Diseases Caused by Bacteria and Phytoplasmas

First Report of Black Spot Disease Caused by *Xanthomonas arboricola* pv. *pruni* on Plumcot in South Korea

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Plumcot is an interspecific hybrid product between Japanese plums (*Prunus salicina*) and apricots (*Prunus armeniaca*) obtained by the National Institute of Horticultural and Herbal Science, Korea, in 1999 (Jun et al. 2009). In early 2017, black spot-like symptoms were observed on plumcot fruits and leaves at cultivation areas in Naju (34.965595, 126.665853) province. Further investigation showed that approximately 60% of the plumcot leaves in the affected orchard were infected, which caused 40% total production loss. At the early stage of infection, disease symptoms appeared as small, angular, and water-soaked spots and developed into circular brown spots at the later stages of infection. As the disease progressed, the leaf tissues around the spots became yellow and the lesions enlarged. When the adjacent lesions merged and the necrotic tissues fell off, shot-hole symptoms appeared on the leaves. To identify the causal agent of this disease, infected leaf tissues were excised and surface sterilized with 1% NaOCl for 30 s prior to rinsing thrice with sterile water. Tissue samples were then placed in sterile water (0.5 ml) for 5 min before its aliquots were streaked onto Luria-Bertani (LB) agar. Plates then were incubated at 28°C. To obtain pure colonies, bacteria were restreaked onto a new LB agar, and colonies showing typical *Xanthomonas* spp. morphology (i.e., convex, smooth, yellow, and mucoid) were subjected to Gram staining assay. For molecular identification, 16S ribosomal DNA (*16S-rDNA*) and gyrase B (*gyrB*) genes were amplified using a 9F/1512r and UP-1/UP-2Sr primers (Hansen et al. 1998; Yamamoto and Harayama 1995), respectively,

from five gram-negative isolates. PCR products were sequenced and analyzed using BLASTN. Result shows that *16S-rDNA* and *gyrB* genes are 99 to 100% identical to a similar genomic region of *Xanthomonas arboricola* pv. *pruni* (*Xap*) isolated in almond (MK156163), peach (MG049922), and apricot (KX950802), respectively (Gerin et al. 2019; Schwarczinger et al. 2017, 2018). *16S-rDNA* and *gyrB* gene sequences were deposited in GenBank (LC485472 and LC576824), and pathogen isolate was deposited into the Korean Agricultural Culture Collection (KACC19949). Pathogenicity testing was performed using *Xap* bacterial suspension (10⁸ CFU/ml) inoculated on the abaxial and adaxial surface of plumcot detached leaves. For inoculation, 10 healthy young leaves were used, and five young leaves mock inoculated with sterile LB broth were used as a control. Both leaf samples were kept in a closed container to maintain 100% humidity before being incubated at 25°C. The water-soaked symptoms were observed visually on the inoculated leaves 2 to 3 days postinoculation. No water-soaked symptoms were observed on the control leaves. Morphology and sequences of molecular markers used showed that the three bacterial colonies reisolated from the inoculated leaves were identical to the original isolate, fulfilling Koch's postulates. Pathogenicity tests were repeated twice, and the results obtained were consistent with the first experiment. As a new variety of stone fruit cultivated in Korea, information about pathogens and registered agrochemicals to control disease outbreak in plumcot are still limited. Therefore, the identification of *Xap* as a causal agent of black spot disease is critical for the development of disease management strategies and to identify appropriate agrochemicals to control the occurrence of this disease in the field. To our knowledge, this is the first report of *Xap* as a causal agent of shot-hole disease on plumcot in Korea.

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