## O13: Simultaneous detection of the 13 viruses and 5 viroids affecting grapevine by molecular hybridization using a unique probe or 'polyprobe'

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### INTRODUCTION

Grapevine is the most economically important fruit crop in the world, which is affected by various diseases of viral and/or viroidal etiology, which may affect the production of grapes with losses of up to 15% (Martelli, 1993). Traditionally, viral infection assays in grapevine have been based on the bioassay or on the ELISA serological technique. However, both techniques have distinct disadvantages derived from 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 up to 13 (low density array, TaqMan RT-PCR, Osman et al., 2008) or 44 (Microarrays; Engel et al., 2010) grapevine 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 polyvalent detection of different viruses/viroids by using a single probe or 'polyprobe' containing, fused in tandem, the different viral/viroidal sequences (Herranz et al., 2005; Janet Zamora-Macorra et al., 2015). This methodology permits the simultaneous detection of different viruses/viroids in one test with limit detection similar to the highest 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 viruses and/or viroids affecting stone fruits (Herranz et al., 2005; Peiró et al., 2012), tomato (Aparicio et al., 2009) and citrus (Cohen et al., 2006). In the present work, we have developed a polyprobe with the capacity to detect 13 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 (MacKenzie et al., 1997; Malinovski, 1997). 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 following viruses and viroids were detected: *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated virus 1, 2, 3, 4* (GLRaV-1, 2, 3, 4), *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), *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). In the case of GLRaV-4, were detected the variants 4, 5 and 6. 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 (Peiró et al., 2012).

### 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 (twelve viruses and four viroids) using a non-radioactive molecular hybridization procedure (Peiró et al., 2012). In the present work we have generated three different polyprobes for the detection of the main viruses (13, Poly15), viroids (5, poly5) or both (poly18) affecting grapevine crops. The individual and the three polyprobes were able to detect up to 5-1 pg/µl of viral/viroidal RNA, comparable to other described probes (Sanchez-Navarro et al., 1999). The analysis of 142

grapevine samples revealed that all positives samples detected by using individual probes were also detected by using the corresponding polyprobe. The infection percentages were: GLRaV-1 (9.1%), GLRaV-2 (39.4%), GLRaV-3 (19.1%), GLRaV-4 variant 5 (8.4%) GLRaV-4 variant 6 (7.7%), GFLV (23.9%), GFkV (36.6%), ArMV (2.8%), GVA (12.7%), GVB (3.5%), GRSPaV (18.3%), GRVFV (92.2%), HSVd (100%), GYSVd-1/-2 (89.4%) y AGVd (0.7%). When the 142 samples were analyzed by the ELISA assay to detect GLRaV-1, GLRaV-3, GFLV, GFkV or ArMV, the infection percentages were similar (GLRaV-1: 9.1%; GLRaV-3: 19.1%), higher (ArMV: 4.2%) or lower (GFLV: 23.2%; GFkV: 31.7%) to that obtained by using the molecular hybridization technique. To our knowledge, this is the first polyprobe described with the capacity to detect eighteen different pathogens.

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