DISEASE NOTE

First Report of Cucurbit chlorotic yellows virus Infecting Cucumber and Zucchini in Algeria

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Cucurbit chlorotic yellows virus (CCYV), a member of the genus *Crinivirus* (family *Closteroviridae*), is a single-stranded, positive-sense plant RNA virus, composed of RNA1 and RNA2. The virus is transmitted by the whitefly *Bemisia tabaci* biotypes MEAM1 and MED in a semipersistent manner (Li et al. 2016). CCYV was first reported on melon plants in Japan in 2004 (Gyoutoku et al. 2009). Later, the viurs was reported on many other cucurbits as well as several non-cucurbit plant species in different countries including China, Taiwan, Sudan, Lebanon, Iran, Greece, Turkey, Egypt and Saudi Arabia (see Kawazu et al. 2018). Recently, CCYV was also reported in Israel (Luria et al. 2019) and in the New World, concretely infecting melon in California (Wintermantel et al. 2019). In 2018 and 2019, zucchini (*Cucurbita pepo*), cucumber (*Cucumis sativus*) and melon (*Cucumis melo*) plants showing virus-like symptoms were observed in northeast Algeria. The presence of whiteflies was observed in all of the investigated fields in the region. The observed symptoms, which included foliar yellowing and veing clearing (e-Xtra 1), were similar to those caused by whitefly transmitted viruses such as the begomovirus (family *Geminiviridae*) tomato leaf curl New Delhi virus (ToLCNDV), the ipomovirus (family *Potyviridae*) cucumber vein yellowing virus (CVYV), and the criniviruses (family *Closteroviridae*) CCYV and cucurbit yellow stunting disorder virus (CYSDV). To investigate the etiology of the disease, a total of 69 symptomatic leaf samples (43 from zucchini, 18 from cucumber and eight from melon) collected in field crops from four agricultural areas in the city of Biskra and its surroundings (Biskra, El ghrouss, Sidi okba and Zribat eloued), and 13 asymptomatic samples from pumpkin (*Cucurbita maxima*) collected from a field located in Sidi-Okba, were analysed by the tissue-printing method (Aparicio et al.

2009). Freshly cut leaf petioles were directly pressed onto nylon membranes and hybridized with individual riboprobes corresponding to the complete CP gene of the four whitefly transmitted viruses described above. Out of the 69 symptomatic samples, 58 were positive for ToLCNDV (38 from zucchini, 12 from cucumber and eight from melon) and five for CCYV (three from zucchini and two from cucumber) (e-Xtra 1), while the viruses CVYV and CYSDV were not detected in any of the analyzed samples. CCYV was detected in all cases in mixed infections with ToLCNDV, which was also recently reported for the first time in this area (Kheireddine et al. 2019). This mixed CCYV-ToLCNDV infection occurred in one sample from El ghrouss (of zucchini) and in four samples from Sidi-Okba (two of zucchini and two of cucumber). In order to confirm CCYV identification, total RNA extracts were obtained with TRIzol reagent (Thermo Fisher Scientific, Carlsbad, CA, USA) from the original zucchini (three) and cucumber (two) samples and from asymptomatic samples (two) and reverse transcribed using random primers. Based on the complete CCYV sequence (accession numbers AB523788 and AB523789 for RNA1 and RNA2, respectively) (Okuda et al. 2010), three pairs of primers were designed. RdRp-up (5´-CCTAATATTGGAGCTTATGAGTAC-3´)/RdRp-do (5´-CATACACTTTAAACACAACCCCCT) was expected to amplify a portion of the RNA dependent RNA polymerase (RdRp) region (754 bp) of RNA1; whereasHsp-up (5´-TGCGTATGTCAATGGTGTTATG-3´)/Hsp-do (5´-ATCCTTCGCAGTCAAAAACC-3´) and CP-up (5´-ATGGAGAAGACTGACAATAAAC-3´)/CP-do (5´-TTATTTACTACAACCTCCCGGTGC-3´) were anticipated to amplify, respectively, a portion of the heat shock protein 70 homologue (Hsp70h) region (462 bp) and the complete coat protein (CP) gene (753 bp) of RNA2. PCR products of the expected sizes were obtained from the five symptomatic samples, but not from the asymptomatic controls (e-Xtra 1). PCR fragments were directly sequenced in both directions by Sanger-sequencing at the Instituto de Biología Molecular y Celular de Plantas (Universitat Politècnica de València, Valencia, Spain). BLAST analysis indicated that the five symptomatic samples shared identical amplicon sequences, and therefore only the sequences of a CCYV isolate from zucchini (CCYV-Sidi) were deposited in GenBank under the accession numbers MN529558 (RdRp), MN529559 (Hsp70h) and MN529560 (CP). The RdRP, Hsp70h and CP sequences demonstrated higher than 99% nucleotide identity with the respective genes of CCYV isolates from Greece, Asia, Africa and California. To our knowledge, this is the first report of CCYV in Algeria.

