International Journal of Recent Advances in Multidisciplinary Topics
Volume 6, Issue 3, March 2025
https://www.ijramt.com | ISSN (Online): 2582-7839

Assessment of the Most Important Viruses Affecting Othello Local Grapevine Variety

Juma Lihwa Nsongoma[1*], Gyorgy Pasztor[2], Andras Takacs[3]
[1]Research Officer, Tanzania Official Seed Certification Institute, Morogoro, Tanzania
[2,3]Hungarian University of Agriculture and Life Sciences, Georgikon Campus, Keszthely, Hungary

***Abstract*: Grape (Vitis vinifera) belonging to the family of Vitaceae, it is one of the most important economic fruit crops in the world. In Hungary, for several thousand years, vine has been grown in the Carpathian basin, where the climatic and soil conditions are favourable for growing grape vine. However, it is facing adversity of multiple virus infections that influenced negatively the performance of the cultivars at a certain extent, which cause premature death of the stocks and generating great yield losses. The study was conducted in a Grapevine plantation from the area of Central Transdanubia (Lesencefalu) (Figure 2). 60 samples were collected from Othello grapevine variety and analyzed in the laboratory to investigate the virus infection using DAS-ELISA. 6 viruses category (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 and GFkV) were found to infect this variety (Appendix 1). Among those 60 samples, 27 samples were infected with viruses while 33 samples were not infected. The highest infection was found to be caused by GLRaV1 while the lowest infection was found to be associated with GLRaV3 (Figure 4). However, 13 samples were found to be infected with more than one virus (multiple infections) (Table 2), while 14 samples were found to be infected with only one virus (single infection) (Appendix1).**

***Keywords*: Viticulture, Virus infection, Grape vine, Cultivar, Varieties, Viral diseases.**

1. Introduction

A. Background Information

Viticulture is the cultivation and harvesting of grapes. It is a branch of science of horticulture. Also, it refers as the studying and growing grapes, either for wine production or for raw consumption. It includes all the agricultural studies, efforts, and actions of growing grapes until the day of harvest.

1) The Viticulture Worldwide

Grape (Vitis vinifera) belonging to the family of Vitaceae, it is one of the most important economic fruit crops in the world (Senthil et al., 2011; Kumar, 2010). It ranges from Western Europe to the Persian shores of the Caspian Sea; the vine has demonstrated high levels of adaptability to new environments. This makes viticulture to be found on every continent except Antarctica. Grapevines are broadly classified into red- and white-berried cultivars based on their fruit skin color, although yellow, pink, crimson, dark blue and black-berried cultivars also exist. Red berried cultivars have anthocyanin pigments in berry skin, while white-fruited cultivars lack this pigment due to nonfunctional of regulatory genes of the anthocyanin biosynthetic pathway (Walker et al., 2007). In worldwide, the major countries which produce grape includes China, (it occupying the top position) 12.85%, followed by Italy with 11.57%, USA with 9.24%, Spain with 9.07 % and France 8.69 % (FAO, 2012). According to FAO (2012), these productions accounting for about 51.42% of total world production.

2) The Importance of the Vine

This fruit is processed into different products including juice, wine, and raisins (dried fruit), it is also consumed as fresh (Buyukbay et al., 2011). Large percentage of grape production of the world is used to make wine. Approximately 71% of grapes produced in the world is used for wine, 27% is consumed as fresh fruits and 2% as raisins (FAO, 2012). Grapes peels are essential source of oil and pectin. They also serve as raw material for production of cattle feed and used for preparation of candies (Kumar, 2010). Grapes raisins are rich source of sugar especially fructose and antioxidants. The juice of raisins is used in cosmetics to bleach and soften skin (Creasy, 2009). Grapes also are very useful in fighting with diseases like dyspepsia, hemorrhoids, stones in the urinary tract and bile ducts. It also activates the functions of the liver, support ease digestion, helps to reduce blood cholesterol level and eliminate uric acid (Kumar, 2010).

3) The viticulture in Tanzania

In Africa, grapes are produced in many countries. The most leading country for grape production in Africa is South Africa (Mpore, 2013). In Tanzania grapes are produced in Dodoma region.

