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Rapid communication

A preliminary study of genetic landscape of grapevine viruses isolated from a western Georgian vineyard: insights from initial sequencing analysis

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Abstract

This study reports the first molecular detection and characterization of four economically important grapevine viruses—Grapevine leafroll-associated virus-1 (GLRaV-1), Grapevine leafroll-associated virus-3 (GLRaV-3), Grapevine fleck virus (GFkV), and Grapevine fanleaf virus (GFLV)—in commercial vineyard of western Georgia. Thirty grapevine samples of the widely cultivated Vitis vinifera cv. Saperavi were collected from a self-rooted vineyard in the Adjara region and analyzed using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and one-step RT-PCR. Single and mixed virus infections were detected. Viral presence was further confirmed by Sanger sequencing of partial genomic regions. Sequence analysis revealed high nucleotide identity (88–100%) with grapevine virus isolates previously reported from Europe, North America, and Asia. Phylogenetic analyses conducted in Geneious Prime software revealed that the Georgian isolates clustered among globally distributed reference strains, without forming distinct regional lineages, indicated limited genetic divergence and a conserved population structure. Typical leafroll symptoms were observed only in GLRaV-1- and GLRaV-3-infected vines, whereas GFkV- and GFLV-infected plants remained asymptomatic. These findings provide the first sequence-based evidence of major grapevine viruses spreading in Georgian vineyards, highlighting the need for systematic virus surveillance and the use of certified virus-free planting material to support sustainable viticulture in the country.

Keywords: Georgia, phylogeny, sequencing, virus, *Vitis vinifera*

The Republic of Georgia, which is the oldest wine-growing region, is the motherland of indigenous grapevine (*Vitis vinifera*) varieties (McGovern *et al*. 2017). Grapevine is a major fruit crop that has the highest socioeconomic importance for the country. Grapevine viral diseases not only play a detrimental role in grapevine longevity and yield production, but they also affect technological properties resulting in the deterioration of wine quality (Martelli 2014; Barba *et al*. 2015; Basso *et al*. 2017). Among the most economically damaging and widespread grapevine viral diseases are: Grapevine leafroll disease (GLRD) caused by the viral species Grapevine leafroll-associated virus-1 (GLRaV-1), Grapevine leafroll-associated virus-3 (GLRaV-3) belonging to the family Closteroviridae (Alabi *et al*. 2016; Naidu *et al*. 2015), Grapevine fanleaf degeneration disease (GFDD) distributed in almost all grapevine cultivated regions (Maliogka *et al*. 2015a) caused by Grapevine fanleaf virus (GFLV) belonging to the Secoviridae family and Grapevine fleck disease (GFkD) reported all over the world caused by Grapevine fleck virus (GFkV) a member of the Tymoviridae family (Martelli 2017). Until now, a survey of the grapevine viruses and phytoplasmas associated with grapevine diseases in the eastern part of Georgian vineyards has been conducted using only double antibody sandwich enzyme-linked immunosorbent assay (Das-ELISA) and polymerase chain reaction (PCR) approaches (Megrelishvili *et al*. 2016, 2022; Elbakidze *et al*. 2022). As far as it is known, the presented study declares and confirms the first record of grapevine viruses in the vineyards of Georgia using a sequencing approach.

Thirty Saperavi cultivar grapevine samples were collected from the self-rooted commercial vineyard in western Georgia in July 2021. They were analyzed by Das-ELISA (Bioreba AG) for the presence of GLRaV-1, GLRaV-3, GFkV, and GFLV according to the manufacturer's instructions. Total RNA was extracted using a Plant RNA Purification kit (OxGEn molecular solutions®) following the manufacturer's instructions. SuperScriptTM IV One-Step RT-PCR System (Invitrogen) using specific primers was applied to target the HSP70-like protein gene for GLRaV-1, GLRaV-3 (Osman *et al*. 2007) with expected amplicon size 320 bp and 546 bp, replicase gene (Naidu and Mekuria 2010) for the GFkV with expected amplicon size 533 bp, and coat protein (CP) gene for GFLV, GLRaV-3 (Sanchez *et al*. 1991; Osman *et al*. 2007) with expected amplicon size 322 bp and 611 bp correspondingly. PCR amplicons were directly loaded into the ABI 3500, Applied Biosystems™, and sequenced in both directions using the Sanger sequencing approach.

