



## First report of Grapevine leafroll-associated virus 4 (GLRaV-4) in Spain

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During phytosanitary certification of three grapevine clones (one of Gorgollosa and two of Mancas de Tibus) received at IMIDA from the Balearic Islands (Spain), we observed discrepancies between symptoms on Cabernet Franc indicator plants and the results of ELISA testing for viruses associated with grapevine leafroll. According to DAS-ELISA analysis (Bioreba, Nyon, Switzerland), the three clones were free from GLRaV-1 and GLRaV-3 but positive for GLRaV-2. However, in four consecutive seasons on indicator rootstocks, symptom severity led to a suspicion that other leafroll viruses might be present; in our experience, symptoms produced by GLRaV-2 infection alone are rarely as severe as those that were observed. Testing of the clones with real-time RT-PCR using TaqMan® probes (Osman *et al.*, 2007) detected *Grapevine leafroll-associated virus 4*. Using similar real-time PCR methods we confirmed the presence of GLRaV-2 but we did not detect either GLRaV-1 or GLRaV-3. Moreover we could also detect *Grapevine fanleaf virus*, GFLV (DAS-ELISA, Bioreba) in the Mancas de Tibus vines; this was confirmed by symptom inspection in the indicator host *Rupestris de Lot*.

In order to confirm the presence of GLRaV-4, RNA extracts were also tested by conventional RT-PCR with consensus primers derived from the GLRaV-4 *hsp70* gene: LR4H89 (5'-CATACCAACCGTTGTGGTA-3') and LR4H327 (5'-TGCAATTACTCTTGTGTTT-3'). Amplicons were of the expected size (238 bp) and were cloned using pGEMT-Easy (Promega). Comparisons showed that sequences were identical in all three isolates (GenBank Accession No. GU735409) and showed 99% nucleotide identity with the homologous *hsp70* genes of GLRaV-4 isolates LS-24 from China (GQ849394) (Pei *et al.*, 2010) and CI-3642 from Chile (EU746619) (Escobar *et al.*, 2008).

To our knowledge this is the first identification of GLRaV-4 in Spain.

Current European regulations state that certified plant material cannot contain GLRaV-1 and GLRaV-3 but do not consider GLRaV-2 and other leafroll viruses (Anonymous, 2008). Our report suggests that other leafroll viruses should be included in the European regulations for grapevine certification. Further analysis of the GLRaV-4 isolates is underway and epidemiological investigations are currently in progress to establish the field incidence of these and other viruses in the provenance area of the grapevines within the Balearic Islands.

### References

- Anonymous, 2008. Pathogen-tested material of grapevine varieties and rootstocks. *OEPP/EPPO Bulletin* **38**, 422-429.
- Escobar PF, Fiore N, Valenzuela PDT, Engel EA, 2008. First detection of *Grapevine leafroll-associated virus 4* in Chilean grapevines. *Plant Disease* **92**, 1474. [doi:10.1094/PDIS-92-10-1474C]
- Osman F, Leutenegger C, Golino D, Rowhani A, 2007. Real-time RT-PCR (TaqMan®) assays for the detection of *Grapevine leafroll associated viruses 1-5* and 9. *Journal of Virological Methods* **141**, 22-29. [doi:10.1016/j.jviromet.2006.11.035]
- Pei G-Q, Dong Y-F, Zhang Z-P, Fan X-D, 2010. First Report of *Grapevine leafroll-associated virus 4* and 5 in grapevines in China. *Plant Disease* **94**, 130. [doi:10.1094/PDIS-94-1-0130A]

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