CCYV could represent a serious threat for valuable cucurbit crops in this and other countries of Mediterranean basin. It is necessary to implement efficient control measures to prevent further spread of the virus and minimize yield losses.

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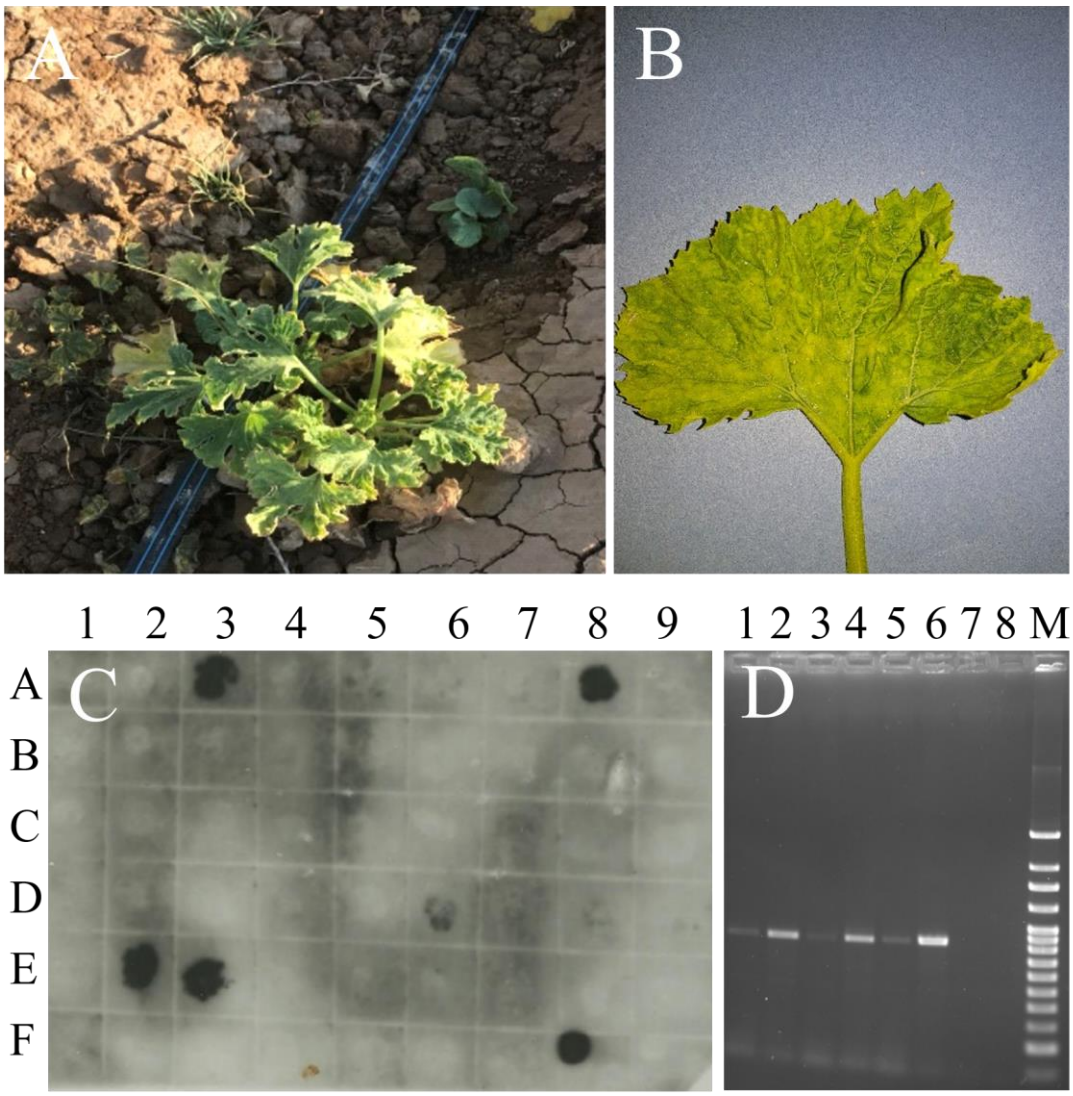

Figure S1. (A) Zucchini (Cucurbita pepo) plants showing virus-like symptoms. (B) Foliar yellowing and veing clearing on zucchini leaf doubly infected with the viruses: cucurbit chlorotic yellows virus (CCYV) and tomato leaf curl New Delhi virus. (C) Tissue-printing approach for the detection of CCYV. Freshly cut leaf petioles from cucurbit plants were directly pressed onto nylon membranes and hybridized with a riboprobe corresponding to the complete CP gene of the CCYV. Positive (F8) and negative (F9) controls were included. Samples A3, A8, D6, E2 and E3 were CCYV-infected. (D) RT-PCR detection of CCYV using primers CP-up/CPdo in the five plants (lanes 1-5) that were positive for CCYV by tissue-printing. Lane 6 positive control from a CCYV-infected plant and lanes 7-8 negative controls from asymptomatic plants. M, molecular size marker.