First Report of Cucurbit Chlorotic Yellows Virus from Cucumber Plants Affected by Intervetinal Yellowing Disease in Jordan

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In April 2017, virus-like symptoms, including chlorosis on the upper leaves and intervetinal chlorotic spots with brittleness on middle and lower leaves, were observed in cucumber cultivar Banan plants grown under three plastic houses in the Jordan River Valley, Jordan. Disease incidence ranged from 30 to 35%, and affected plants were infested by dense whitefly populations. The observed symptoms were similar to those caused by whitefly-transmitted viruses of the genera *Crinivirus* (family *Closteroviridae*) and *Ipomovirus* (family *Potyviridae*) and by the aphid-transmitted *Polerovirus* (family *Luteoviridae*). To identify the potential agent(s) causing these symptoms, 185 samples from symptomatic plants and 29 from asymptomatic ones were subjected to total RNA extraction using the SV-Total RNA Extraction kit (Promega, U.S.A.). The samples were tested by RT-PCR using species-specific primers and protocols (Boubourakas et al. 2006; Lotos et al. 2014; Orfanidou et al. 2014; Papayiannis et al. 2005). Cucurbit chlorotic yellows virus (CCYV) was detected in 152 of 185 symptomatic samples with primers targeting a fragment of the RNA-dependent RNA polymerase (RdRp) (Orfanidou et al. 2014), and cucurbit yellow stunting disorder virus and cucumber vein yellowing virus were detected in 48 and 10 samples, respectively. All 214 samples were negative for beet pseudo-yellows virus and cucurbit aphid-borne yellows virus, and no virus was detected in the asymptomatic plants. To confirm the presence of CCYV, eight selected RdRp-specific PCR amplicons (757 bp) were

purified and ligated into pGEM T-Easy Vector (Promega), and two clones from each product were sequenced (GenBank accession nos. MT248933 to 40). BLASTn analysis showed that the nucleotide sequences shared 93 to 99% identity to RdRp of 32 CCYV isolates from East Asia, the Middle East, North Africa, and the United States. Moreover, transmission of CCYV by *Bemisia tabaci* MEAM1 was carried out from singly infected cucumbers cultivar Banan. Approximately 1,000 whiteflies that were maintained on cucumber (cv. Beta) in isolated insect-proof cages were given an acquisition access period (AAP) of 48 h on cucumber plants singly infected by CCYV. Groups of 30 whiteflies per plant were transferred onto 27 healthy cucumber plants (cv. Cetiriole Marketmore) in insect-proof cages at the three- to four-leaf stage for an inoculation access period (IAP) of 48 h. A group of 180 whiteflies without an AAP were transferred onto six healthy cucumber plants of the same cultivar to serve as negative controls. After the IAP, all plants were treated with the insecticide Confidor, maintained in a growth chamber, observed weekly for symptom development, and tested by RT-PCR for the presence of CCYV 30 days postinoculation (dpi). Seventeen of the 27 test plants developed yellowing symptoms on lower leaves and tested positive for CCYV by RT-PCR at 21 dpi. The virus specificities of the amplicons were verified by sequencing. No symptoms appeared on the six control plants, which also tested negative for CCYV by RT-PCR. The presence of CCYV in Jordan may have serious epidemiological implications for the cultivation of cucurbits in Jordan and neighboring countries where the virus is prevalent (Abrahamian et al. 2012; Al-Saleh et al. 2014; Orfanidou et al. 2017). Further research is necessary to determine the incidence of CCYV in cucurbit and noncucurbit plant species in Jordan and consequently improve the knowledge about its epidemiology, including its natural host range.

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