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NEW DISEASE REPORT

First report of Cucumber vein yellowing virus in Iraq

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Courgette (*Cucurbita pepo*) is one of the main cucurbit crops grown in Iraq for local consumption. Viruses are a major threat to cucurbit production in Iraq, particularly whitefly-transmitted viruses (Mohammed et al., 2021). In the 2022 growing season, courgette plants with extensive leaf vein-yellowing symptoms (Figure 1), associated with whitefly infestation, were observed in fields around Al-Yusufiyah, Baghdad Province, Iraq. The disease incidence was 40–50%. Thirty leaf samples with vein-yellowing symptoms were collected randomly.

One sample was sent in RNAprotect Tissue Reagent (Qiagen, Germany) for high throughput RNA sequencing (RNA-seq) at Macrogen Inc. (Seoul, South Korea). Overall, 39,549,599 paired-end reads (151 bp length) were obtained. The reads were first aligned to the complete genome sequence of *Cucurbita pepo* via Bowtie2 (v. 2.4.5) to deplete host sequence. The Velvet assembler (v. 1.2.10) was operated for *de novo* assembly of the unmapped reads (Lahuf, 2021). The contigs produced were compared with plant virus sequences retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genome/viruses/) using BLASTn algorithms (Abass & Lahuf, 2023). The BLASTn analysis showed ≥99% pairwise nucleotide identity with the full genomes of five *Cucumber vein yellowing virus* (CVYV) isolates. Other viruses were also detected including *Squash leaf curl virus* (SqLCV) and *Tomato leaf curl Palampur virus* (ToLCPMV). Reference-based genome assembly was conducted using a sequence of CVYV (NC_006941.1). A total of 86,245 reads mapped to NC_006941.1 with 100% coverage and a depth coverage of 1.335× and BLAST analysis showed that the assembled genome of CVYV Iraq-1 shares 99.86% pairwise nucleotide identity with this isolate. The genome sequence of the CVYV isolate Iraq-1 was deposited in GenBank (Accession No. OQ685957.1). A phylogenetic analysis showed a close relationship between CVYV isolate Iraq-1 and isolates from diverse origins, particularly those from Spain (AY578085.1 and MK777994.1) and Portugal (MZ130935.1 and OK181771.1) (Figure 2).



FIGURE 1 Courgette leaves displaying vein-yellowing symptoms and confirmed to be infected with *Cucumber vein yellowing virus*

Total RNA was extracted from the thirty diseased courgette leaf samples, including the sequenced sample, using a Viral RNA/ Viral Nucleic Acid Mini Kit (Favorgen, Taiwan) and kept at -20°C for further analysis. RT-PCR was done using a OneStep RT-PCR kit (Qiagen, Germany) with CVYV-specific coat protein primers (CV+ and CV-) (Cuadrado et al., 2003). The amplicons were directly sequenced and CVYV infection was confirmed in all thirty leaves. Another fifty diseased leaf samples were collected randomly from different fields of courgette in Baghdad Province to confirm the presence of the virus in the region and 38 also tested positive using the CVYV-specific RT-PCR.

This is the first report of CVYV in Iraq. Additional work is needed to reveal the distribution and prevalence of CVYV, SqLCV and ToLCPMV and to determine their role in causing disease in courgette and other curcurbit crops in Iraq.

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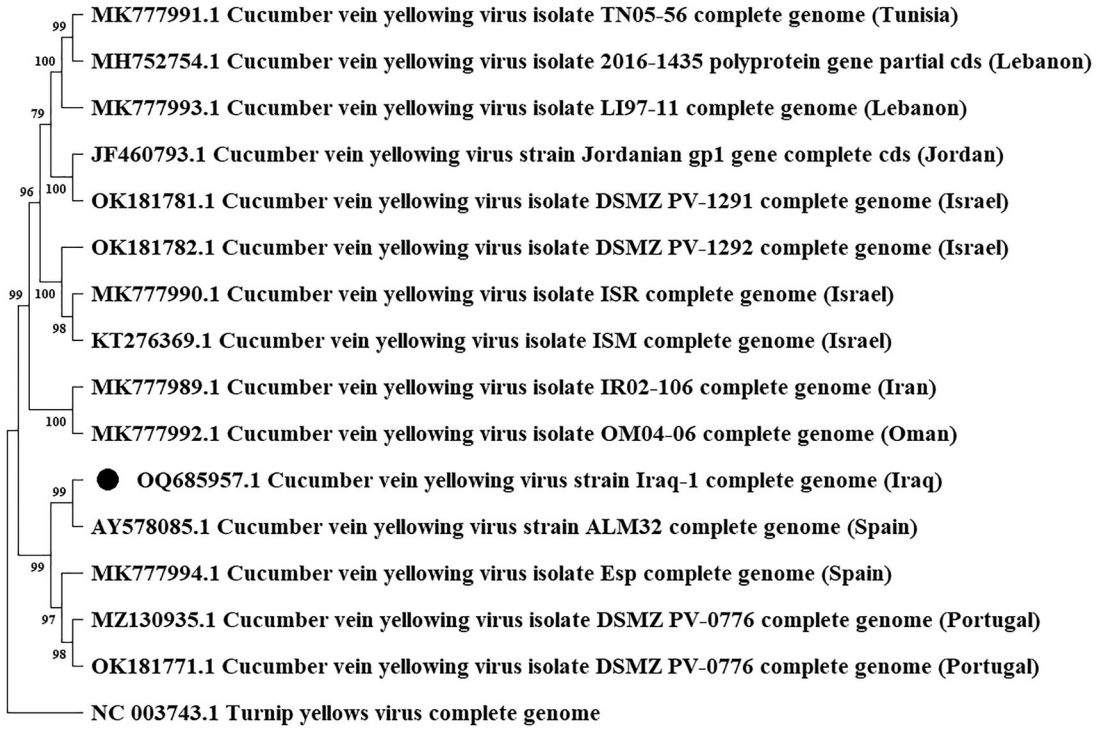

FIGURE 2 Neighbour-joining tree based on the whole genome sequence of *Cucumber vein yellowing virus* (CVYV). Bootstrap values (n = 1000) above 75% are indicated for each node. The position of the Iraqi CVYV isolate within the phylogenetic tree is indicated with a black dot. *Turnip yellows virus* was used as the outgroup.

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