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Additional Information

First report of cucurbit chlorotic yellows virus infecting watermelon and zucchini in the Canary Islands, Spain

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In July 2019, eleven watermelon (*Citrullus lanatus*) plants, six ‘Augusta negra’ and five ‘Kasmira’, and seven zucchini (*Cucurbita pepo*) ‘Marcado’ plants showing yellowing and vein clearing on the leaves were collected in Arico, in Tenerife Island of the Canary Islands. Analysis with enzyme-linked immunosorbent assay (ELISA) with polyclonal antibodies using kits from (LOEWE® Biochemica GmbH, Sauerlach, Germany) detected cucurbit chlorotic yellows virus (CCYV) in four watermelon (two ‘Augusta negra’ and two ‘Kasmira’) plants and one zucchini plant. Reverse transcription-polymerase chain reaction (RT-PCR) with primers CCYV-CPs (5'-ATGGAGAAGACTGACAATAAACAA-3') and CCYV-CPas (5'-TTTACTACAACCTCCCGGTG-3') (Hamed et al. 2011) yielded 750 bp DNA fragments, which were purified, and directly sequenced by Sanger sequencing. The four CCYV sequences obtained from watermelon plants were identical and showed a 99.6% nucleotide identity with the CCYV isolate from Zucchini. The nucleotide sequences of CCYV isolates 351/19 from watermelon and 361/19 from zucchini were deposited in GenBank under accessions OK562588 and OK562589, respectively. BLASTn analysis showed high nucleotide identity, greater than 99 % with most worldwide CCYV isolates (AB523789, HM581658, JF502222, JF807053, JN126045-6, JQ904629, JX014262, KC990503-5, KC990507, KJ735450, KJ149806, KT946809, KT946816, KU507602, KX118632, KY400632-4, KY618799, LT716000, LT716003-4, MH477612, MH806868, MH819191, MN529560, MN815012-3, MT396249, MT048669, MW033301, MW584337, MW251342, MW521380, MW521381, MW584335-6, MW629380, MZ325848-9 and MZ405664), about 98% with two Saudi Arabian isolates (KT946810 and KT946815) and about 95 % with three Iranian isolates (KC577201-3). These genetic relationships were confirmed with a phylogenetic tree (Supplementary Fig. 1) inferred with the maximum likelihood method with 500 bootstrap replicates and the nucleotide substitution model HKY+G implemented in the program MEGA-X (Kumar et al., 2018). There is no correlation between geographic distance and genetic distance. CCYV is related to viruses in the genus *Crinivirus*, family *Closteroviridae* (Okuda et al., 2010). It is widespread and causes high economic losses (yield reduction and unmarketable fruits) in cucurbit crops from East Asia: Japan, China, Taiwan and South Korea;

North America (the USA states of California, Texas, Georgia and Alabama); Middle East: Sudan, Saudi Arabia and Iran; and the Mediterranean basin: Egypt, Israel, Lebanon, Turkey, Greece, Algeria and peninsular Spain (Kheireddine et al., 2020; Chynoweth et al., 2021; Jailani, et al., 2021; Kwak et al., 2021). This is the first report of CCYV infecting cucurbits in the Canary Islands. The emergence of this virus poses a threat to the cucurbit crops in the Canary Islands and requires control measures to prevent its spread in Tenerife and the other Canary Islands.

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Supplementary Figure 1. Unrooted maximum likelihood phylogenetic tree of the coat protein gene of cucurbit chlorotic yellows virus from different geographic origins and hosts. Branch lengths are proportional to the genetic distances and bootstrap values are indicated

