 days. By a single-spore isolation technique, a set of 10 isolates produced floccose and violaceous colonies. Macroconidia were sparse, slightly falcate with three to five septa, and measured 24.9 to 38.3×2.1 to $4.2 \,\mu$ m. Elliptical to oval shaped microconidia were produced in short chains from

monophialides and polyphialides and measured 4.5 to 9.2×1.4 to $3.4 \mu m$. No chlamydospores were observed. These morphological features match those of Fusarium proliferatum (Leslie and Summerell 2006). In order to validate the fungus's identity, representative isolate FC-01 was prepared for PCR amplification and partial sequencing of the translation elongation factor gene (TEF-1a; GenBank accession no. MW655796). A query of this sequence against the Fusarium-ID database (Geiser et al. 2004) showed 100% similarity (511/511) with F. proliferatum (MN784814.1). Phylogenetic analysis based on maximum likelihood was performed using RAXML-HCP2 v.8.2.12 and grouped the isolate with other F. proliferatum sequences with a high bootstrap value (98%). We also performed a pathogenicity test following the method described by Sousa et al. (2008). In summary, isolate FC-01 was grown on PDA amended with mannitol to adjust the osmotic potential to -1.0 MPa. Cultures were incubated at $25 \pm 2^{\circ}$ C for 7 days, and then 100 sterile cowpea seeds of BR-17 Gurguéia were placed on them. Seeds distributed over the PDA + mannitol without the fungus were used as a control. After 48 h of exposure, the seeds were sown into 250-ml plastic cups filled with sterile substrate and maintained under greenhouse conditions. Sixteen days after inoculation (DAI) the plants began to show gradual yellowing and wilting symptoms, and a stalk section at 36 DAI revealed darkened vascular bundles. In contrast, plants from the control group remained healthy. This test was repeated twice and F. proliferatum was reisolated from the roots and vascular bundle of cowpea seedlings, thus confirming Koch's postulates. Vascular wilt is one of the most important fungal diseases of cowpea (Shrestha et al. 2016). Considering that F. proliferatum is a cosmopolitan fungus that affects a wide host range (Yan 2020), its occurrence on cowpea fields may cause both yield and economic losses for Brazilian farmers. Therefore, precise identification of F. proliferatum is needed to develop effective management strategies. To the best of our knowledge, this is the first report of F. proliferatum causing vascular wilt in cowpea plants in Brazil.

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