First Report of Pilidiella granati Causing Dieback and Fruit Rot of Pomegranate (Punica granatum) in Iran

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Iran is the largest producer of pomegranate (Punica granatum) in the world, with more than 60,000 ha currently in production. In the spring of 2011, a decline and dieback of young pomegranate trees (7 to 10 years old) were observed in the Kheir area of Fars Province. Dieback and twig blight developed toward the lower part of the stem, resulting in death of aerial tree parts and growing suckers from roots. Surface-disinfected tissues of diseased plants were plated on potato dextrose agar (PDA) and malt extract agar media. Isolates were separated into two groups that had either pale green or white aerial mycelia and sporulated after 5 to 7 days at 25°C. Pycnidia were globose and black with thin, membranous, pseudoparenchymatic walls, 80 to 140 µm in diameter. Conidia were hyaline, one-celled, elongate to fusiform, straight, and 11 to 17 × 4 to 6 µm (average 14 × 4.7 µm). Cardinal minimum growth temperatures were 8 to 10°C, optimum at 27 to 30°C, and maximum at 35°C. Radial growth rate at 30°C was 8 to 9 mm per day. Representative isolates were deposited in the CBS-KNAW Fungal Biodiversity Centre, the Netherlands (CPC 19625 = CBS 130974 and CPC 19626 = CBS 130975; GenBank JN815312 and JN815313, respectively). Genomic DNA was extracted with the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA) and the internal transcribed spacer (ITS) region of the nrDNA operon of two isolates were sequenced as described previously (1). On the basis of morphology (3), the causal organism was identified as Pilidiella granati Sacc. This identification was corroborated by the ITS sequence data, which was identical for both colony types to GenBank HQ166057 (identities = 614 of 614 [100%]). Pathogenicity tests were conducted using two representative isolates from each group on 5-month-old P. granatum trees with 10 replicates under greenhouse conditions; 5-mm mycelial plugs from the edge of 7-day-old colonies on PDA were placed under the bark of twig wounds. Uncolonized PDA plugs were used as noninoculated controls. Pathogenicity was also tested on nonwounded fruit by placing colonized 5-mm-diameter mycelial plugs on surface-disinfected pomegranate fruits; noncolonized PDA plugs were used as controls. All treated fruit were placed in plastic bags and maintained at 25°C for 10 days. Isolates were found to be pathogenic on twigs after 2 months, giving rise to brown lesions that were 2 to 5 cm long. No lesions
were observed on the controls. Furthermore, the fungus was reisolated from all infected tissues, satisfying Koch's postulates. On pomegranate fruit, the fungus colonized the fruit after 5 to 8 days, followed by the appearance of fruit rot symptoms leading to the formation of abundant pycnidia covering the skin after 10 days. No decay was observed in control inoculations. Pilidiella granati has previously been reported as a pathogen of *P. granatum* fruit from Europe, Asia, and the United States (2). To our knowledge, this is the first report of this pathogen causing dieback and fruit rot of pomegranate in Iran.