Disease Note

OCCURRENCE OF GRAPEVINE VIRUS A IN WILD GRAPEVINES (*VITIS VINIFERA* subsp. *SYLVESTRIS*) IN TUNISIA

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Wild grapevine (*Vitis vinifera* subsp. *sylvestris*) populations exist in the northern mountain forests of Tunisia (Harbi Ben Slimane *et al*., 2010). These populations can be natural reservoirs of pathogens including viruses (Pacifico *et al*., 2016). *Grapevine virus* A (GVA) is the type member of the genus *Vitivirus* in the family *Betaflexiviridae*. GVA has a worldwide distribution, is associated with Kober stem grooving and Shiraz diseases (Minafra *et al*., 2017), and is widely distributed in Tunisian vineyards (Mahfoudhi *et al*., 1998). To study the presence of this virus in wild grapevines, a survey was carried out in the mountain forests of northern Tunisia in spring and autumn of 2015. Dormant canes from 84 accessions (male and female specimens) were collected and tested for the presence of GVA by RT-PCR using specific primers (MacKenzie, 1997) to amplify a 236 bp fragment of the coat protein gene. Results showed that 13% (11 of 84) of the tested samples were infected by GVA. To confirm the presence of GVA in wild Tunisian grapevines, RT-PCR amplicons from three positive accessions were sequenced and sequences were compared with those from GenBank. The nucleotide sequence identity of Tunisian GVA isolates VS15 (LT906663), VS16 (LT906664) and VS178 (LT906665) ranged from 92 to 96% among themselves and from 89 to 93% with other isolates for which information is available in GenBank. To our knowledge, this is the first report of GVA in wild grapevines in Tunisia.

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Disease Note

FIRST REPORT OF *PSEUDOMONAS AERUGINOSA* CAUSING INTERNAL BROWN ROT OF STORED ONION BULBS IN TAIWAN

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Onion (*Allium cepa* L.) may suffer from various bulb rot diseases during postharvest storage, including internal brown rot caused by *Pseudomonas aeruginosa* (Cother *et al*., 1976). Onion bulbs, stored at room temperature, were sampled in Chiayi city, Taiwan in July 2017. The bulbs were externally asymptomatic but symptoms of internal brown rot occurred in 6% of bulbs. Repeatedly a bacterium was isolated on nutrient agar from surface-sterilized tissue of diseased onion bulbs and fluoresced under UV light while cultured on King's B plates. Several isolates were purified and maintained on King's B plates, and produced fluorescence. Two isolates, OP01 and OP03, were stored in Luria-Bertani broth with 20% glycerol at −80°C. They were identified as *Pseudomonas aeruginosa* by API 20 NE system (Biomerieux) and phenotypically similar to a reference strain of *P. aeruginosa* (ATCC 27853). Furthermore, the 16S rDNA sequence of the strain OP01 was amplified using the primers fD1/rP1 (Weisburg *et al*., 1991) and sequenced (GenBank accession No. MF946565). Blastn analysis of 16S rDNA indicated that the onion-pathogenic strain was *P. aeruginosa* (100% identity to *P. aeruginosa* ATCC 27853, AB594760). For pathogenicity assay, surface-sterilized onion bulbs were injected with a bacterial suspension (Schwartz and Otto, 2000). *P. aeruginosa* ATCC 27853 was included as a control strain. After incubation at 28°C for 14 days in closed plastic bags in the dark, the artificially inoculated bulbs exhibited symptoms indistinguishable from those observed in natural infections, but not the control bulbs. The same fluorescent bacterium could be consistently re-isolated from the inoculated bulbs, fulfilling Koch's postulates. To our knowledge, this is the first report of *P. aeruginosa* causing internal brown rot of stored onion in Taiwan.

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