Diseases Caused by Bacteria and Phytoplasmas

First Report of *Agrobacterium tumefaciens* Causing Crown Gall Disease of Roselle (*Hibiscus sabdariffa*) in Taiwan

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Roselle (*Hibiscus sabdariffa* L.) plants, whose calyces are used for production of beverages or jams, are mainly cultivated in Taitung County of eastern Taiwan. Since 2016, large crown galls were observed on the roselle plants in the commercial plantations at Taimali and Jinfong Townships of Taitung County. A follow-up survey in July and August of 2017 revealed spreading of this disease to the neighboring areas including Beinan and Dawu Townships. Disease incidence was estimated to be 0.6 to 10%. Galls of varying sizes (2 to 15 cm in diameter) were usually found on the roots and crowns of the roselle plants, starting with small swellings at the infection sites. Galls were light-colored and smooth and tender in texture at the early stage, but they later turned dark-colored and appeared rough and woody. In some cases, adventitious roots extruding from the larger crown galls could be seen. Isolation of the causal agent was performed by quadrantally streaking bacterial suspension made from surface-sterilized, macerated galls on trypticase soy agar (TSA). After incubating at 28°C for 5 days, single colonies were transferred onto new TSA plates for further cultivation at 28°C. Finally, circular, convex, viscous, and milky white colonies with a smooth surface similar to colony morphology of *Agrobacterium tumefaciens* C58 were obtained for further identification. First, all six candidate isolates (TZ-1, TL1-2, TL2-1, TD1-1, TD1-24, and TD2-1) were identified as *Agrobacterium* spp. using the

partial sequences of the 16S rRNA gene (accession nos. MW205820 to MW205825 in the GenBank database). The selected isolates also showed some biochemical and physiological characteristics similar to *A. tumefaciens*, including oxidase positive, growth at 35°C and in 2% NaCl, and alkalinity from litmus milk. Moreover, they tested negative for utilization of citrate and acid production on potato dextrose agar supplemented with calcium carbonate (Young et al. 2015). Under a transmission electron microscope, the bacterium was rod-shaped and possessed peritrichous flagella. By means of multiplex PCR using primers designed for differentiation of *Agrobacterium rubi*, *Agrobacterium vitis*, and *Agrobacterium* biovars 1 and 2 (Puławska et al. 2006), a 184-bp product was detected in all six isolates, indicating that they all belong to *Agrobacterium* biovar 1. Furthermore, the *recA* allele of each isolate was PCR amplified using primers F2898/F2899 (Costechareyre et al. 2010), and *recA* sequence analysis assigned all six isolates to *A. tumefaciens* genomospecies G7 (GenBank accession nos. MZ570905 to MZ570910). A pathogenicity assay was carried out by inoculating the stems of 2-week-old roselle seedlings through wounds made with a sterile needle with bacteria on it. The inoculated seedlings were kept in plastic bags to maintain high humidity. Symptoms similar to those observed in the field developed at the inoculation sites after 7 days, and Koch's postulates were fulfilled when the bacteria reisolated from the galls were also identified as *A. tumefaciens* genomospecies G7 using *recA* gene sequence analysis. To our knowledge, this is the first report of crown gall disease caused by *A. tumefaciens* on *H. sabdariffa* in Taiwan. This disease may potentially damage the roselle industry if no action is taken to stop its spreading. Identification of the causal agent of roselle crown gall disease could help us further investigate its ecology and develop integrated pest management strategies for prevention of this disease in the future.

References:

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