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First Report of Watermelon Crinkle Leaf-associated Virus 1 and 2 Infecting Watermelon (*Citrullus lanatus*) in Greece

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In 2025, surveys were conducted to assess the incidence of virus diseases on cucurbit crops (*Citrullus lanatus*, *Cucumis sativus*, *Cucurbita melo* and *Cucurbita pepo*) in 13 regional units of Northern (Kavala, Serres and Thessaloniki), Central (Aetolia-Acarnania, Euboea, Magnesia/Sporades and Trikala) and Southern (Chania, Corinthia, Heraklion, Laconia, Lasithi and Messenia) Greece. Cucurbit leaf samples exhibiting typical virus symptoms, such as mosaic, mottling, yellowing, curling and crinkling (Figure 1), were collected and analysed with RNA-Seq. Total RNA was extracted from 2 grammes of leaf tissue derived from 5 to 10 diseased plants (two leaves per plant) from each sampled field or greenhouse. An RNeasy Plant Mini Kit (Qiagen, Germany) was used following the manufacturer's instructions. Ribosomal depletion was performed with the RiboCop rRNA Depletion Kit for Plants (Lexogen, Austria) in composite samples comprised of an equimolar mixture of total RNA originating from five to six locations. In total, nine composite samples were analysed. The NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs, USA) was used for library preparation, and the samples were analysed in an Illumina Novaseq 6000 platform (Greek Genome Center, BRFAA). The in silico analysis of the obtained 50 – 60 M reads per library was performed with Geneious v. 11.1.5. Briefly, adaptor sequence and low-quality reads were trimmed with *BBDuk*, duplicate reads were removed with *Dedupe*, and the the reference host genomes subtracted. Finally, the obtained reads were *de novo* assembled into contigs with Geneious assembler. BLASTn annotation of the contigs revealed the presence of a plethora (>7) of viruses known to be widespread in Greece. Most notably, in three of the nine composite samples which contained watermelon (*Citrullus lanatus*) plants, two new viruses were detected, namely watermelon crinkle leaf-associated virus 1 (WCLaV-1, *Coguvirus citrulli*) and watermelon crinkle leaf-associated virus 2 (WCLaV-2, *Coguvirus henanense*). Both viruses belong to the *Coguvirus* genus and their genomes consist of one negative and one ambisense single stranded RNA. Further analysis of one of the composite samples containing a single watermelon sample allowed acquisition of the near complete genome of the WCLaV-1 Greek isolate (RNA1: GenBank Accession No. PX445771, RNA2: PX445772) which showed the highest identity at nucleotide level with the TR-D63 isolate derived from Turkey [99.98% RNA1 (PV701212), 97.31% RNA2 (PV701220)]. The nearly complete genome of the WCLaV-2 isolate (RNA1: PX445769, RNA2: PX445770) resembled the WCLaV2-ITA1-1006 isolate from Italy [99.96% RNA1 (PQ869160), 97.82% RNA2 (PQ869161)].

The nucleotide sequences of each virus obtained from the RNA-Seq were verified by Sanger sequencing the PCR products of the movement protein (*MP*), nucleocapsid (*NP*) and RNA-dependent RNA-polymerase (*RdRp*) genes (Hernandez et al. 2021) and 100% identity was obtained for both viruses. Subsequently, the prevalence of the two viruses was assessed in the original cultivated sites from which the composite samples were derived. In total eight watermelon, 17 courgette, 12 cucumber and 12 melon samples were tested by RT-PCR targeting the *MP* genes (Hernandez et al. 2021). WCLaV-1 was detected in all eight watermelon samples tested; these samples originated from the Chania, Euboea, Kavala, Lakonia, and Serres, while WCLaV-2 was detected in six samples from Chania, Euboea, Kavala and Serres. No other cucurbit species tested positive for either of the two viruses (Fig. S1).

To the best of our knowledge this is the first report of WCLaV-1 and WCLaV-2 infecting watermelon in Greece. The viruses have

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FIGURE 1 | Representative symptoms of leaf mosaic, mottling, yellowing, curling and crinkling in watermelon samples in which watermelon crinkle leaf-associated virus 1 and 2 were detected.

been reported, individually or in mixed infections, in China (Xin et al. 2017), USA (Hernandez et al. 2021; Murcia Bermudez et al. 2025), Brazil (de Moya-Ruiz et al. 2025), Australia (Xin et al. 2017), Italy (Parrella 2025), Spain (de Moya-Ruiz et al. 2025) and Slovenia (Vučurović et al. 2025). Our results indicate that they are possibly widespread in Greece often coinfecting watermelon. Both viruses are included in the EPPO Alert List since 2023 due to their possible impact on cucurbits. However, further research is needed to assess their potential risk since their biology, host range and mode of transmission are yet unclear.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supplementary Figure: ndr270097-sup-0001-FigS2.tif