

# Occurrence of Grapevine Leafroll-Associated Viruses in the East Part of Georgian Vineyards

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**Abstract** – Grapevine leafroll associated viruses (GLRaV) in the family of Closteroviridae are the most widespread and dangerous viral pathogens of severe grapevine viral infection - Grapevine leafroll disease (GLD). Among this viruses GLRaV-1 and GLRaV-3 are the main causative agents of GLD. Economic impact of grapevine viral agents can be dramatic for the grape industry. Only a limited number of viruses infecting grapevine have been identified in Georgian vineyards which is the ancient and one of the main wine-producing region. It is important to focus on increasing the quality of wines produced from the widely distributed indigenous grape varieties in Georgian vineyards such as white-berried cultivar-Rkatsiteli and red-berried cultivar-Saperavi in order to produce wine that meets international quality standards. Main goals of the proposed study were to determine which member of Grapevine leafroll associated viruses is prevalent in Georgian vineyards also application of serological methods for the detection of different serological groups of the viruses. Sampling for the detection of GLRaV-1 and GLRaV-3 viruses was conducted from Rkatsiteli and Saperavi grapevine cultivars growing in vineyards located in traditional wine-producing region Kakheti, the east part of Georgia. A total of 600 samples (300-Rkatsiteli and 300 Saperavi) were collected and analyzed from twenty different locations during 2016-2017 growing seasons. The study showed the distribution of GLRaV-1 and GLRaV-3 as either single or mixed infections. Virus profiles were determined for individual vines. The overall analysis showed that GLRaV-3 virus was the prevalent also mixed infections were found when the grapevine was infected with both of the viruses. Viral disease control strategies that involves the use of virus-free planting material for further propagation in nurseries from tested vines by use of comprehensive laboratory assays will be the outcome in order to be sure in quality of new plant material.

**Keywords** – *Vitis Vinifera*, Virus, GLD, GLRaV, Elisa.

## I. INTRODUCTION

Plant viral diseases present a global threat to the maintenance of the environment and unceasing economical development of the countries. Economic impact of the viruses that infects grapevine can be considerable for the grape industry. Nowadays more than 60 infectious intracellular agents including viruses and viroids are disseminated globally and cause severe grapevine diseases with infliction of damage on vine health and grape quality resulting in detrimental effect on brix and wine color and heavy yield reduction in the most important grapevine-growing landsides all over the world [1; 2]. Worldwide some common dangerous economically important viral agents affecting wine grapes (*Vitis vinifera* L.) are *Grapevine leafroll associated virus 1* (GLRaV-1) and -3 (GLRaV-3). These viruses from the genus *Ampelovirus* are the most important species among the vector-borne virus species complex in the family of *Closteroviridae* that may cause grapevine leafroll disease (GLD) that might be one of the major factors affecting grapevines regarding their quality [3]. Only a limited number of viruses infecting grapevine have been identified in Georgia. Last studies showed that evidence of viticulture during the early Neolithic Period was obtained from Georgia [4]. According to its importance as a wine-producing region Georgia is focused on increasing the quality of wines produced from indigenous grape varieties (white-berried cultivar-Rkatsiteli and red-berried cultivar-Saperavi), making them in a clean international style therefore estimation of the prevalence of grapevine viral agents in Georgian vineyards

is of great importance. Within the Georgia, Kakheti region is the number one producer of grapevine including wine grapes. Despite what is known about viruses in wine grapes, virtually no studies have focused on the distribution of grapevine viruses in Georgian vineyards. To further understand the epidemiology of viruses in the east part region of Georgia a survey was conducted through the growing seasons during 2016-2017. Six hundred grapevine samples were collected and tested for the presence and occurrence of Grapevine leafroll viruses -1 and 3. Widely distributed two grapevine cultivars were sampled and tested for the detection of viral agents: 1. Rkatsiteli (literally “red stem”) is a kind of grape used to produce white wine. This ancient vinifera originates in Georgia and is one of the oldest grape varieties [5]. According to an area of vineyards Rkatsiteli is a primary variety in Georgia and it is widely distributed in Kakheti and Kartli region. 2. Saperavi (literally “paint, dye”) is a grape variety native to Georgia, where it is used to make many of the region’s distinctive red wine. According to an area of vineyards Saperavi is a primary red grape variety in Georgia and mostly distributed in Kakheti region. The primary goals of proposed study was a survey of Georgian vineyards in order to document the presence of grapevine viruses GLRaV-1 and GLRaV-3 by knowing exactly what viruses are distributed in Rkatsiteli and Saperavi grapevine cultivars also application of reliable serological approaches in order to develop and apply a better diagnostic tool for the analysis of grapevine viral agents.

