Diseases Caused by Viruses

First Report of *Garlic virus A*, *Garlic virus B*, and *Garlic virus C* on Garlic (*Allium sativum*) in Tunisia

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Mite-borne viruses in the genus *Allexivirus* (family *Alphaflexiviridae*) commonly occur on garlic (*Allium sativum* L.) in many parts of the world. They are usually asymptomatic and cause little damage to the plants, but often occur in mixed infection with potyviruses and carlaviruses, with synergistic effects reducing crop quality and leading to higher losses (Taglienti et al. 2017). Their occurrence on Tunisian garlic crops was studied here in the same garlic germplasm collection mentioned in a previous first report (Ayed et al. 2019). Leaf samples from 66 garlic accessions were tested by DAS-ELISA using specific antibodies (DSMZ, Germany) against garlic virus A (GarV-A), garlic virus B (GarV-B), and garlic virus C (GarV-C). These serological tests showed individual virus incidence of, respectively, 56.4, 67.7, and 10%. Our findings corroborate the results of Chodorska et al. (2012). In order to confirm the presence of these viruses, RT-PCR assays were performed using total RNAs extracted using two silica-capture extraction procedures according to Foissac et al. (2005) and specific primers targeting the coat protein genes of the various viruses. These primers, designed for the present study, are GarV-A-F (5'-YCTYTTCTCHYTDGCHTG-GACYTG-3') and GarV-A-R (5'-RCCYTTCTCCTAGACCARTTRGCRGG-3') for GarV-A; GarV-B-F (5'-TGGGCGYGTACCAACAAYGGATC-3') and GarV-B-R (5'-TCTGCGCGVGTGGADACCATRTT-3') for GarV-B; and GarV-C-F

(5'-ARGAYCTYTTYTCMCTYGCRTGGGC-3') and GarV-C-R (5'-GGAG-GYTCRTGAATYTGTTGTTG-3') for GarV-C. The viruses were detected by two-step RT-PCR as described by Marais et al. (2015). PCRs consisted of one cycle at 95°C for 5 min; followed by 40 cycles of denaturation at 95°C for 45 s, annealing at 45°C for 45 s, and elongation at 72°C for 45 s; and a final extension step at 72°C for 10 min. Products of the expected size (214 bp for GarV-A, 363 bp for GarV-B, and 439 bp for GarV-C) were amplified from 58 (88%), 47 (71%), and 56 (85%) accessions, respectively. Forty-three samples (65%) were coinfecting by the three viruses. Higher numbers of positives revealed by RT-PCR, especially in the case of GarV-C, may reflect the higher sensitivity and efficiency of this technique compared with ELISA. Direct sequencing of selected amplicons of the expected size obtained for GarV-A, -B, and -C Tunisian isolates was performed and the sequences submitted to GenBank, validating the specificity of the three RT-PCR assays. The two sequenced GarV-A isolates (MK599147 and MN995836) shared 98% nucleotide (nt) sequence identity with each other, and 93 to 94% identity with the closest isolate in GenBank, the G118 isolate from China (MN059320). The three sequenced GarV-B isolates (MN995829 to MN995831) shared 88 to 98% nt identity with each other. For GarV-B 18.1 (MN995830) and GarV-B 36.2 (MN995831) the closest isolate was 1109.1 (JX682828) from Spain (92 to 93% nt identity). For GarV-B 17.2 (MN995829), the closest isolate was B-Sp-3 (LC97167) from Spain (90% nt identity). The sequenced GarV-C isolate (MN995834) showed the highest sequence nt identity (93%) with the GarV-9 isolate (HQ724848) from Spain. To our knowledge this is the first report of the presence of GarV-A, -B, and -C in Tunisia. The presence of these allexiviruses may pose a threat to the preservation of the Tunisian garlic germplasm and, more broadly, to garlic production in Tunisia. For this reason, the scrupulous identification of viruses occurring in garlic plants will help to determine the appropriate strategy to decrease viral incidence in the garlic growing area.

References:

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