Materials and Methods

Since the discovery of Grapevine fanleaf virus as the first virus-infecting grapevines over 50 years ago, about 70 different viruses have now been identified from this plant host. Next generation sequencing (NGS, high throughput sequencing, or deep sequencing) has become an important diagnostic tool to detect known, but also novel viruses. In this study we selected four grapevine samples from the Croatian region of Kaštela, in Central Dalmatia: VB-108 (variety Babica), VD-102 (var. Dobričić), VLJ-178 (vari. Ljutun) and VVL-101 (vari. Vlaška). Kaštela region has a long viticultural tradition and numerous grapevine varieties locally grown that are considered to be autochthonous. In addition, previous studies suggested deteriorated sanitary status of varietals from mentioned region with common mixed infections with different viruses.

Results and Discussion

De novo assembly of data from grapevine accessions VB-108, VD-102, VLJ-178, and VVL-101 generated 436, 467, 459, and 251 contigs longer than 1000 nucleotides, respectively. Detailed analysis of these contigs identified two novel grapevine-associated viruses, tentatively named Grapevine virus G and Grapevine badnavirus-1.

Grapevine virus G (GVG)
- in all grapevine accessions
Genome: linear RNA, 5 ORFs, 7475 nts + poly(A) tail (Fig. 1).
- distinct species in the genus Vitivirus (Fig. 2).

Grapevine badnavirus 1 (GBV-1)
- grapevine accessions VLJ-178 and VVL-101 (identical sequence)
Genome: circular DNA, three ORFs, 7145 nts (Fig. 3).
- distinct species in the genus Badnavirus (Fig. 4).

Conclusion

We report the de novo assembly of two novel virus-like sequences from total host plant RNA as a template. It is clear that NGS data will lead to the discovery of new plant viruses, as shown here with only four samples. However, it is important to stress the fact that biological information about most grapevine viruses is very limited or non-existent, and the ability to detect a large number of putative pathogens is of limited applied value if there are no studies describing their respective roles, or how to reduce their impact to a crop of economic importance.

Acknowledgments

The research leading to these results received funding from the European Union Seventh Framework Programme (FP7 2007-2013) under grant agreement n° 291823 Marie Curie FP7-PEOPLE-2011-COFUND (The new International Fellowship Mobility Programme for Experienced Researchers in Croatia - NEWFELPRO). This article was prepared as a part of a project "EcoPlevo - Ecology of an emerging grapevine virus in Croatia and California" which received funding through NEWFELPRO under grant agreement n°51. The work used the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley, supported by NIH S10 OD018174 Instrumentation Grant.