Disease Note

Diseases Caused by Bacteria and Phytoplasmas

First Report of *Ralstonia pseudosolanacearum* Causing Wilt Disease in Tomato (*Solanum lycopersicum*) Plants in Mexico

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Mexico produces more than four million tons of tomato (*Solanum lycopersicum* L.) fruits annually and is ranked 10th worldwide in tomato production. In February 2022, tomato plants grown in a greenhouse in Culiacan, Sinaloa State, were affected by wilt disease with an incidence of 20%, and irreversible wilting and death of the infected plants (with disease severity up to 70%) were also observed. Upon dissection, infected plants exhibited reddish to brown discoloration of the vascular tissues. Stem samples were dissected, disinfected with 1% NaClO for 5 min, and then kept in a humid chamber. Characteristic milky-white stem exudates were obtained. From the exudate, irregular, mucoid, white colonies with pink centers were obtained on casamino peptone glucose plates supplemented with 1% 2,3,5-triphenyl tetrazolium chloride (TZC); these morphological characteristics are typical of the *Ralstonia solanacearum* species complex (RSSC) (Garcia et al. 2019). Molecular identification of the pathogen was done by PCR using specific primer pairs reported by Paudel et al. (2022): RssC-wF3 (5′-TATATATCCTCGACTTTTCCATGAAGCTGTG-3′)/RssC-wR3 (5′-CTATATATATACCCCACTTGTTGAGGAACTG-3′) and Rpseu-wF5 (5′-TTTTATTTTTTTGGTGTCCGGGCCAAGATAG-3′)/Rpseu-wR5 (5′-TTATATTACTCGAACGTGCTGCAAAACCACT-3′), which amplified fragments of 162 and 251 bp for RSSC and *R. pseudosolanacearum*, respectively. Additionally, 759 (5′-GTCGCCGTCAACTCACTTTCC-3′)/760 (5′-GTCGCCGTCAGCAATGCGGAATCG-3′) (Opina et al. 1997) and Nmult21:1F (5′-CGTTGATGAGGCGCGCAATTT-3′)/Nmult22:RR (5′-TCGCTTGACCCTATAACGAGTA-3′) (Fegan and Prior 2005) were used to generate 282- and 144-bp amplicons for RSSC and phylotype I, respectively. Then, the representative strain ClnMx was used to generate a sequence for the endoglucanase (*egl*) gene for separation into sequevars by using the primers Endo-F (5′-ATGCATGCCGCTGGTCGCCGC-3′) and Endo-R (5′-GCGTTGCCCGGCACGAACACC-3′), which amplified a fragment of 750 bp (Fegan et al. 1998). The *egl* sequence (GenBank ON542479) showed 100% identity with the well-defined *R. pseudosolanacearum* sequevar 14 (UW763, I-14, GenBank CP051174), which was isolated from tomato plants from Senegal (Steidl et al. 2021), as well as with the strain MAFF 301070 (GenBank AB508612), which was isolated from tomato plants from Japan. For testing pathogenicity, four 1-month-old tomato plants were inoculated with a pure bacterial suspension (approximately 2×10^8 CFU/ml) using a sterile syringe. For each plant, 20 µl of the suspension was infiltrated into the axil of the third upper leaf, and for untreated controls, tomato plants were infiltrated with sterile water. All plants were kept at 28°C under greenhouse conditions. Six days after inoculation, inoculated plants exhibited symptoms resembling those observed in the field, and the plant pathogen was recovered on the TZC medium. To confirm the identity of the pathogen, PCR was conducted using the aforementioned primer pairs. In contrast, water-treated control plants remained healthy. Koch's postulates were fulfilled twice with similar results. RSSC causes severe economic losses in many countries because of its ability to infect a wide range of host plants, including potato, tomato, eggplant, tobacco, and banana. *R. pseudosolanacearum* has been reported to cause tomato wilt disease mainly in the Afro-Eurasian countries such as Senegal, Cambodia, and Japan (Klass et al. 2019). To our knowledge, this is the first report of *R. pseudosolanacearum* causing bacterial wilt disease on tomato in Mexico. As the control of *R. pseudosolanacearum* is challenging because of its long survival time in soil, water, and infected plant tissues, the identification of this important pathogen could provide relevant information for developing management strategies.

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