Diseases Caused by Viruses

First Report of Natural Infection of Zucchini by Tomato Chlorosis Virus and Cucurbit Chlorotic Yellows Virus in China

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Zucchini (*Cucurbita pepo*) is an extensively cultivated and economically important cucurbit crop in China. In September 2018 and 2019, interveinal chlorosis and yellowing symptoms, suspected to be caused by either tomato chlorosis virus (ToCV; genus *Crinivirus*) or cucurbit chlorotic yellows virus (CCYV; genus *Crinivirus*), or by their coinfection, were observed on zucchini in a greenhouse in Shandong Province, China. The incidence of the disease in the greenhouse was 20 to 30%. To identify the causal agents of the disease, leaf samples from 66 zucchini plants were collected in 14 greenhouses in the cities of Shouguang ($n = 12$), Dezhou ($n = 36$), Qingzhou ($n = 12$), and Zibo ($n = 6$) in Shandong. Four whitefly (*Bemisia tabaci*) samples and four symptomatic tomato samples were also collected from these sampling sites (one each for each site) because numerous whiteflies were observed in the greenhouses and ToCV was previously reported in greenhouse tomato plants from these regions (Zhao et al. 2014). To determine whether the symptoms were associated with *Crinivirus* infection, reverse transcription polymerase chain reaction (RT-PCR) using *Crinivirus*-specific degenerate primers (CriniRdRp251F/CriniRdRp995R) (Wintermantel and Hladky 2010) was performed on total RNA extracted using the TRIzol protocol (Jordon-Thaden et al. 2015). Then, the RNA samples were subjected to RT-PCR with ToCV- or CCYV-specific primers (Gan et al. 2019; Sun et al. 2016). Of the 66 zucchini samples, 54 tested positive by the degenerate crinivirus primer pair; among them, 10 tested positive for ToCV only, 40 positive for CCYV only, and 4 positive for both viruses. Interestingly, while both viruses were detected in all *B. tabaci* samples, only ToCV was detected in the tomato samples ($n = 4$). To

confirm the identity of the viruses, the amplicons of ToCV (four samples each of tomato, *B. tabaci*, and zucchini) and CCYV (four samples each of *B. tabaci* and zucchini) were Sanger sequenced (Tsingke Biotechnology Co., Ltd., Beijing, China) after cloning into pMD18-T vectors (Takara, Shiga, Japan). BLASTn analysis demonstrated that all sequences were identical to their respective amplicons. The ToCV sequences (GenBank accession nos.: tomato, MN9444406; *B. tabaci*, MN9444404; zucchini, MN9444405) shared 100% sequence identity with isolates from Beijing (KT751008, KC887999, KR184675, and KP335046), Hebei (KP217196), and Shandong (KX900412). The CCYV sequence (GenBank accession no. MT396249) shared 99.9% sequence identity with isolates from China (JN126046, JQ904629, KP896506, KX118632, KY400633, and MK568545), Greece (LT716000, LT716001, LT716002, LT716005, and LT716006), and Cyprus (LT992909, LT992910, and LT992911). To assess the transmissibility of ToCV and CCYV, virus-free *B. tabaci* ($n = 30$) were placed in ToCV or CCYV-infected zucchini for one day for virus acquisition. Then, the whiteflies were transferred to virus-free zucchini seedlings (cv. Zaoqingyidai, 4-leaf-stage, $n = 6$ for each of the control, ToCV, and CCYV treatment) for 1 day. Three weeks after inoculation, all plants that were inoculated with either ToCV or CCYV displayed the same symptoms as those observed in the greenhouses, and plants in the control group remained symptom free. RT-PCR analysis using ToCV- and CCYV-specific primers confirmed the infection of the plants with the respective viruses, and control plants were free from the viruses. CCYV has been previously reported on zucchini in Algeria (Kheireddine et al. 2020), Iran (LR585225), and Cyprus (LT992910). To our knowledge, this is the first report of CCYV infection in zucchini in China, and the first report of ToCV infection in zucchini in the world. Clearly, stringent management is needed to minimize the losses caused by these viruses in greenhouse operations in the region.

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