Grapes are one of the major economic importance fruit crops in Tanzania. In Dodoma, grape production is one of the biggest cash crops in the region. It is the main stay for many farmers in Dodoma Municipal and the nearby districts of Chamwino and Kongwa. The urban Dodoma produces 70% of the grapes and rural Dodoma produces 30% (SNV Tanzania a report on fresh fruits, 2005). This crop has multi-usage such that it can be eaten raw or can be used for making jam, juice, jelly, wine, grape seed-extracts, raisins, vinegar and grape -seed oil. The crop is considered as a symbol crop for Dodoma region. According to the history, grapevines are believed to be introduced in Dodoma region by missionaries in the year of 1940 (MAFS, 2006).

The first small wineries were started in Bihawana and Hombolo missions. The missionary started to produce wineries

*Corresponding author: nsongomam@gmail.com

as communal wine for church purposes only. In 1963, grape production started to expand, when the Isanga prison started growing grapevine. They started with only four acres, and three years later the crop was gradually introduced to the five villages namely Mpunguzi, Msalato, Nala, Nkulabi and Mundemu. Then, the National Service Camp at Makutupora - Dodoma accepted the idea and increasing the acreage of vine production. This made the yields rising high from the grapes to be consumed fresh as table grapes to wine production. Since its introduction, vine cultivation has become fully adapted and contributes significantly to household income (Safari et al., 2015). This is due to the fact that, grapes are considered as one of the most important cash crops, raw materials in wine industry and a source of employment to the people living in Dodoma region.

4) The Viticulture in Hungary

In Hungary, for several thousand years, vine has been grown in the Carpathian basin, where the climatic and soil conditions are favourable for growing grape vine (HAJDU, 2018). The first grape vine was introduced by the Romans to Pannonia, and by the 5th century AD, there are records of extensive vineyards in what is now Hungary (Smithsonian, 2013). After introduction, vine growing has been developing until it reached its current status. Viticulture has been operated for the purpose of propagation material, table grape and wine grape. The largest area for viticulture is used to produce wine grape varieties, of which 72% is white wine and 25% is red wine (HAJDU, 2018). The remaining area (3%) is used for table grapes. The total area of vineyards is about 63000 ha, and this area is seems to be divided in 22 wine regions (HAJDU, 2018). Among these regions, 75% of the vineyards are on hills and mountains and 25% of them are on the Great Hungarian Plain (HAJDU, 2018). Hungary is very rich in vine biodiversity. It has a lot of native and valuable bred varieties and clones in cultivation (Smithsonian, 2013). Due to continental climate, resistant and winter frost resistant vine varieties have an important role. Currently, a lot of varieties are available for producers and consumers. For example (Fanny, Nero, Teréz, etc.) are varieties resistant to insects damaging. These table grape varieties are in focus because they are suitable for environmental viticulture and for bio-products. By adapting to the continental climate, frost resistant and easy-to-bred varieties took advantage while preserving the traditions of the respective vine region. The main varieties in Hungary are White wine, they includes: Furmint, Welschriesling, Bianca, Chardonnay, Cserszegi fűszeres and Rajna Riesling and red wine varieties are: Cabernet Franc, Blaufränkisch, Blauer Portugieser, Merlot and Zweigelt (HAJDU, 2018). These modern vineyards which are plated in the wine regions, are not only favourable from the aspect of economy but also, they have a beautiful landscape.

B. Problem Statement and Justification

Grapevines are, like any other plant species, exposed to environmental influences, and several pests. It can be affected by living (biotic) and non-living (abiotic) factors. As a result of those factors, is disrupting of the key processes in plants such as: photosynthesis, respiration, transportation of water and nutrition, growth and reproduction.

Non-living factors are unfavorable weather conditions (cold, heat, drought, heavy rains, hail, strong winds), lack or excess of nutrients, poor soil conditions (compaction of the soil, inadequate pH), toxic substances in the soil, water and/or air, inappropriate treatment of the plants; for example, phytotoxic of the pesticides (Martelli, 2014). Also living factors, which threaten plants spices, are fungus, fungi-like organisms, bacteria, phytoplasma, viruses and viroids.

