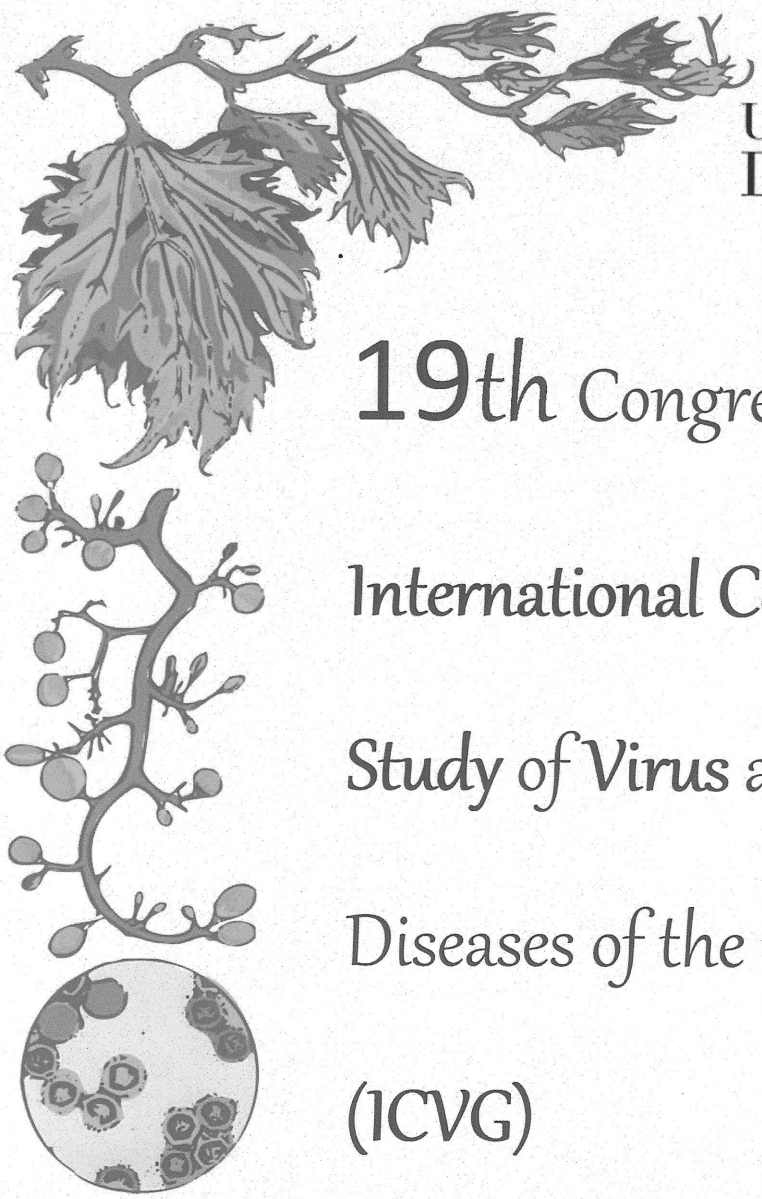

UNIVERSIDAD DE CHILE

19th Congress of the International Council for the Study of Virus and Virus-Like Diseases of the Grapevine (ICVG)

PROCEEDINGS

April 2018 - Santiago, Chile

O9: Nanobody-based reagents as diagnostic tools for Grapevine viruses

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INTRODUCTION

With more than 70 different viruses species identified so far, grapevine (*Vitis spp*) is the crop with the highest number of infecting viruses (Martelli, 2014). Although the pathogenicity of all of these viruses has not been established, a number of them are considered as severe grapevine pathogens such as the emerging *Grapevine red blotch virus* or *Grapevine Pinot gris virus* in addition to the well described viruses responsible for rugose wood, leafroll- and fanleaf degeneration-diseases (Basso et al., 2017; Maliogka et al., 2015). These viruses cause substantial crop losses, reduce berry quality and shorten the longevity of grapevines, hampering for the soil-borne nepoviruses infesting high-value vineyards, the cultivation of grapevines. No germplasm resistance to these viruses has been reported so far in *Vitis* species. However, while many efforts are being made, no effective and economically acceptable solution to eradicate or efficiently limit the disease is yet established. One of the most efficient approaches to limit the spread of the virus is the release by the nurseries of virus-free grapevine material through systematic and reliable certification schemes.

The certification of propagative material is mainly based on double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using immunochemical reagents derived from polyclonal or monoclonal antibodies. Their production can be expensive and time-consuming, requiring specific structures and skills, and the availability and quality of antibodies produced can be prone to variations in performance. These classical ELISA reagents could favorably be replaced by Nanobodies (Nbs). Nbs are small peptides derived from heavy-chain-only antibodies found in camelids (Fig.1) (Muyldermans, 2013). They are the smallest naturally occurring intact antigen-binding domains known to date. They are monovalent, stable, soluble, and recognize cryptic epitopes inaccessible to common antibodies. They can be easily tailored and produced to almost unlimited amounts in bacteria such as *E. coli*. We recently also established that Nb possx ess antiviral activity by showing that constitutive expression of Nanobodies directed against *Grapevine fanleaf virus* (GFLV) conferred resistance to the cognate virus in *N. benthamiana* and grapevine rootstocks (Hemmer et al., 2017).

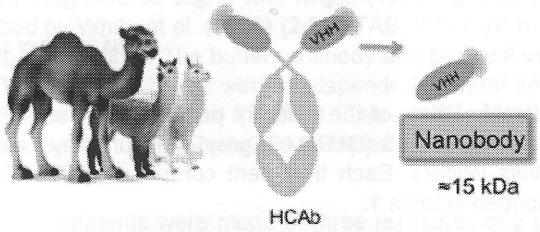


Fig.1 Nanobodies derived from heavy-chain-only antibodies.

