# Simultaneous Detection of the Main Viruses and Viroids Affecting Grapevine by Molecular Hybridization Using a Unique Riboprobe or 'Polyprobe'

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## INTRODUCTION

Grapevine is economically the most important fruit crop in the world, which is affected by various diseases of viral and/or viroidal etiology, whose symptoms range from asymptomatic, through chlorosis, interveinal reddening or yellowing, delayed ripening of the grapes, etc. which may affect the production of grapes with losses of up to 15% (7). Traditionally, viral infection assays in grapevine have been based on the bioassay or ELISA serological technique. However, both techniques have distinct disadvantages associated with the space/time required, the inability to identify the pathogen (bioassays), the absence of antibodies against important pathogens or the inability to detect viroidal agents (e.g., ELISA). In recent years, the incorporation of detection techniques based on molecular components of pathogens (RT-PCR, real time PCR –TaqMan-, etc.) has significantly increased the detection limit but also the cost of the analysis. For this reason, trends in detection techniques have been focused on reducing the costs/time of the analysis by performing the simultaneous detection of several pathogens, allowing the analysis of 13 (low density array, TaqMan RT-PCR, 8) or 44 (Microarrays, 3) vine viral pathogens. However, the cost resulting from these methods is incompatible with large-scale surveys, one aspect to consider in cultures with many years of planting. In this sense, the technology based on the nonradioactive molecular hybridization is a fast, simple and reliable methodology for routine diagnosis of viruses and viroids.

In our laboratories, we have developed a molecular nonradioactive hybridization for simultaneous detection of different viruses/viroids by using a single probe or 'polyprobe' containing, fused in tandem, the different viral/viroidal sequences. This methodology permits the simultaneous detection of different viruses/viroids in one test with limit detection similar to the greater obtained by ELISA (in the case of viruses). This technology has proved to be an efficient and cheap methodology for the detection of the main virus and/or viroids affecting stone fruit (4, 9), tomato (1) and citrus (2). In the present work, we have developed a polyprobe with the capacity to detect 15 viruses and 5 viroids affecting grapevine plants.

## MATERIALS AND METHODS

Infected plants with the different virus and viroids were subjected to total nucleic acids extraction (TNA) by the silica capture method (5, 6). RT-PCR reactions were performed using the TNA and the specific primers containing the 5′ and 3′ *Xho*I and *Sal*I restriction sites respectively. The amplicons corresponded to the following viruses and viroids: *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated virus 1*, *2*, *3*, *4*, *5*, *6*, *9* (GLRaV-1, -2, -3, -4, -5, -6, -9), *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), *Grapevine virus D* (GVD), *Grapevine fleck virus* (GFkV), *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Grapevine rupestris vein feathering virus* (GRVFV), *Arabis mosaic virus* (ArMV), *Citrus exocortis viroid* (CEVd), *Grapevine yellow speckle viroid 1* (GYSVd-1), *Grapevine yellow speckle viroid 2* (GYSVd-2), *Hop stunt viroid* (HSVd), and *Australian grapevine viroid* (AGVd). The incorporation of the PCR fragments in the pKS + plasmid and the subsequent fusion in tandem was performed by using the restriction sites *Xho*I-*Sal*I as described previously (9).

## RESULTS AND DISCUSSION

The use of riboprobes carrying partial sequences of different plant viruses and viroids fused in tandem, has permitted the simultaneous detection of up to ten different pathogens (eight viruses and two viroids) using a non-radioactive molecular hybridization procedure (9). In the present work we have generated three different

polyprobes for the detection of the main viruses (15, Poly15) viroids (5, poly5) or both (poly20) affecting grapevine crops. Actually, we are analyzing the detection limit and the specificity of the new polyprobes. To our knowledge, this is the first polyprobe described with the capacity to detect twenty different pathogens.


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## REFERENCES


1. Aparicio, F., Soler, S., Aramburu, J., Galipienso, L., Nuez, F., Pallás, V. And López, C. 2009. Simultaneous detection of six RNA plant viruses affecting tomato crops using a single digoxigenin-labelled polyprobe. European Journal of Plant Pathology 123 (1): 117-123.
2. Cohen O., Batuman O., Stanbekova G., Sano T., Mawassi M. And Bar-Joseph M. 2006. Construction of a multiprobe for the simultaneous detection of viroids infecting citrus trees. Virus Genes. 2006 Dec;33(3):287-92.
3. Engel E., Escobar P.F., Rojas L.A., Rivera P.A., Fiore N., Pablo D.T. Valenzuela P.D.T. 2010. A diagnostic oligonucleotide microarray for simultaneous detection of grapevine viruses. Journal of Virological Methods 163: 445-451.
4. Herranz M.C., Sanchez-Navarro J., Aparicio F., Pallás V. 2005. Simultaneous detection of six stone fruit viruses by non-isotopic molecular hybridization using a unique riboprobe or 'polyprobe'. Journal of Virological Methods 124: 49-55.
5. MacKenzie D.J., McLean M.A., Mukerij S., Green M. 1997. Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcriptase-polymerase chain reaction. Plant Disease 81: 222-226.
6. Malinovski T. 1997. Silicacapture-reverse transcription-polymerase chain reaction (SC-RT- PCR): application for the detection of several plant viruses. In: Dehne HW, Adam G, Diekmann M, Frahm J, Mauler-Machnik A, van Halteren P. (eds) Proc of IV Intern Symposium of the European Foundation for Plant Pathology, 9–12. September 1996.
7. Martelli G. 1993. Leafroll, in Martelli, G., ed., Graft-transmissible diseases of grapevines. Handbook of detection and diagnosis. FAO. Rome.
8. Osman F., Rowhani A. 2008. Real-time RT-PCR (TaqMan®) assays for the detection of viruses associated with Rugose wood complex of grapevine. Journal of Virological Methods 154 (1–2): 69-75.
9. Peiró A., Pallás V., Sánchez-Navarro J.A. 2012. Simultaneous detection of eight viruses and two viroids affecting stone fruit trees by using a unique polyprobe. European Journal of Plant Pathology 132(4), 469-475.