Molecular Characterization and Detection of Redglobe-Isolated Closterovirus

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Introduction

Grapevine is hosting several distinct viruses belonging to the family Closteroviridae mainly associated with leafroll disease complex. Up to date, nine serologically distinct viruses associated with grapevine leafroll disease complex and designed as Grapevine leafroll associated virus type 1 to 9 in the family Closteroviridae had been reported. Recently, researchers' giving the molecular biology approaches an increasing weight in vitivirus research especially describing virus structures and their genomic organization. In the last decades, many variants had been identified mostly based on their molecular characteristics (Martelli, 2006). Biological assessment of one Redglobe vine was noticed to show grooving and stem pitting symptoms on woody cylinder of V. rupestris, cv. St. George; an indicator host for GRSPaV (Uyemoto *et al*.,2001). In the field the canopy developed solid red coloration while the examined woody stems appeared with necrotic lesions only on symptomatic test plants. For that, the trivial name *Grapevine rootstock stem lesion associated virus* (GRSLaV) was given to that virus.

Material and methods

An own-rooted table grapevine (Vitis vinifera cv. Redglobe); tested negative for all known grapevine viruses except for Grapevine rupestris stem pitting associated virus (GRSPaV); was used as a source for virus molecular characterization and maintained in the Foundation Plant Service vineyard. In cloning, dsRNA was used as a template for cDNAs synthesizing following the manufacturer's procedures (Invitrogen Inc., Carlsbad CA). Gaps in the sequence were bridged by designing specific PCR primers for the missing regions, amplifying and sequencing the products. Complementary DNA clones were sequenced and the sequences were analyzed using the Wisconsin GCG software package (Genetic Comupter Group, Madison, WI) and other bioinformatics programs available at the NCBI website. Oligonuclotide primers were designed against the sequence of the HSP70 region of GRSLaV and used for specific viral detection in infected plants. It was designed to detect nothing but GRSLaV. These primers were used for detection of the virus: "RGHSP227 forward primer, 5'-GCGACTCCAGCAACTTTAGTGA-3' and RGHSP777 reverse primer, 5'-GTCTAACGAAAGATCGGGTTCTAAG-3'" that was able to amplify a product of 551 nucleotides. Over 920 samples from varies commercial vineyards and nurseries, collected through 2001-2007, including over 384 different grapevine varieties, were tested for the presence of GRSLaV.

Results

A high molecular weight dsRNA band, typical for closteroviruses, was extracted and purified from bark tissue of infected Redglobe grapevine. The cDNA was cloned into plasmid TOPO TA, and 50 clones were selected for sequencing Gaps in the sequence data were bridged using specific primers to generate overlapping coverage over the length of the viral genome. The genome of GRSLaV was found to be composed of nine open reading frames (ORFs) (Fig. 1). ORF1a encodes a polyprotein (327 kDa predicted size) with PRO, MTR, and HEL domains. ORF1b, translated via ribosomal frameshift (Jelkmann *et al.*, 1997) encodes a polypeptide of 459 amino acids length with predicted molecular weight of ca. 53 kDa. The ORF2 of the virus genome translates into a protein of 57 amino acids, with a molecular weight of 6,442 Da with unknown function. ORF3 translates to a 599 amino acid polypeptide of molecular weight

65,439 Da, with homologies to the heat shock 70 protein (HSP70). ORF4 translates to a 534 amino acid polypeptide of molecular weight 61,769, with homologies to the heat shock 90 protein (HSP90) sequences. ORFs 5 and 6 were identified as genes for the minor coat protein (CPm; molecular weight 24,693 Da) and major coat protein (CP; molecular eight 21,620) respectively. ORFs 7 and 8, common among members of genus Closterovirus, were designated based on their predicted product size of 19 kDa and 24 kDa, composing 161 and 205 amino acid residues, respectively.
These data were to be used for comparative analysis of the virus to other closely related ones. Amino acid sequences showed similarities among GRSLaV and GLRaV-2 as much as 89% and 90%, respectively, for CPm and CP.

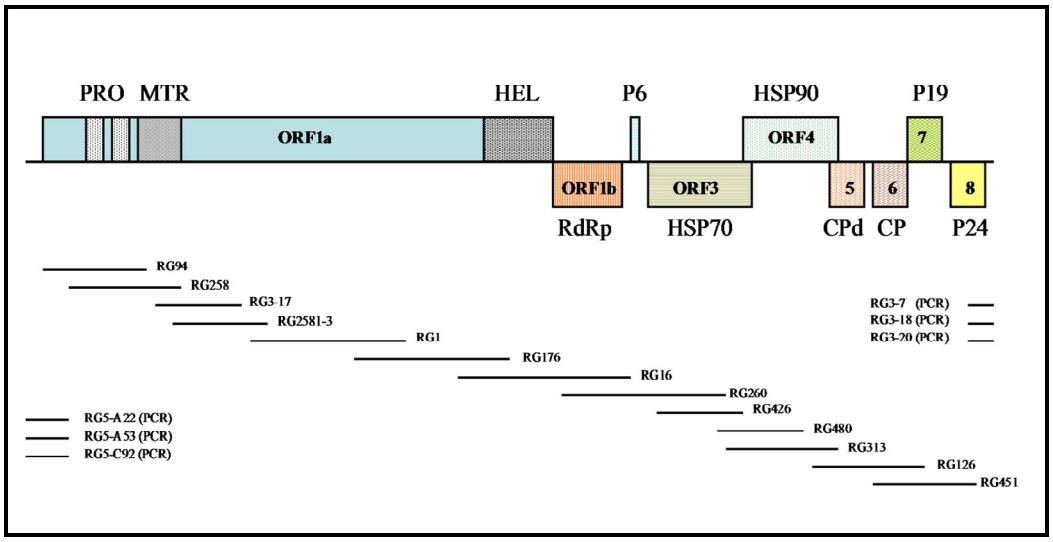

Fig.1. Genomic sequence strategy of GRSLaV

Samples testing using RT-PCR for GRSLaV was revealed low incidence of the virus. Over 920 samples collected from commercial vineyards, *ca*. 1.7% tested positive for GRSLaV, while GLRaV-2 (the closely related virus) was showing higher incidence (13.5% positive). The presence of virus mixtures in a diseased vine is quite common in many viruses, but surprisingly, only two varieties (*Pribidrag* and *Chardonnay*) found to be infected with the both viruses, suggesting the different aetiology for each one. Considering the existence of mixed infection (GRSLaV and GRSPaV) at the early discover of the virus, *ca*. all the 500 samples from different rootstocks found to be infected with GRSPaV were GRSLaV free. Thus, no correlation on symptom manifestation could be drawn due to that.
Although all members of family *Closteroviridae* found to be associated with leafroll disease;*Grapevine rootstock and stem lesion associated virus* is the first reported one that is not associated with that, instead it manifest stem lesions on Couderc 3309 and Kober 5BB rootstocks.

References

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