Viruses are microscopic pathogens living inside the living cells. After entering into the vines, they spread into all underground and above ground plant parts. In the nature, viruses are transmitted through vectors – insects, mites and nematodes. The most reported viruses of grape-vine disease in Hungary are: Grapevine Feanleaf virus (GFLV), Grapevine Leafroll Associated Virus (GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-6, GLRaV-7), Grapevine fleck virus (GFkV), Arabis mosaic virus (ArMV), Tomato black ring virus (TBRV), Grapevine chrome mosaic virus (GCMV), Alfalfa mosaic virus (AMV), Grapevine Bulgarian latent virus (GBLV), Grapevine virus A (GVA), Grapevine virus B (GVB), Grapevine rupestris stem pitting- associated virus (GRSPaV) and Grapevine line pattern virus (GLPV) (Cseh et al., 2012). Due to continuous vegetative propagation and breeding new vine varieties. Hungarian grape plantations are permanent targets of different viruses. The constant viral infections of Hungarian vine, lead to reduced yield and quality, shortening in productive period, weakening in rooting of propagation materials, reduction in disease resistance to abiotic and biotic stressors (Cseh et al., 2012). Because of this reason, it was therefore an essential investment to study (to perform virological examination) using Serological methods to check the presence of new viruses or current six viruses (Grapevine leafroll associated virus (GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-6, GLRaV-7) and Grapevine fleck virus (GFkV) infecting Hungarian grapevine.

C. Objectives

1) General Objective

The aim of the experiment was to find out whether the direct-growing grape varieties can be infected with the most important domestic grape viruses, and the rate of infection in a plantation.

2) Specific Objectives

i. To identify the local or Hungarian grapevine variety for samples collection.
ii. To determine the rate of virus infection in 60 samples of grapevine.
iii. To identify the most important viruses affecting Hungarian vineyard.

2. Literature Review

A. Morphology of Grapevine

Grapevine is a vigorous and climbing woody vine; it may reach a height of 15-20 m in the wild. For cultivated vines, the development is regularly reduced through pruning of shoots and leaves every season. The fruit is a perennial, polycarpic, and deciduous species (Keller, 2010). Since it is a woody

procumbent plant, it uses its tendrils to climb and elongate to branches by bearing lobed leaves and clusters of flowers (Figure 1). The upper structure of the plant is termed as canopy and it is shaped by training the vine into specific arrangement, this lead to favorable growth and better production, but depending on the seasons and the grapevine varieties (Hellman, 2003).

1) Rootstock and Scion

Grapevine grows on its own rootstock known as self-rooted vines (Hellman, 2003). On the other hand, due to their combination of desirable features, grafted vines are more preferred. The scion consists of the shoot portion which has the desired fruit properties, and the rootstock is commonly used for resistance against phylloxera and mildews diseases. For graft compatibility, the correct positioning of the graft union is very important. The vascular cambium of the stock and scion must be connected to each other in order to enable nutrient and water channels work properly Keller, 2010).

2) Root System

Grapevine root system is normally multi-branched and spreads horizontally or vertically (Figure 1). But it can be affected by soil fungi (mycorrhizae) that enable to decrease their growth and influence their nutrient uptake (Hellman, 2003).

3) Trunk and Shoot

The aboveground part (stem) of the grapevine is formed by the trunk, the arms and shoots (Figure 1). In order to support shoot development, specific training systems are used by using a cordon and wire to support the trunk (Keller, 2010). The shoots always generate tendrils which hold other growing shoots of the vine.

4) Leaf

The broad leaves of the vine plant are produced on the apical meristem. Grapevine has four types of leaves which are: Bracts (are the small leaves usually found at branch points), Cotyledons (embryonic leaves), foliage leaves and Scales leaves which grow around the buds (Keller, 2010).

5) Flowers and Fruit

In general grapevine cultivars express perfect hermaphroditic flowers and usually a productive shoot generates about one to three flower clusters (Hellman, 2003).

6) Viral Diseases of the Grape

Grapevines can be subject to attacks by many different pests and pathogens, including graft-transmissible agents such as viruses, viroids, and phytoplasmas (Martelli, 2014). Currently, approximately 65 different viruses belonging to nearly 30 different genera, eight viroids and four satellite RNAs belonging to different families have been reported and documented infecting grapevines (Martelli, 2012; Oliver and Fuchs, 2011). More viruses have been identified in grapevines than in any other woody perennial crop in the whole world. Grapevines viral diseases can be classified according to disease they cause or are associated with. Currently there are four major groups based on the disease they cause or are associated with which have been documented in the worldwide. These major groups includes, viruses involved in the degeneration/decline disease complex, viruses associated with the leafroll disease complex, viruses associated with the rugose wood complex, and viruses associated with the fleck disease complex. There some other grapevine viruses are apparently not associated with disease but they are suspected to have a minor impact (Martelli, 2000; Martelli, 2014 and Oliver and Fuchs, 2011). The majority of grapevine viruses have a RNA genome. There are also two viruses with a DNA genome have been reported recently. These viruses are termed as a badnavirus associated with vein-clearing and vine decline syndrome and a geminivirus associated with red blotch symptoms (Zhang *et al*., 2011; Al Rwahnih *et al*., 2013; Krenz *et al*., 2012 and Poojari *et al*., 2013)**.** These viruses formerly they have an emerging economical important constraint to grapevine production. Most virus-induced grapevine disorders are regarded as complex diseases, because they show different symptoms and they are caused by different viral species. For example, "infectious degeneration" is characterized by two syndromes, malformation (fanleaf) and leaf yellowing, and decline, both are caused by several species of the genus *Nepovirus*; "leafroll" caused by viruses of the family *Closteroviridae* and "rugose wood" (pitting to deep longitudinal grooves of the woody cylinder) which is caused by viruses of the genera *Vitivirus* and *Foveavirus* (Martelli 2014).

