Survey of emerging viruses in Switzerland

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Introduction

In recent years, next-generation sequencing (NGS) has significantly improved our understanding of viral pathogens. The NGS approach has allowed the identification of several new viruses. For most of these newly described pathogens, there is still little information available about their spread and prevalence in commercial vineyards. Therefore, we conducted a study to monitor the occurrence of three emerging viruses in Swiss vineyards: *Grapevine red blotch-associated virus* (GRBaV), Grapevine redglobe virus (GRGV) and *Grapevine Pinot gris-associated virus* (GPGaV). GRBaV, a tentative member of the family *Geminiviridae*, is a recently discovered viral pathogen associated with the red blotch disease (Al Rwahnih *et al*., 2013). GRBaV has not yet been reported outside North America. Grapevine redglobe virus (GRGV) belongs to the family *Tymoviridae* and was first described in southern Italy (Sabanadzovic *et al.*, 2000). GRGV was detected later on in Greece, California and France. *Grapevine Pinot gris-associated virus* (GPGaV), a trichovirus, was first identified on Pinot gris plants showing leaf mottling (Giampetruzzi *et al.*, 2012). Glasa *et al.* (2014) also identified GPGaV, yet did not observe an association between GPGaV and any specific symptom. Recently, Saldarelli *et al.* (2015) showed that different GPGaV lineages possibly had different biological properties: some isolates were associated with symptoms, and others were not.

Materials and Methods

The vineyards in the La Côte region cover a surface of *ca.* 2000 ha and are located on the edge of Lake Geneva, between Lausanne and Geneva. Fifty commercial vineyards were randomly selected in the La Côte appellation. Vineyards were at least ten years old. Within each vineyard, a plot (500m^2) was defined and 20 individual grapevines were sampled at random. Samples, consisting in dormant canes, were collected in January 2012. To account for the possible uneven distribution of the virus within a vine, three dormant canes per plant were collected. All samples collected from one location were then bulked for nucleic acid extraction, using RNeasy Plant Mini Kits (Qiagen). GPGaV and GRGV infection were assessed by RT-PCR and GRBaV by PCR. One-step reverse transcription-polymerase chain reaction was performed with the AMV reverse transcriptase (Promega, Germany), GoTaq polymerase (Promega, Germany) and total RNA as template. The primers used in this study were as follows: CPfor/ CPrev for detecting GRBaV (Krenz *et al.*, 2014); DetF/DetR for GPGaV (Saldarelli *et al.*, 2015) and RG-CF-F1/ R1 for GRGV (Beuve *et al.*, 2015). To confirm viral infection, amplicons were cloned into pGEM-T easy vector (Promega, Germany) and sequenced at Fasteris SA (Switzerland). Nucleotide alignments were created using ClustalW. The phylogenetic relationships were determined using the software MEGA (version 6). Phylogenetic trees were generated using the maximum likelihood algorithm with 1000 bootstrap replicates.

Results and Discussion

GRBaV was not found during this survey, whereas GRGV was frequently detected in grapevines in the vast majority of studied locations (83 %). Preliminary observations showed no specific symptoms associated with this virus. Further work will be necessary to clarify the effect of GRGV infection in grapevine.
GPGaV was found in seven locations (*i.e.* prevalence of 15%). The resulting 598 bp amplicons were sequenced in order to evaluate the phylogenetic relationship within GPGaV isolates. Three sequences obtained from our viral collection were added, and the 10 sequences were labeled GPGaV_CH1 to 10.These Swiss isolates are all closely related, the maximum genetic variability being only 6 % in the MP/CP region. The low heterogeneity of GPGaV was also reported for Slovak and Italian isolates. When these sequences were compared with publicly available ones, the identity score ranged from 94 to 99%. According to phylogenetic analyses, Swiss isolates segregated into two clades (Figure 1). All, except one, Swiss isolates clustered with French and Slovak isolates. The exception, GPGaV_CH1, grouped into a different clade, along with symptomatic isolates described by Saldarelli *et al.* (2015). C/T polymorphism in the MP stop codon was observed among Swiss isolates (Figure 2), as previously mentioned by others (Glasa *et al.*, 2014; Saldarelli *et al.*, 2015). So far, all GPGaV isolates identified in Switzerland were found on cultivar Chasselas and no specific symptoms were noted. Biological indexing has been initiated to evaluate if these different isolates can induce leaf mottling symptoms, when inoculated on cultivar Pinot gris. Further studies are clearly needed to evaluate the impact of GPGaV on grape and on wine production.

