Disease Notes

First Report of *Agrobacterium vitis* as the Causal Agent of Grapevine Crown Gall in Serbia

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Abstract

In November 2010, a serious outbreak of crown gall disease was observed on 3-year-old grapevine (*Vitis vinifera* L.) cv. Cabernet Sauvignon grafted onto Kober 5BB rootstock in two commercial vineyards located in the South Banat District in Serbia. Large, aerial tumors were visible above the grafting point on grapevine trunks, and in most cases, the tumors completely girdled the trunk. From the gall tissues, white, circular, and glistening bacterial colonies were isolated on yeast mannitol agar medium. Eight, nonfluorescent, gram-negative, and oxidase-positive strains were isolated from seven tumor samples and selected for further identification. PCR assays with A/C′ (1) and VCF3/VCR3 (4) primers corresponding to the *virD2* and *virC* genes yielded 224- and 414-bp fragments, respectively, confirming that the strains harbored the plasmid responsible for pathogenicity. The strains were differentiated to the species/biovar level with a multiplex

PCR assay targeting 23S rRNA gene sequences (3) and were identified as *Agrobacterium vitis*. The 16S rDNA gene sequence from one isolate (GenBank Accession No. JN185718) showed 99% identity to the sequences of *A. vitis* previously deposited in NCBI GenBank database. The physiological and biochemical test results corresponded to the results of genetic analysis (2). The strains grew at 35°C and in nutrient broth supplemented with 2% NaCl. They were negative in 3-ketolactose, acid clearing on PDA supplemented with $CaCO_3$, and ferric ammonium citrate tests; nonmotile at pH 7.0; pectolytic at pH 4.5; utilized citrate; produced acid from sucrose and alkali from tartarate. Pathogenicity w confirmed by inoculation of three plants per bacterial strain on grapevine cv. Cabern Franc and on a local cultivar of tomato (*Lycopersicon esculentum* L.). The plants were inoculated on the stem by pricking one to three times through a drop of inoculum (10^8 CFU/ml) at three inoculation sites. Sterile distilled water was used as a negative control. Inoculated plants were maintained in a greenhouse at 24 ± 3°C. Typical tumors developed at the inoculation sites on tomatoes 3 weeks after inoculation and on grapevine 6 weeks after inoculation. No symptoms were observed on the control plants. Bacteria were reisolated from tumorigenic tissues and identified as pathogenic *A. vitis* by PCR. Crown gall disease was sporadically observed in vineyards in Serbia in previous years, but did not cause significant damage. Therefore, the causal agent was not studied in detail. To our knowledge, this is the first report of *A. vitis* determined as the causal agent of grapevine crown gall in Serbia.

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