## **II. MATERIALS AND METHODS**

### *A. Survey and Sampling*

Survey was conducted in the vineyards located in the east part of Georgia in the traditional wine-producing region of Kakheti. As the concentration of GLRaV-1 and 3 in grapevine tissue might be irregular and varies according to time of the year and tissue source well-developed mature leaves including petioles and veins, from the lower part (basal leaves) late in the growing season as well as bark (phloem) scrapings on mature canes, were the most relevant tissue sources for the detection of viral infection [6]. In late Spring and early Autumn twenty vineyards ten for each season, respectively were selected for the survey of GLRaV-1 and 3 viruses during 2016-2017 years. A total of 600 samples, 30 samples for each vineyard (300-Rkatsiteli and 300 Saperavi) were collected. Symptoms of Grapevine leafroll disease (GLD) varies greatly with the grape cultivar, season and climatic conditions. Some grapevine varieties can be completely symptomless. Samples were collected according to the typical symptoms of leafroll disease on the red grape leaves, including reddening of the interveinal areas while the primary and secondary veins remain green. In white cultivars symptoms of GLD exhibit chlorotic appearance of the interveinal area and leaf margins downward rolling [7]. Besides the sampling according to the expressed symptoms sample collection were also conducted randomly as GLD symptoms may not be recognizable. Considering the possibility of uneven distribution of the virus within the plants, several subsamples from different canes were sampled. One plant sample that was tested for the GLD disease was equivalent to a one grapevine and consisted of over 5 leaves including petioles and cambial scrapings.

### *B. Methods*

Many different serological diagnostic formats have been developed for the screening of GLD disease. Among these techniques enzyme-linked immunosorbent assay (ELISA) remains robustness, cost effective and popular detection method for routine testing capable of large-scale screening for the GLD associated viruses GLRaV-1

and 3 [8; 9]. In the proposed study GLRaV-1 and 3 presence in collected samples were tested throughout serological analysis using the DAS-ELISA assay (Bioreba DAS ELISA complete Kit; Switzerland). DAS-ELISA reagents contain poly- and monoclonal antibodies developed against purified viruses of different isolates as well as a recombinant coat protein of the viruses. The mixture of these selected antibodies were capable of broad-spectrum detection of all scientifically recognized and published isolates so far, notably isolates of genetic variant groups I, II, III and VI, including serologically distinct isolates in case of GLRaV-3 [10]. Collected samples of grapevine cambial scrapings or petioles of basal (mature) leaves were homogenized using semi-automated homogenization (Homex 6 by Bioreba, AG, Switzerland) in the special extraction buffer “Grapevine” (0.2 M TRIS, pH 8.2) (Bioreba) to provide plant extract containing viral proteins for further analysis. Each extracted sample and positive and negative controls were run in triplicate while extraction buffer as a standard was run eight times. Das Elisa assay was performed according to manufacturers’ instructions. Absorption value of mean was calculated using cut-off formula to determine positive and negative results based on the positive and negative controls that come with the kit and standards presented by the extraction buffer with an appropriate OD value. Plant samples were stored at -80°C freezer for further molecular analysis.

### III. RESULTS

Survey for the detection of GLRaV-1 and GLRaV-3 viruses was conducted from widely distributed grapevine cultivars (Rkatsiteli and Saperavi) growing in vineyards located in traditional wine-producing region Kakheti, the east part of Georgia. A total of 600 samples (300-Rkatsiteli and 300 Saperavi) were collected from twenty vineyards during 2016 and 2017 growing seasons (Fig. 1). The study determined the incidence of both virus serotypes (34.2%) as either single or mixed infections (Fig. 2). Virus profiles were determined for individual vines. Analyses showed that GLRaV-3 virus was predominantly distributed in Saperavi cultivar (20.7%) (Fig. 3) while in case of Rkatsiteli both viruses were presented (GLRaV-1-18%, GLRaV-3-17.7%) equally (Fig. 4). The overall analysis showed that GLRaV-3 virus was the prevalent and contributed 19.2% of total samples while GLRaV-1 virus was presented in 15% case respectively. Mixed infections were found in 3% of samples.

