Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium equiseti* Causing Wilt of *Cajanus scarabaeoides*, a Wild Relative of Pigeonpea, in India

Raj K. Mishra,^{1,†} Monika Mishra,¹ Abhishek Bohra,¹ Sathesh Naik SJ,¹ P. R. Saabale,¹ Krishna Kumar,¹ Prakash G. Patil,¹ D. K. Srivastava,² and N. P. Singh¹

¹ICAR-Indian Institute of Pulses Research, Kanpur, India

²Council of Science and Technology, Lucknow, Uttar Pradesh, India

Current address of P. G. Patil: ICAR-National Research Centre on Pomegranate, Solapur, India.

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Wild species or crop wild relatives provide an opportunity to introduce novel traits and expand the genetic base of cultivated pigeonpea (Bohra et al. 2020). Among its wild relatives, *Cajanus scarabaeoides* is cross-compatible with cultivated pigeonpea (*C. cajan*). To identify resistant sources for use in breeding, a study was conducted using 79 wild pigeonpea accessions at ICAR-Indian Institute of Pulses Research, Kanpur, India, in 2016–17 and 2017–18. The accessions belonged to *Cajanus*, *Rhynchosia*, and *Flemingia*. During field scouting, seedlings were observed with foliar chlorosis and wilting. Infected stem tissue exhibited brown to black discoloration, followed by gradual plant drying, and ultimately plant death. Infected plants were collected, and pathological examination was performed. Wilted plant parts were surface disinfected with 1% NaOCl for 2 min, and 5.0-mm pieces of stem tissue were transferred to Petri dishes containing 90 mm of *Fusarium* specific medium (FSM) (Nash and Snyder 1962) and incubated at 27°C. After 48 h of incubation, white to orange aerial mycelial growth was observed. The fungus was transferred to fresh FSM and purified by the single-spore technique (Choi et al. 1999). Macroconidia had four to six septa, slightly curved at the apex, and 20.0 to 25.0 × 3.0 to 5.5 µm. Microconidia were absent. The isolated fungus was putatively identified as belonging to the *Fusarium equiseti* species complex based on colony morphology and macroconidia characteristics (Leslie and Summerell 2006). A pathogenicity test was conducted on 15-day-old healthy wild pigeonpea seedlings using root dip inoculation and soil inoculation (Haware and Nene 1994). Plant roots were immersed in a 6 × 10⁶ conidia/ml suspension for 3 to 4 min (Marley and Hillocks 1996); roots of control plants were immersed in sterilized distilled water. A single-spore culture of *F. equiseti* was grown on PDA-containing Petri dishes. Two actively grown mycelia discs (5 mm diameter) from the periphery of

7-day-old pure culture of *F. equiseti* were separately inoculated in 500-ml conical flasks containing 100 g of pigeonpea meal medium. The flasks were incubated at 28 ± 2°C for 10 days. A fungus/soil mixture was prepared by mixing 200 g of inocula with 2 kg of autoclaved sand/soil mixture (3:7). Earthen pots (15-cm diameter) were sterilized by formalin (0.1%). Pots were then filled with fungus/soil mixture. Seeds sterilized with HgCl₂ (1%) were sown in each pot. Seeds sown in uninoculated pots served as a control. Five seeds were sown in each pot with three replications. Disease symptoms developed 10 days after inoculation of wild pigeonpea plants in the greenhouse. Symptoms were identical to those observed in the field. No symptoms were observed in the control. Reisolating the *F. equiseti* pathogen from the inoculated wild pigeonpea seedlings corroborated Koch's postulates. Reference cultures of three isolates were deposited in the Indian Type Culture Collection, ICAR-Indian Agricultural Research Institute, New Delhi, under ITCC8413, ITCC8414, and ITCC8415. Fungal genomic DNA was extracted through a modified CTAB method (Murray and Thompson 1980). The ITS regions 1 and 2, including 5.8S rDNA region, and part of translation elongation factor 1-α (TEF) were amplified by using the ITS6F and ITS4R and *tef* (F: ATGGGTAAGGAAGACAAGAC; R: GGAAGTACCAGTGAATCAT GTT) primers. BLASTn analysis of the sequences generated showed 98.78% homology with *F. equiseti*. The sequences were deposited in GenBank (ITS: MF351849, MF351850, MF351851; TEF: MK259963, MK264345, MK264346). Phylogenetic analysis of the ITS and TEF sequences revealed that all *Fusarium* isolates belong to the *F. equiseti* species complex and other available sequences of *Fusarium* spp. Occurrence of *F. equiseti* on various plant species is reported worldwide (Liang et al. 2011; Prasad et al. 2017; Ramchandra and Bhatt 2012). This is the first report of wilt disease on wild pigeonpea in India, caused by *F. equiseti* (Corda) Sacc.

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[†]Indicates the corresponding author.
Raj K. Mishra; rkmishraiipr@gmail.com