MATERIALS AND METHODS

Virus isolate and virus purification: *Strawberry latent ringspot virus* (SLRSV) -T29 was originally isolated from naturally infected grapevines and maintained by mechanical inoculation on *Chenopodium quinoa* or *N. benthamiana*. The viral particles were purified from infected *C. quinoa* and purified using standard nepovirus purification procedure consisting of clarification and sucrose gradients.

Nanobodies production: *Camelidae* were immunized with purified virus particles at weekly intervals for 6 weeks. SLRSV specific single domain antibodies (Nbs) or (VHH) were generated according to Ghassabeh *et al.*, 2010. The resulting VHH libraries were screened by phage display for virus-specific binders against SLRSV purified virions. Nbs were tailored with appropriate tags (ie His6 tag, alkaline phosphatase or fluorescent proteins) using standard molecular biology protocols. Large-scale production of Nbs was performed by expression in *E. coli* and soluble Nbs further purified by affinity and size exclusion chromatography.

DAS-ELISA assessment of Nbs reactivity: Virus detection was performed from SLRSV infected grapevine extracts by DAS-ELISA using anti-SLRSV IgG as capture/trapping antibodies and the tagged Nbs as detection antibodies. For the coating step, tailored Nbs were used as capture/trapping antibodies and anti-SLRSV IgG or tailored Nbs as detection antibodies. Negative control consisted of healthy plants.

RESULTS AND DISCUSSION

Two Nanobodies (Nb 1.1 and Nb 1.8) belonging to two different families, able to recognize SLRSV-T29, were identified from the screening step. To evaluate their ability to detect SLRSV in plant crude extracts, the two anti-SLRSV Nbs were tagged either with fluorescent protein or alkaline phosphatase. The tailored Nbs were successfully produced in *E. coli* and semi-purified (Fig. 2)

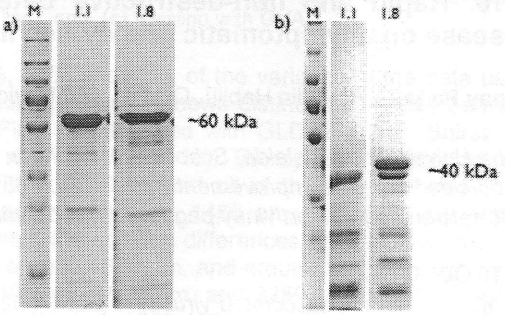
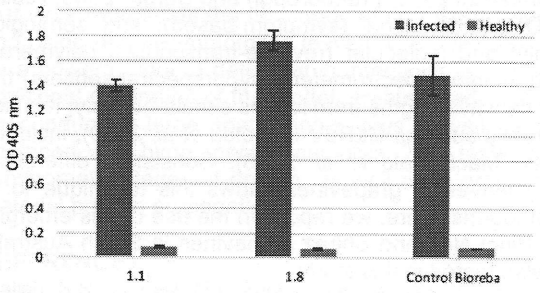


Figure. 2 SDS-PAGE analysis of the semi-purified Nanobodies tagged with alkaline phosphatase (a) and fluorescent protein (b). 1 µg from each tailored Nb after the purification process was separated by SDS-PAGE and stained with Coomassie blue. M: Ladder

The SLRSV Nanobody-based reagents were able to detect the virus from a solution containing purified particles and from infected *Ch. quinoa* and blackberry crude extracts (Fig. 3). They performed similarly to the commercial antibodies or even slightly better.

Figure 3: DAS-ELISA on SLRSV infected blackberry.



Further competition experiments were performed in order to see if the two Nbs recognize different epitopes. Even though the two Nbs have two completely different CDR3, our experimental data show that they partially share a common epitope. Finally, the anti-SLRSV Nbs were tested on the SLRSV-infected plant collections of the INRA Colmar, Agroscope and BIOREBA.

A similar approach was initiated to develop Nanobody-based reagents for the detection of *Raspberry ringspot virus* (RpRSV), *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), *Grapevine leafroll-associated virus* 1 and 3 (GLRaV-1 and GLRaV-3), and *Grapevine Pinot gris virus* (GPGV), which are associated to major grapevine virus diseases. The performance of DAS-ELISA tests using Nanobody-based reagents for other major grapevine viruses detection from leaves and woody grapevine material will be presented.

Table 1: Recognition spectrum of Nb 1.1 and Nb 1.8 mixed together and Nb 1.1 and Nb 1.8.individually in comparison to anti-SLRSV conventional antibodies. Green and red colors correspond to positive and negative SLRSV detection, respectively. "+" relates to detection levels. Note that Nb 1.1 and Nb 1.8 do not recognize the peach isolates. Nb 1.8 performs better than Nb 1.1.

Plant	Grapevine			Ch. quinoa		Blackberry	Peach
Isolate	34198+34199	38004	T35 Sylvaner	1005 (peach)	997 (blackberry)	11092	27862
ELISA	++	+	+	++	++	++	+++
Nb 1.1	+	-	-	-	+++	++	-
Nb 1.8	++	+	++	-			-
Nb Mix	+	+	+	-	+++	++	-

ACKNOWLEDGMENTS

This work is partly supported by the European grant H2020-MSCA-RISE-2016 VirFree project #734736 and ANRgrant 14-CE19-0018-02. Magdalène Kosfitskas is funded by a PhD fellowship from the Région Grand-Est and BIOREBA AG.

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