Received: 19 May 2025 | Accepted: 5 June 2025

DOI: 10.1002/ndr2.70041

New Disease Reports Open Access WILEY

NEW DISEASE REPORT

First report of Cistus incanus RNA virus 1 associated with *Cistus monspeliensis* in Greece

D. Beris | **A. Galeou** | **O. Kektsidou** | **C. Varveri**

Laboratory of Virology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, Kifissia, Athens, Greece

Correspondence
D. Beris, Laboratory of Virology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, 8 S. Delta Str. 145 61 Kifissia, Athens, Greece. Email: d.mperi@bpi.gr

KEYWORDS
Amalgaviridae, high throughput sequencing, Montpellier cistus

In spring 2023, a sample of Montpellier cistus (*Cistus monspeliensis*) from a garden in Attica, Greece, exhibiting virus-like symptoms such as chlorotic lesions, yellowing and leaf mottling (Figure 1) was subjected to RNA-Seq. Total RNA was extracted from leaf tissues with an RNeasy Plant Mini Kit (Qiagen, Germany) and ribosomal RNA was removed using the RiboMinus™ Plant Kit for RNA-Seq (Thermo Fisher Scientific, USA). The resulting ribo-depleted RNA was sent to the Greek Genome Center (Biomedical Research Foundation of the Academy of Athens, Greece) for library preparation and high throughput sequencing analysis in an Illumina Novaseq 6000 platform. The obtained 25 M, 100 bp reads were *de novo* assembled using Geneious (v. 11.1.5) and the resulting contigs were annotated with BLASTn. The analysis revealed the presence of a single virus, Cistus incanus RNA virus 1 (CiRV1), an unclassified viral species belonging to the *Amalgaviridae* family that was initially identified in silico in a *C. incanus* transcriptome dataset (Goh *et al*., 2018). The nearly complete genome of the Greek isolate was 3318 nucleotides long (GenBank Accession No. PP658211) and shared 77% identity at the nucleotide level with the full-length genome of the AV-1 isolate (MG833407) from Italy. When comparing their putative RNA-dependent RNA-polymerase protein sequences, an amino acid identity of 81% was obtained To verify the RNA-Seq results an RT-PCR assay was developed and a 634 bp fragment of the viral genome was amplified using the CIRV1-F (5′-ATCAGGGACAGACAGATCAG-3′) / CIRV1-R (5′-CTGGTTCCTGGGAATAAGAG-3′) primer pair. The obtained PCR product was Sanger sequenced and the resulting sequence was identical to that derived from the RNA-Seq.

To further study the properties of CiRV1, leaves from the original symptomatic cistus sample was used for sap inoculation of indicator



FIGURE 1 Virus-like symptoms of chlorotic lesions, yellowing and leaf mottling observed in the *Cistus monspeliensis* plant in which Cistus incanus RNA virus 1 was identified.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2025 The Author(s). *New Disease Reports* published by British Society for Plant Pathology and John Wiley & Sons.

species, namely *Nicotiana benthamiana*, *N. tabacum* cv. Xanthi NC, *N. rustica* and *Chenopodium quinoa*. Plants were monitored for symptom development; however, no symptoms were recorded up to 30 days post inoculation suggesting that either the virus is not mechanically transmitted, or it has a narrow host range. Virus absence in the inoculated plants was also confirmed by RT-PCR. Furthermore, five *C. monspeliensis* and five *C. creticus* asymptomatic plants, derived from a nursery and a private garden respectively, were analysed with the developed RT-PCR for the presence of the virus and all tested negative indicating a putative association of CiRV1 with the observed symptoms.

To the best of our knowledge this is the first report of the virus in Greece and the first record of CiRV1 in living plants. Although the Greek and Italian isolates shared a relatively low nucleotide and amino acid identity, they possibly belong to the same species, since the current species demarcation criteria for the *Amalgavirus* genus were not met. Our efforts to transmit the virus mechanically were unsuccessful, a fact that is in accordance with the current data that amalgaviruses are only vertically transmitted via seeds or pollen. Although the majority of amalgaviruses are found in latent infections, CiRV1 was detected in the only symptomatic plant suggesting that its presence may contribute to the appearance of the symptoms observed. However, the finding of only one symptomatic plant indicates that further studies are needed to elucidate CiRV1 prevalence in the Cistaceae family as well as the virus effect on symptom development, plant physiology and metabolite content since *Cistus* species are widely used in the pharmaceutical industry.

ORCID

D. Beris https://orcid.org/0000-0003-3308-8742

REFERENCE

Goh, C.J., Park, D., Lee, J.S., Sebastiani, F. and Hahn, Y. (2018) Identification of a novel plant amalgavirus (*Amalgavirus*, *Amalgaviridae*) genome sequence in *Cistus incanus*. *Acta Virologica*, 62, 122–128. https://doi.org/10.4149/av_2018_201

How to cite this article: Beris, D., Galeou, A., Kektsidou, O. & Varveri, C. (2025) First report of Cistus incanus RNA virus 1 associated with *Cistus monspeliensis* in Greece. *New Disease Reports*, vol.51, e70041. https://doi.org/10.1002/ndr2.70041