Figure 1. Maximum likelihood phylogenetic tree using nucleotide sequences of MP/CP genes present in 10 swiss GPGaV isolates and 9 other published isolates (*e.g.* French_GPGaV_Mer = KM491305; reference_ Italian_ GPGaV = NC_015782). Branches are condensed and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches.

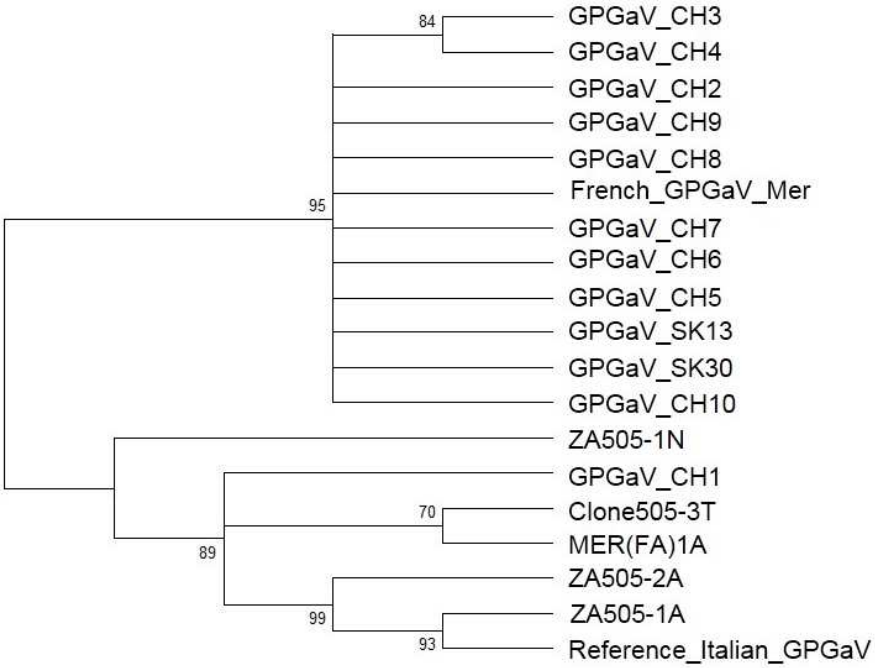

Figure 2. Alignment of 14 MP/CP nucleotide sequences used in phylogenetic analyses. The T/C polymorphism in position 441 is indicated by an arrow.

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French_GPGaV_"Mer"          TGAGCGAGGCGAATCAAGTACTTCATGGGTTGACAGAAGGCAACAAAGAT 450
GPGaV_CH7                   TGAGCGAGGCGAATCAAGTACTTCATGGGTTGACAGAAGGCAACAAAGAT 450
GPGaV_CH6                   TGAGCGAGGCGAATCAAGTACTTCATGGGTTGACAGAAGGCAACAAAGAT 450
GPGaV_SK13                  TGAGCGAGGCGAATCAAGTACTTCATGGGTTGACAGAAGGCAACAAAGAT 450
GPGaV_SK30                  TGAGCGRGGCGAATCAAGTACTTCATGGGTTGACAGAAAACAACAAAGAT 450
GPGaV_CH5                   TGAGCGAGGCGAATCAAGTACTTCATGGGTTGACAGAAGGCAACAAAGAT 450
GPGaV_CH10                  TGAGCGAGGCGAATCAAGTACTTCATGGGTTGACAGAAGGCAACAAAGAT 450
ZA505-1N                    TGAGCGAGGCGAATCAAGTACTTCATGGGTTGACAGAAGGCAACAAAGAT 450
Clone505-3T                 TGAGCGAGGCGAATCAAGTACTTCATGGGCTGACAGAAGGTAACAAAGAT 450
MER_FA_1A                   TGAGCGAGGCGAATCAAGTACTTCATGGGCTGACAGAAGGTAACAAAGAT 450
ZA505-1A                    TGAGCGAGGCGAATCAAGTACTTCATGGGCTGACACAAGGTAACAAAGAT 450
Reference_Italian_GPGaV     TGAGCGAGGCGAATCAAGTACTTCATGGGCTGACACAAGGTAACAAAGAT 450
ZA505-2A                    TGAGCGAGGCGAATCAAGTACTTCATGGGCTGACAGAAGGTAACAAAGAT 450
GPGaV_CH1                   TGAGCGAGGCGAATCAAGTACTTCATGGGCTGGCAGAAGGTAACAAAGAT 450
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