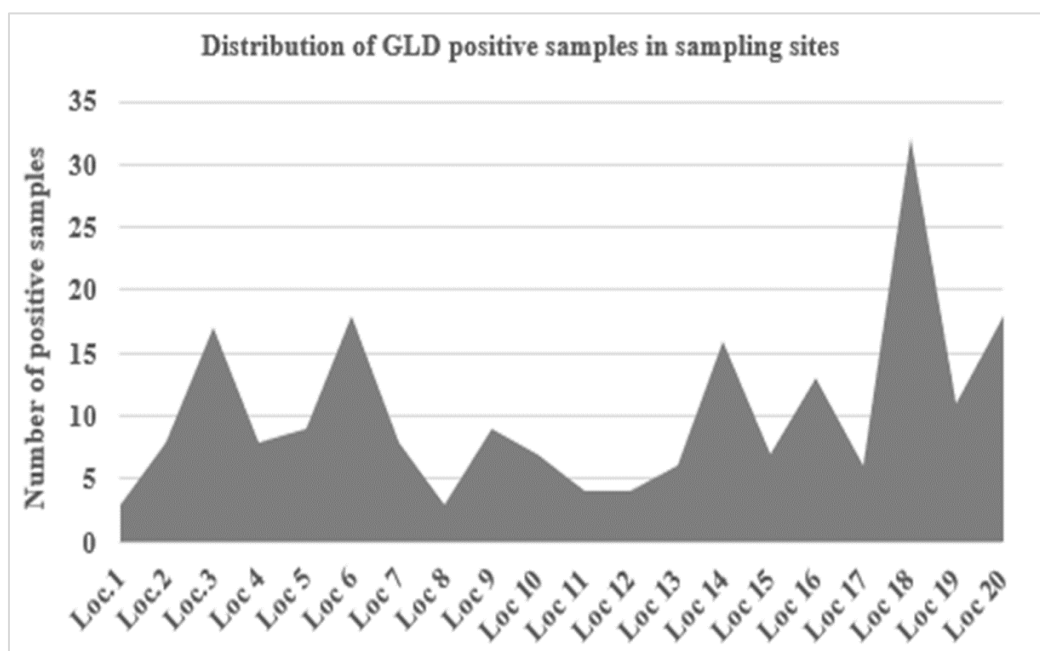


Fig. 1. Distribution of Positive samples in sampling sites.

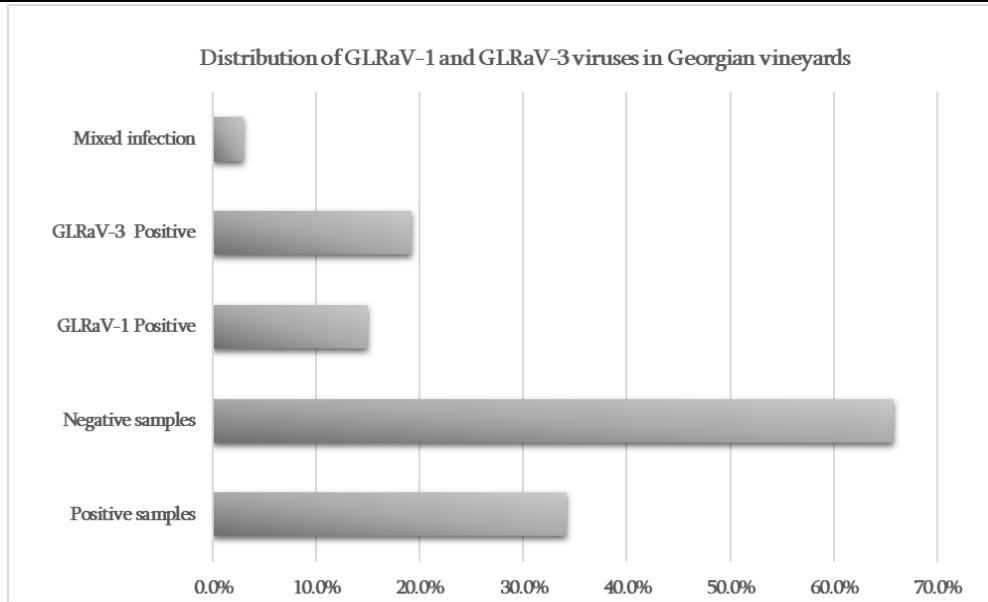


Fig. 2. Incidence of GLRaV-1 and GLRaV-3 viruses.



Pic.1. GLRaV-3 positive sample in Sapervi Grapevine cultivar. Pic. 2. GLRaV-3 positive sample in Rkatsiteli Grapevine cultivar.