Sequencing data quality analysis and data collection from the ABI3500 were performed in SeqA6 software, reviewing chromatograms and data quality scores. Partial nucleotide sequences

corresponding to the HSP70-like protein gene (GLRaV-1 and GLRaV-3), coat protein gene (GFLV and GLRaV-3), and replicase gene (GFkV) were aligned using the alignment tools implemented in Geneious Prime software (Biomatters Ltd.) to perform phylogenetic analyses. Phylogenetic trees were constructed in Geneious Prime using a distance-based approach based on nucleotide sequence data. The reliability of the inferred tree topologies was assessed by bootstrap analysis. Exported data were deposited in GenBank under the following accession numbers: ON014523 (GLRaV-1 isolate 09 (HSP70)), ON014524 (GLRaV-1 isolate 10 (HSP70)), ON009178 (GLRaV-3 isolate 11(HSP70)), ON009179 (GLRaV-3 isolate 11(CP)), ON014525 (GFLV isolate 05 (CP)), ON035730 (GFkV isolate 01 (rep)), and ON035731 (GFkV isolate 03 (rep)).

The obtained results revealed both mixed and single infections (Table 1). BLAST analysis revealed that sequences of "09" (ON014523) and "10" (ON014524) isolates shared 93.5-95.0% nucleotide identity with GLRaV-1 isolates from Canada and France (MH807221 and MG925331) within the HSP70 gene. ON009178 revealed 100% nt identity in the HSP70 gene with a number of GLRaV-3 sequences, including isolates from Austria, Slovenia, the USA, and Poland (AJ748511.1, KP867017.1, KF417609.1, KF029732.1). ON009179 revealed 100% nt identity in the CP gene with the GLRaV-3 isolates from China, Iran, Australia (JX088189.1, OK274305.1, OR614439.1). ON014525 displayed 88-89% nt identity with GFLV isolates from Crimea and Tunisia (MW970077.1, PQ736799.1, MH545679.1) in the CP gene. For the ON035731, the similarity was found with GFkV isolates from Canada and the USA (KT694349.1, KF417611.1, GU372369.1) with 97% nt identity in the replicase gene. ON035730 revealed 96-98% nt identity with GFkV isolates from the USA, Russia, and South Korea (GU372368.1, ON584178.1, LC897302.1) in the replicase gene, respectively.

This study provides the first molecular confirmation based on sequencing analysis of GLRaV-1, GLRaV-3, GFkV, and GFLV in Georgia. Sequence analysis revealed high nucleotide identity between Georgian virus isolates and those reported from Europe, North America, and Asia. Phylogenetic analyses were conducted to determine the genetic relationships between grapevine virus isolates identified in this study and reference sequences retrieved from GenBank. Phylogenetic analyses performed in Geneious Prime confirmed the close genetic relationships between grapevine virus isolates detected in western Georgia and previously reported isolates from other grapevine-growing regions worldwide. In all constructed phylogenetic trees, Georgian isolates clustered among reference sequences retrieved from GenBank and did not form distinct,

geographically separated lineages (Fig. 1). In the GFkV phylogenetic tree based on partial replicase gene sequences, isolates identified in this study clustered closely with isolates originating from North America, Europe, and Asia. Short branch lengths observed within this cluster indicated low genetic divergence and a high level of sequence conservation in the analyzed genomic region. This phylogenetic pattern, together with the high nucleotide identity values obtained through BLAST analysis, reflected the conserved genetic structure of GFkV and suggested limited regional diversification. Similar clustering patterns were observed for GLRaV-1, GLRaV-3, and GFLV, whose Georgian isolates grouped with previously reported international strains. In particular, GLRaV-3 isolates exhibited up to 100% nucleotide identity with international strains and were presumptively assigned to genetic variant group I, one of the most globally distributed GLRaV-3 groups (Diaz-Lara, A. *et al.* 2018).

