Diseases Caused by Bacteria and Phytoplasmas

First Report of Crown Gall Disease Caused by *Agrobacterium radiobacter* on Olive Plants in Layyah, Punjab, Pakistan

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Crown galls were observed on 1-year-old olive plants (*Olea europaea* cv. Manzanilla) in the District Layyah (30.9693° N, 70.9428° E) of Punjab, Pakistan. Large tumors were evident in the collar region, causing stunted growth, leaf yellowing, and overall plant dieback. A total of 900 olive plants were grown, including 300 young plants, in a 5-ha orchard. Around 25% of the young plants in the orchard had gall formation of varying size (2 to 15 cm); the majority of infected plants were grown near the water channel, where soil moisture levels were high (90 to 100%). Other olive orchards in the same area have not had a crown gall problem. Tumorigenic strains of bacteria can cause crown gall on plants (Kuzmanović et al. 2015). This study aimed to determine the pathogen of disease. Randomly collected samples were rinsed with tap water and galls were sterilized with 10% sodium hypochlorite solution for 1.5 to 3.0 min, washed with sterilized distilled water (SDW), chopped, and immersed overnight in SDW at room temperature. Isolations were carried out by plating the internal gall tissues on fresh Luria Bertani agar (LBA) supplemented with natamycin. After incubating at 28°C for 5 days, 10 single colonies were transferred to new LBA plates for further cultivation at 28°C. After 48 to 72 h, three strains showed white to cream-colored, smooth, convex, glistening, circular with entire edges, and mucoid

bacterial colonies resembling *Agrobacterium* spp. These three strains (BAT01, BAT02, BAT03) also showed biochemical and physiological characteristics similar to *A. radiobacter*, including oxidase positive, growth at 35°C and in 2% NaCl, and alkalinity from litmus milk. They tested negative for utilization of citrate and acid production on potato dextrose agar supplemented with CaCO₃ (Young et al. 2015). Amplification and sequencing of the three strains' 16S rRNA region and chromosomal *recA* gene with the universal primers rD1/rP2 and F2898/F2899 verified the identification at species level (Weisburg et al. 1991). BLAST analysis revealed 100% identity for the 16S rRNA and *recA* gene between the olive crown gall strains. Accession numbers of deposited sequences are given in the supplementary table. Reference sequences of *A. radiobacter* had GenBank accession numbers of AB247615 for 16S rRNA and KY913787 for *recA*. Phylogenetic analysis based on 16S rRNA and the chromosomal *recA* gene of the strains from the crown gall and reference strains of various species of *Agrobacterium* using the maximum-likelihood method with Tamura's three-parameter model in the MEGA X software program confirmed the strain from olive was *A. radiobacter* (*Agrobacterium* genomic species G4). The crown part of plants was inoculated through wounding with sterile needles plunged into young (2 to 3 day) bacterial culture (10⁷ CFU/ml) and SDW and was screened for pathogens on 10 1-year-old olive plants cv. Manzanilla. Plants were grown at 23 ± 3°C, and tumor formation was observed 4 weeks after inoculation. Typical tumors formed, no symptoms were found in control plants at inoculation sites, and Koch's postulates were fulfilled with reisolation and amplification of the *recA* gene region of bacteria. These data show that *A. radiobacter* causes crown gall in olive plants. Although this has been reported previously in different olive-growing regions of the world, this is the first report in Layyah, Punjab, Pakistan.

References:

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The author(s) declare no conflict of interest.

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