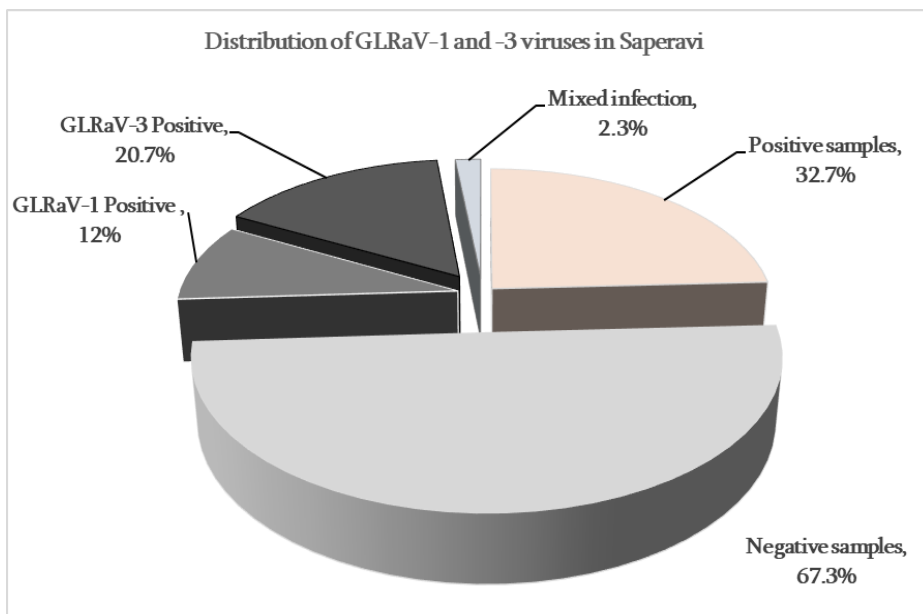


Fig. 3. Distribution of GLRaV-1 and GLRaV-3.

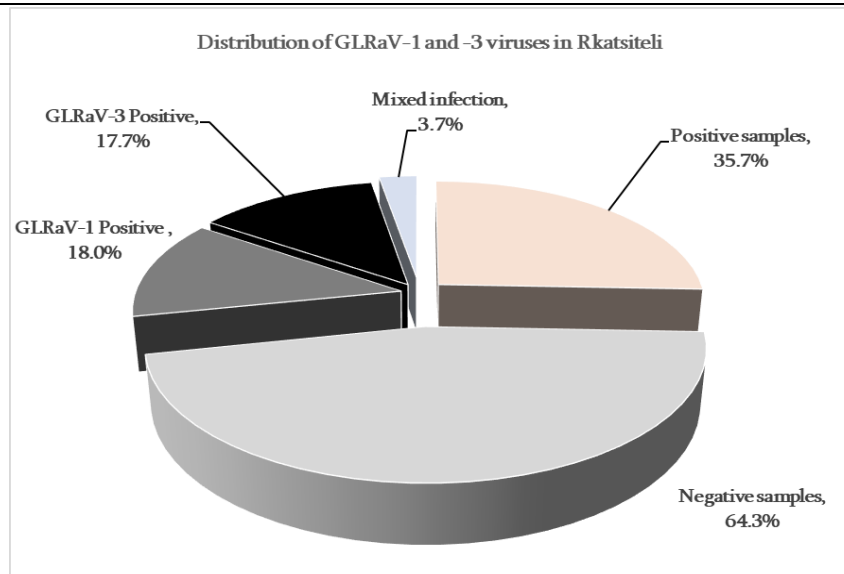


Fig. 4. Distribution of GLRaV-1 and GLRaV-3 viruses in Saperavi Grapevine cultivar viruses in Rkatsiteli Grapevine cultivar.

#### IV. CONCLUSION

The presence of viruses in Georgian vineyards is of great concern for growers due to likely movement and spread of virus diseases within the vineyards that might result in economic losses to the grape industry. The overall outcome of this study will be valuable to develop integrated decision matrices for vineyard managers to implement profit-maximizing disease control strategies based on using virus-free planting material from tested vines for further propagation in nurseries in order to be sure in quality of new plant material or define whether infected vine is removed from the vineyard or virus free rootstocks are used for establishing new vineyards by use of comprehensive laboratory diagnostic procedures. Elisa assay still remains a valuable and reliable method for high-throughput and large scale screening of samples. With this knowledge reliable detection systems will be developed and put in place in order to detect all known viruses in Georgian vineyards. This study will greatly improve awareness of virus diseases of grapevine in Georgia and will aid grower decision making regarding ailing vines and thus reducing virus spread.

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