Diseases Caused by Oomycetes

First Report of Root Rot and Damping-Off Disease in Soybean (*Glycine max*) Caused by *Pythium deliense* in India

Sanjeev Kumar,^{1,†} Laxman S. Rajput,¹ Rajkumar Ramteke,² Vennampally Nataraj,² Milind B. Ratnaparkhe,² Hemant S. Maheshwari,¹ and Maranna Shivakumar²

¹Division of Plant Protection, ICAR-Indian Institute of Soybean Research, Indore 452001, India

²Division of Crop Improvement, ICAR-Indian Institute of Soybean Research, Indore 452001, India

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In India, soybean (*Glycine max* [L.] Merr.) is an essential oilseed crop whose production is limited by several biotic stresses (Nataraj et al. 2020). During the 2019 rainy season, root rot and seedling rot were observed at the Research Farm of ICAR-Indian Institute of Soybean Research, Indore, India. Infected seedlings had water-soaked lesions on the cotyledons and hypocotyls that gradually developed into brown lesions and further progressed to soft rot. Seedlings could be easily pulled from the soil. Diseased seedling samples were rinsed thoroughly in flowing tap water and then double-distilled water (DDW) and were surface sterilized with NaOCl (1%). Samples were washed thrice with sterilized DDW. Root fragments were sterilized and placed on V8 juice agar and PDA plates. Plates were incubated at 27 ± 2°C for 48 h. After incubation, white fluffy mycelial growth was observed on both media. The oomycete was observed to produce brown round vesicles with mycelial attachment when observed under a compound microscope magnification of 20×. Subcultures of these isolates were placed on PDA and incubated for 7 days at (27 ± 2°C). The pure culture along with PDA was cut into small pieces and mixed with sterilized soil (70% soil, 20% sand, 10% vermicompost) at one Petri dish per pot (plastic pots of 10 cm depth) and covered with tin foil. Pots were subjected to substrate colonization for 10 days at room temperature, and the substrates were shaken occasionally to improve infection efficiency by enhancing inocula production. Seeds of soybean variety Gaurav were sown in three replicates, each with 10 seeds in the inoculated pots. The control was seeds sown in the soil mix amended with plain PDA. Pots were maintained at 25 to 30°C with 45 to 50% soil moisture content under glass-house conditions. In the inoculated pots, the pathogen killed soybean seeds

before and after germination. Some plants that emerged developed lesions that were initially yellow and gradually turned necrotic. Lesions were found on the roots of the plant and at the base of the hypocotyl region. Seeds planted in uninoculated soil emerged but did not develop necrotic lesions. The causal organism reisolated from the diseased plant was morphologically and culturally similar to the original culture. The pathogen was classified as *Pythium deliense* based on morphological and cultural characters and the pathogenicity test (van der Plaats-Niterink 1981). To confirm identity, complete genomic DNA was extracted using the HiPurA Fungal DNA Purification Kit (HiMedia, India). The nuclear rDNA region of the internal transcribed spacer and 5.8S rDNA was amplified by universal primers ITS 1 and ITS 4 (White et al. 1990). Amplification was performed in a 12.5-µl reaction volume containing 1.5 µl of 10× PCR buffer, 3 µl of 25 mM MgCl₂, 1.2 µl of 2.5 mM dNTPs, 0.7 µl of 10 pM each primer (ITS 1 and ITS 4), 1 µl of DNA template, and 0.3 µl of 1 U Taq DNA polymerase. The thermal cycle consisted of 4-min initial denaturation at 94°C, followed by 35 cycles of 1-min denaturation at 95°C, 30-s primer annealing at 57°C, 1-min extension at 72°C, and a final 10-min extension at 72°C. PCR products were sequenced and submitted to NCBI (GenBank MT2665888). BLAST of the isolate showed 100% similarity with reference sequences of *P. deliense* (MT126658.1) in GenBank. The isolate was identified as *P. deliense* on the basis of molecular analysis, corroborating the above morphological identification. The beta-tubulin gene (Bt) was amplified with primers BtF and BtR and the cytochrome oxidase (COI) gene with COXF and COXR. The PCR products were sequenced and submitted to GenBank with accession numbers MW196444 and MW196445, respectively. In BLAST analysis, Bt and COI exhibited 100% sequence homology with *P. deliense* (MK752986.1 and HQ708566.1, respectively). *P. deliense* has been recorded worldwide causing disease in many agricultural crops including soybean, but this is the first study in India of the genus *Pythium* and *P. deliense* causing root rot and damping-off of soybean.

References:

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[†]Indicates the corresponding author.
S. Kumar; sanjv007@gmail.com