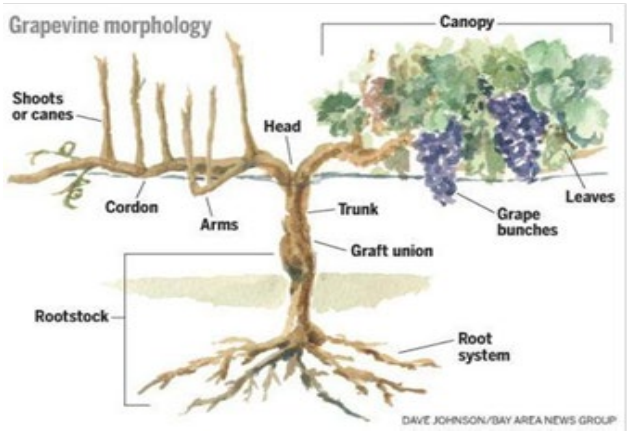

Fig. 1. Grapevine morphology structure
Source from: http://www.mercurynews.com/2015/04/07/wine-trivia-anatomy-lesson-from-rootstalk-to-tendrils/

Among the virus and virus-like diseases, grapevine leafroll disease (GLD) is by far the most widespread and economically damaging viral disease of grapevines in many regions around the world (Freeborough and Burger, 2006; Naidu *et al*., 2008 and Nimmo-Bell, 2006). A recent economic study indicated that GLD, depending on the level of disease incidence, yield reduction, and impact on fruit quality, can cause an estimated loss of approximately $25,000 to $40,000 per hectare in the absence of any control measure (Atallah *et al*., 2012).

7) Viruses Associated with Grapevine Leafroll Disease

The grapevine leafroll associated viruses (GLRaVs), these are viruses which are similar morphologically but serologically and genetically are distinctive viruses. They usually numbered in a serially way as GLRaV-1,-2, -3, -4,-6-7 and so on (Martelli *et al*., 2012). These viruses are belonging to the plant virus family *Closteroviridae* (Karasev, 2000 and Martelli *et al*., 2002). The virions properties of GLRaV are flexuous rods, with

lengths ranging between 1,400 nm and 2,200 nm and a diameter of approximately 12 nm. They are encapsidate a linear, positive-sense, single stranded RNA genome with a possible cap structure at the 5′ terminus and no poly (A) track at the 3′ terminus (Karasev, 2000). The genomic RNA of GLRaVs may constitute around 5% of the total mass of virions (Karasev, 2000). The GLRaVs are like other member of the family *Closteroviridae* which can have bipolar virions and most of the genome is encapsidated by the coat protein and the 5′ extremity of virions, they have a segmented "tail" structure which is made up of several other virus-encoded proteins (Peremyslov *et al*., 2004 and Satyanarayana *et al*., 2004). The segmented tail of a closterovirus plays a role as a movement device which guiding directional transport of viral genomes between neighboring cells (Dolja *et al*., 2006). This indicates that the segmented tails of GLRaVs might have similar functions as other members of this family. Based on the current understanding of the functional roles of proteins and *Citrus trizteza virus* (Dawson *et al*., 2013), Proteins encoded by GLRaVs could be multifunctional, involved in various aspects of the virus life cycle, this is based on the protein encoded by *Beet yellows virus* (Dolja and Koonin, 2013) and *Citrus trizteza virus* (Dawson *et al*., 2013). The grapevine leafroll associated viruses are limited to phloem-associated cells, they replicate in companion and phloem parenchyma cells. They have effect on the cytology of differentiating sieve tubes, parenchyma, and companion cells (Castellano *et al*., 2000, Faoro, 1997). So, this is the characteristic of plant infection by members of the family *Closteroviridae* (Zhou et al., 2002). Generally, GLRaVs exist in low concentrations and are unevenly distributed in grapevines (Tsai *et al*., 2012). The infection of these viruses in grapevine occurs as mixed infections of several GLRaVs or GLRaVs with other viruses. This mixed infection is due to continual vegetative propagation of grapevines for long time and grafting between different scion and rootstock materials (Fuchs *et al*., 2009; Naidu, 2011 and Prosser *et al*., 2007). So mixed infection of GLRaVs it can lead to either synergistic or antagonistic interactions (Syller, 2012). In the area where grapevine leafroll disease is present, GLRaV-3 appears to be the most widespread, as single or mixed infections with other GLRaVs, grapevine viruses and viroids (Charles *et al*., 2006 and Maree *et al*., 2013).

