Diseases Caused by Viruses

First Report of Cucurbit Chlorotic Yellows Virus Infecting Cucumber Plants in Spain

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Plant Dis. 105:2258, 2021; published online as <https://doi.org/10.1094/PDIS-12-20-2553-PDN>. Accepted for publication 25 February 2021.

During the winter of 2018, symptoms of leaf chlorotic spots followed by symptoms of leaf interveinal chlorosis and severe chlorosis in basal leaves were observed in cucumber cultivar Laredo (*Cucumis sativus*) plants in three independent greenhouses, sited in distinct locations in southern Spain. In all cases, *Bemisia tabaci* populations were observed on infected plants. The symptomatology observed was similar to that caused by whitefly-transmitted *Cucurbit yellow stunting disorder virus* (CYSDV, genus *Crinivirus*, family *Closteroviridae*), which is usually found infecting cucumber plants in this geographical area (Rubio et al. 2001). Samples from four different cucumber plants of distinct greenhouses were collected and tested for the presence of CYSDV. Total RNA was extracted from the samples using the NucleoSpin RNA Plant kit (Macherey-Nagel, Germany). Molecular detection of CYSDV was performed using the multiplex and degenerate primer RT-PCR method (Wintermantel et al. 2010), specific to the region of the highly conserved RNA-dependent RNA polymerase (RdRp) gene of criniviruses, which also detects other criniviruses such as lettuce infectious yellows virus (LIYV) and beet pseudo-yellows virus (BPYV). Results indicated that the viral species CYSDV, LIYV, and BPYV were not detected in the four cucurbit plant

samples. In 2004, an emergent crinivirus (*Cucurbit chlorotic yellows virus*, CCYV), inducing symptoms similar to those caused by CYSDV, was described infecting cucurbits in Japan (Okuda et al. 2010). Recently, CCYV was detected in 2011 in Greece (Orfanidou et al. 2014) and in 2014 in Egypt (Amer 2015) and Saudi Arabia (Al-Saleh et al. 2015). Therefore, the four RNA samples were tested for the presence of CCYV by a RT-PCR method previously described (Hamed et al. 2011). Specific primers were designed to amplify 336 nt of the capsid protein (CP) gene and 680 nt of the RdRp gene, located on CCYV genomic RNA 1 and RNA 2, respectively. In all cases, clear cDNA bands of both expected sizes were detected for each cucumber sample, which were then purified and sequenced via Sanger technology. BLAST analysis of those sequences showed 99% identity with the nucleotide sequence of the CP and RdRp genes from the CCYV isolates from Greece (LT992911, LT992910), China (KY400633.1, KX118632), and Taiwan (JF502222). To our knowledge, this is the first report of CCYV infecting cucurbits in Spain. It is possible that CCYV has spread throughout the Mediterranean basin, remaining undetected due to the yellowing symptom similarities between CYSDV and CCYV. Detection of the emergent virus CCYV in Spain represents a new threat for the horticultural area of southern Europe.

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The author(s) declare no conflict of interest.

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Keywords: Crinivirus, cucumber, CCYV, epidemiology, disease development and spread, pathogen detection, whiteflies, vegetables, *Bemisia tabaci*

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