Notably, visible disease symptoms were observed only in samples infected with GLRaV-1 and GLRaV-3 showing reddish downward leaf roll with veins remaining green while GFkV- and GFLV-infected vines remained asymptomatic. This observation was in agreement with previous studies reporting latent or mild infections for certain grapevine viruses and highlighted the limitations of symptom-based diagnosis (Fajardo *et al.* 2012; Kubina *et al.* 2022) as the absence of visible symptoms in infected samples underscores the limitation of visual inspection for virus diagnosis in grapevine and therefore, reinforces the need for routine laboratory-based diagnostics to accurately assess virus prevalence and prevent inadvertent dissemination through planting material. These findings provided essential baseline data for the development of national grapevine virus monitoring and certification programs aimed at safeguarding the sustainability and quality of Georgian viticulture.

Previous surveys of grapevine viruses in Georgia have been limited to eastern regions and relied primarily on serological and PCR-based diagnostics without sequence validation. The present findings, therefore, can fill a significant geographical and methodological gap and indicate that economically important grapevine viral pathogens are probably more widely distributed across Georgian viticultural regions than previously documented. The detection of both single and mixed infections among the analyzed Saperavi samples is consistent with reports from other grapevine-growing countries, where co-infections are common due to vegetative propagation and long-term vineyard establishment. Mixed infections involving GLRaV species are of particular concern, as they have been associated with increased disease severity, cumulative yield losses, and

deterioration of grape and wine quality (Alabi *et al.* 2016; Basso *et al.* 2017). The occurrence of such infections in western Georgia highlights the potential risk for sustained virus spread and emphasizes the necessity of comprehensive virus monitoring programs.

Phylogenetic analyses conducted using Geneious Prime further corroborated the molecular identification of grapevine viruses detected in western Georgia and provided insight into their genetic relationships with globally distributed virus populations. Across all analyzed viruses, Georgian isolates clustered within established phylogenetic groups and did not form distinct monophyletic clades, indicating the absence of strong phylogeographic structuring. These findings suggest that grapevine viruses present in Georgian vineyard are genetically related to globally distributed virus populations and were likely introduced through infected propagation material rather than arising from localized evolutionary divergence. The observed genetic relatedness underscores the importance of implementing certified virus-free planting material and strengthening phytosanitary control measures in grapevine nurseries.

Georgia, as one of the world's oldest wine-producing countries, places strong emphasis on wine quality and vineyard sustainability. The confirmation of multiple grapevine viruses in western Georgian vineyards provides essential baseline data for the development of national grapevine health certification programs and targeted disease management strategies. Further investigations focusing on the genetic diversity, population structure, and spatial distribution of grapevine viruses across different viticultural regions of Georgia are warranted to better evaluate their epidemiological impact and support long-term viticulture sustainability.

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Table 1. Vineyard surveyed for GLRaV-1, GLRaV-3, GFkV, and GFLV viruses. Samples tested by Das-ELISA and One-Step RT-PCR are presented

Region	No. of vineyards surveyed	Tested viruses	No. of Das-ELISA positive samples/ tested samples	No. of One-step RT-PCR positive samples/ tested samples	Positive plant samples/single and mixed infections
Adjara; Western Georgia	1	GLRaV-1	2/30	2/2	Sample N3; N4
		GLRaV-3	1/30	1/1	Sample N1
		GFkV	2/30	2/2	Sample N1; N5
		GFLV	1/30	1/1	Sample N3