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Comparative pathogenic effects of distinct *Grapevine fanleaf virus* strains on *Vitis vinifera* cvs Gewurztraminer and Chardonnay.

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Introduction

Grapevine fanleaf virus (GFLV) is the main etiological agent of fanleaf disease, one the most severe and widespread virus diseases of grapevine, worldwide. This virus causes serious economic losses (up to 80% yield reduction) and reduces the productive lifespan of vineyards (Andret-Link *et al.*, 2004). Symptoms consist of a progressive degeneration with variable symptoms affecting leaves (yellow mosaic, deformation, vein clearing), canes (short internodes) and clusters (flower abortion, ripeness disturbance). Disease symptom expression depends on the virus isolate, the susceptibility of the variety/rootstock combination, and environmental factors.

GFLV has a bipartite RNA genome (RNA1 and RNA2) and is transmitted by the ectoparasitic nematode, *Xiphinema index*. In recent years, the structure and genetic variability of GFLV populations have been elucidated in several grape-growing regions (Liebenberg *et al.*, 2009, Oliver *et al.*, 2010, Palomares-Rius *et al.*, 2012, Vigne *et al.*, 2009). Mixed infections with genetically distant variants and recombinants are frequent in vineyards, preventing a precise association between genetic variability and symptom expression (Elbeaino *et al.*, 2014).

In order to determine the effect of distinct GFLV isolates on symptoms expression, healthy and infected grapevines were tested in an experimental vineyard. Our main objectives consisted in: i) Monitoring symptom development of five GFLV strains for which the full-length genome sequence was determined, and ii) Analyzing the effect of these strains on fruit yield, as well as on fruit and wine quality. Our findings will shed light on putative viral domains associated to the fanleaf symptoms expression, enabling the selection of GFLV strains with reduced pathogenicity that could benefit future cross-protection experiments aiming at reducing the impact of fanleaf disease in vineyards.

Material and methods

GFLV strains F13, GHu, B844, CO1(A17b) and CO2(A17d) were isolated from *Vitis vinifera* cvs Muscat de Frontignan, *Gloria Hungariae*, Cabernet franc and Chardonnay respectively (Komar *et al.*, 2008, Legin *et al.*, 1993, Vigne *et al.*, 2005), biologically cloned by multiple passages on herbaceous hosts. They were subsequently transferred to the rootstock Kober 5BB by *in vitro* heterologous grafting.

The experimental vineyard was established in a *X. index*-free plot in 2006 at INRA in Colmar, France. Test plants consisted of *Vitis vinifera* cvs Gewurztraminer (Gw) and Chardonnay (Ch) grafted onto healthy or mono-infected Kober 5BB. For each of the six treatments (infection with one of the five virus strains and mock inoculation), eight Gw vines and eight Ch vines were obtained, for a total of 96 vines that were planted 1 m apart in groups of four vines within three rows.

The nucleotide sequence of the complete genome of the GFLV strains was obtained by RNA Seq (Next Generation Sequencing facility, IGBMC, Illkirch, France) and *de novo* assembly. The verification of the GFLV content in the single-infected vines was confirmed by IC-RT-PCR-RFLP (immunocapture - reverse transcription - polymerase chain reaction - restriction fragment length polymorphism).

Symptoms on plant development, leaves, canes, and clusters were monitored on individual vines. The number of clusters was counted for each plant at harvest, and the clusters weighted. For each treatment, fruit juice chemistry was analyzed and fruits were processed for micro-vinification. Aromatic molecules were detected and quantified by gas chromatography followed by mass spectrometry on the INRA Colmar metabolomics platform. Data were collected from 2012 to 2014. Statistical significance of the results was assessed using the 3.2 R software for ANOVA analyses.

Results and discussion

The five GFLV strains displayed at least 9 % nucleotide sequence diversity, regardless of whether RNA1 or RNA2 sequences were analyzed. Unlike strains F13, GHu, CO1(A17b) and CO2(A17d), the genome of B844 is composed of one RNA1 molecule and two genetically distant RNA2 molecules. This original genomic composition with two or more molecular species of RNA1 or RNA2 is novel for GFLV but was already described for other *Secoviridae*, such as *Bean pod mottle virus* strains and *Arabis mosaic virus* (Gu and Ghabrial, 2005, Marmonier *et al.*, 2009).

Regarding symptoms expression, GFLV-B844 causes a severe stunting on Gw cultivar, while the others strains only caused faint mosaic symptoms on Gw leaves. In contrast, all five GFLV strains caused only rare/mild mosaic symptoms on Ch leaves. Significant flower abortion was observed for all strains regardless of the year and cultivar.

Yield impact was similar as already described for other GFLV strains (Walter and Martelli, 1996): crop losses were higher on Ch (- 63%) than on Gw (- 45%), independently of the strain used with the exception of B844 on Gw (- 77%). The effect of the five GFLV strains on wine quality, as measured by must composition, aromatic molecules composition, sensory analyses and comparative tastings, is under way.

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