Partial characterization of a new divergent variant of GLRaV-4

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Introduction

Grapevine leafroll disease (GLD) is one of the most economically important viral diseases. GLD is associated with a complex of filamentous viruses referred to as Grapevine leafroll-associated viruses (GLRaVs). All GLRaVs identified so far belong to the family *Closteroviridae*. Up to now, 11 different GLRaVs have been identified: one in the genus *Closterovirus* (GLRaV-2), nine in the genus *Ampelovirus* and one in the new-defined genus *Velarivirus* (GLRaV-7) (Al Rwahnih *et al.* 2012). The genus *Ampelovirus* is further divided into subgroup I (GLRaV-1 and GLRaV-3) and subgroup II containing the short ampeloviruses GLRaV-4, -5, -6, -9, GLRaV-De, GLRaV-Pr and GLRaV-Car. A recent taxonomic revision of the genus *Ampelovirus* proposed that GLRaV-5, -6, -9, GLRaV-De, GLRaV-Pr and GLRaV-Car are all molecular variants of a single species, GLRaV-4 and not, as has been assumed previously, distinct species in the genus *Ampelovirus* (Martelli *et al.* 2012).

Here we report the detection of a new variant of the GLRaV-4 and examine the relationship with other members of the genus *Ampelovirus*. For convenience, all variants belonging to the reference species GLRaV-4 will be referred in this work as to "GLRaV-4 group".

Materials and Methods

- During field inspection of the grapevine collection at Agroscope ACW in Nyon, a vine was detected with clostero-like particles but it was not infected by one or more of the known Grapevine leafroll-associated viruses.
- Nested RT-PCR with degenerate primers (Dovas and Katis, 2003) and the CODEHOP (Consensus Degenerate Hybrid Oligonucleotide Primers) approach has been used to characterize a part of the viral genome (Boyce *et al.* 2009).
- Multiple alignments with published sequences were carried out and the percent amino acid sequence identity were calculated using the program Clustal W. The phylogenetic relationships were determined with the maximum likelihood algorithm of the MEGA 5 package.

Results

Initial molecular characterization of this virus was carried out. An hHSP 70 amplicon of 502 bp was obtained from the nested RT-PCR with degenerate primers. Using TBLASTX algorithm, the obtained sequence showed a limited amino acid identity with the hHSP70 genes of P*ineapple mealybug wilt-associated virus* 1 and 3 (PMWaV-1 and 3) and of viruses belonging to "GLRaV-4 group" and. Further sequence data were obtained using a CODEHOP approach based on the sequences of "GLRaV-4 group" already available in GenBank. The generated sequence data consisted of 5500 nucleotides and putatively encodes 5 complete ORFs: p5, hHSP70, p60, CP and p23 (Fig. 1). The genomic arrangement was typical of members of the "GLRaV-4 group". However, the sequence data showed that this virus was fairly different from other ampeloviruses of subgroup II. For example, amino acid identity in the gene of hHSP70 between the new variant detected in this study and other viruses of "GLRaV-4 group" vary from 63-66% (Table 1).

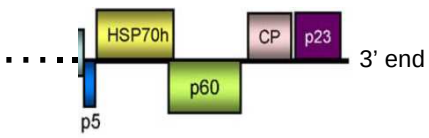

Figure 1. Genome organization of the obtained partial sequence (5,5 kb). Modified from Abou Ghanem-Sabanadzovic *et al.* 2012

Table 1. Heat shock protein 70 homologue amino acid sequence identity (%) of members of the "GLRaV-4 group".

	GLRaV-9	GLRaV-5	GLRaV-4	GLRaV-6	GLRaV-Car	GLRaV-De	GLRaV-Pr	New variant	Mean Divergence
GLRaV-9	100	89	81	84	67	84	79	65	**22**
GLRaV-5		100	84	84	67	85	80	65	**19**
GLRaV-4			100	78	66	81	77	66	**24**
GLRaV-6				100	66	92	78	65	**22**
GLRaV-Car					100	66	69	63	**34**
GLRaV-De						100	78	66	**21**
GLRaV-Pr							100	64	**25**
New variant								100	**35**

In phylogenetics analyses, performed on amino acid sequences of hHSP 70 and CP, the new variant always clustered with viruses of the subgroup II in the genus *Ampelovirus* (Fig. 1). GLRaV-Car and the new variant described here appeared to be the most distinct members of the GLRaV-4 cluster. Based on our results, the virus identified during the inspection of our collection appears to be a new divergent strain of the "GRLaV-4 group". The description of this new divergent variant confirmed the high genetic diversity within the species GLRaV-4.

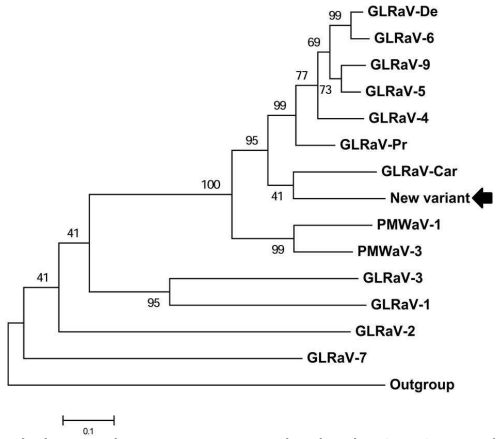

Figure 2. Phylogenetic tree constructed using hHSP70 complete amino acid sequences of the new variant of GLRaV-4 and some other species in the family *Closteroviridae*. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

Summary

A putative new member of the GLRaV-4 group was detected during a field inspection of our grapevine collection. Initial molecular characterization was carried out and provided 5,5 kb of the genome sequence. Five complete potential ORFs (p5, hHSP70, p60, CP and p23) were identified in the data set. The partial viral genome showed a structure similar to that of members of the subgroup II in the genus *Ampelovirus* (fam. *Closteroviridae*). Our preliminary analyses showed that the detected virus represents, together with GLRaV-4 strain Car, the most distinct variants of the GLRaV-4 group. Thus, it is proposed that the virus described hereby represents a novel strain of the GLRaV-4 species.

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