New Disease Reports

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NEW DISEASE REPORT OPEN ACCESS

First Outbreak of Citrus Viroid V (*Apscaviroid epsiloncitri*) in Sweet Orange in Italy

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Received: 13 June 2025 | **Accepted:** 5 August 2025

Keywords: CVd-V | citrus viroid

Citrus viroid V (CVd-V, *Apscaviroid epsiloncitri*) was first identified in Spain (Serra, Barbosa, et al. 2008) and has subsequently been detected in other countries of the Mediterranean basin, Asia and America (Serra, Eiras, et al. 2008; Cao et al. 2010; Hamdi et al. 2015). A wide range of asymptomatic citrus hosts has been reported. Foliar and stem symptoms have been observed in experimentally inoculated Etrog citron, which are more severe in synergistic infections with other viroids. Yield reduction and significant dwarfing have been reported in clementine trees grafted on trifoliate orange, in association with additional viroids (Serra, Barbosa, et al. 2008). Like other citrus viroids, CVd-V is graft-transmitted, and spreads through the propagation of infected material. Therefore, it is crucial that the mother plants used for nursery propagation are free of CVd-V.

To evaluate the risk of CVd-V infections in Italy, 137 samples randomly collected from commercial citrus orchards of the main citrus areas of Sicily (Catania, Messina and Siracusa provinces) were tested in March and April 2025. Sweet orange trees were grafted on different citranges and some exhibited non-specific viroid-like symptoms, such as leaf yellowing and stunted growth, whereas lemon trees, grafted on sour orange or volkameriana lemon, were asymptomatic. Total RNA was extracted from the petioles of the collected samples and used for RT-PCR amplification with CVd-V specific primers: 5′-GACGAAGGCCGGTGAGCAGTAAGCC-3′ and 5′-GACGACGACAGGTGAGTACTTTC-3′ corresponding to CVd-V nucleotide positions 90–114 and 69–89, respectively (Bani Hashemian et al. 2010). RNA extracts of 10 sweet orange plants produced a viroid-specific 293 bp amplicon following RT-PCR analysis, while the remaining samples tested negative. The varieties tested were cv. Sanguinello (*Citrus* × *sinensis* (L.) Osbeck) grafted onto Troyer citrange (*Citrus* × *sinensis Osbeck* × *Poncirus trifoliata* Raf.) and Tarocco Meli Nuc. C8158 grafted on citrumelo (*Poncirus trifoliata* × *Citrus* × *paradisi*). Both infections were asymptomatic. PCR amplicons of two samples were purified using ExoSAP-IT (Thermo Fisher Scientific, USA) and sequenced in both directions by Macrogen Europe (the Netherlands) to confirm their identity as CVd-V. The resulting sequences were deposited in GenBank as isolates DW18 (Genbank Accession no. PV657136) and DW61 (PV657137). BLASTn analysis revealed that both isolates shared 99.89% nucleotide identity between them and 99.66% with the Spanish reference isolate (NC_010165), Figure 1. To our knowledge, these findings report for the first time the presence of CVd-V in commercial citrus orchards in Italy and surveillance is needed to determine its distribution, incidence, and potential impact on citrus cultivation.

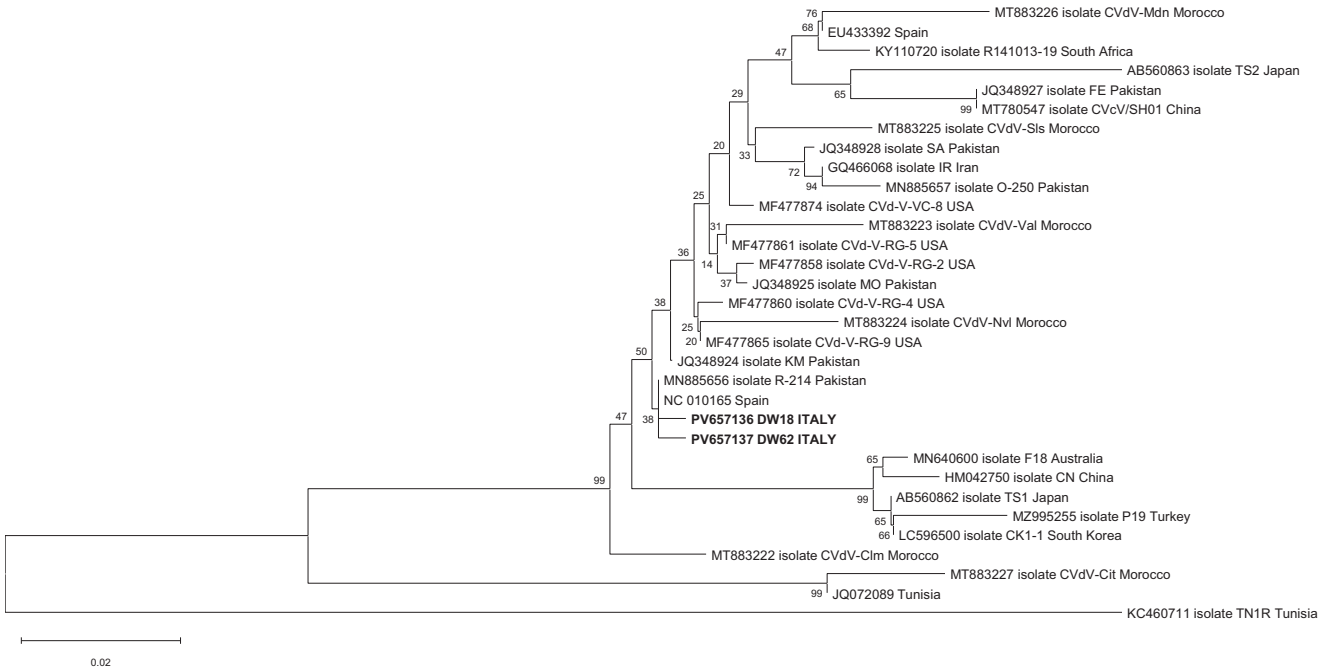

FIGURE 1 | Evolutionary history was inferred using the neighbour-joining method. The optimal tree with the sum of branch length = 0.565 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch length in the same units as those of the evolutionary distance used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes–Cantor method and are in the units of the number of base substitutions per site. The analytical procedure encompassed 32 nucleotide sequences. The pairwise deletion option was applied to all ambiguous positions for each sequence pair resulting in a final data set comprising 299 positions. Evolutionary analyses were conducted in MEGA12 utilising up to three parallel computing threads.

Acknowledgements

The authors thank the 'SiciliAn MicronanOTecH Research and Innovation CEnter SAMOTHRACE (MUR, PNRRM4C2, ECS_00000022), spoke 3 – University of Palermo S2-COMMs-Micro and Nanotechnologies for Smart and Sustainable Communities'.

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