a) Taxonomy, Genome Organization, and Diversity of GLRaVs

The grapevine leafroll associated viruses (GLRaVs) is belonging to the distinct genera in the family of *Closteroviridae*. The GLRaV-1, -3, and -4 are assigned to the genus *Ampelovirus* (Martelli *et al*., 2012). This genus is derived from ampelos, which means grapevine in Greek, with GLRaV-3 as the type species. The GLRaV-2 is belongs to the genus *Closterovirus*, this genus is also derived from Greekword ''kloster'' which means thread exemplified by *Beet yellows virus* (Al Rwahnih *et al*., 2012). There is also the new proposed genus *Velarivirus* where GLRaV-7 is assigned (*Velarivirus* is from velari, meaning cryptic in Latin) (Al Rwahnih *et al*., 2012). Current taxonomic reviews have designated GLRaV-5, -6, -9, GLRaV-Pr, GLRaV-De, and GLRaV-Car as genetically divergent strains of GLRaV-4 because of the similarity in their overall genome size and genetic organization (Abou Ghanem-Sabanadzovic *et al*., 2010). Therefore, these former virus species are now grouped under the umbrella term "GLRaV-4-like" viruses (Martelli *et al*., 2012). Currentlly, the full-length genome sequence of almost all known GLRaVs is available in public databases. The GLRaVs are genetically diverse with open reading frames (ORFs) encoded by each virus varying in size and number. Nowdays, sequence data indicate that GLRaV-4 strain 'Car' is the smallest of the GLRaVs with 13,626 nucleotides (nt) encoding six ORFs (Abou Ghanem-Sabanadzovic *et al*., 2010), and GLRaV-3 is the largest and the most complex with 18,671 nt encoding 12 ORFs (Fei *et al*., 2013 and Jarugula *et al*., 2010,). The genome organization of GLRaVs has similarities to the gene modules characteristic of closterovirids, although some differences are notable and appear to be unique to viruses associated with grapevine leafroll. Comparatively to the members of the genus *Closterovirus*, all GLRaVs have a signature replication gene block (RGB) that covers a large portion of the genome toward the 5′ terminus (Dolja *et al*., 2006). So the replication gene block is made up of replication-associated proteins encoded by Open Reading Frames 1a and 1b. The ORF1a of GLRaV-3 and GLRaV-4 and its strains contains an AlkB domain within the large inter-domain region, this makes them to differ from other GLRaVs (Abou Ghanem-Sabanadzovic *et al*., 2012), and Thomson *et al*., 2012). All GLRaVs, ORF1b expresses the RNA-dependent RNA polymerase-like domain via a +1 frameshift translation (Thomson *et al*., 2012). But the other ORFs are located downstream of replication gene block toward the 3′ terminus of the genome. A signature quintuple gene module, consisting of a 6-kDa protein (p6 or its homologue), a heat-shock protein 70 homolog (HSP70h), a ~60-kDa protein (p55 or its homologue) (Thomson *et al*., 2012). The CP and a minor CP (CPm), is present in GLRaV-1, -2, and -3, but not in GLRaV-4 and its strains -5, -6, -9, -Pr, -De, and -Car, and GLRaV-7 (Thomson *et al*., 2012). The arrangement of CP and CPm in GLRaV-2 is similar to that in the members of the genus *Closterovirus* while the GLRaV-1, -3, and -7 genomes have CP and CPm Open Reading Frames in the reverse order relative to GLRaV-2 (Abou Ghanem-Sabanadzovic *et al*., 2012). Further, GLRaV-1 shows an additional peculiarity in that its genome has two divergent copies of CPm. But also, CPm is absent in GLRaV-4 and its strains -5, -6, -9, -Pr, -De, and –Car (Maliogka *et al*., 2009). Therefore, all characterized GLRaVs indicate remarkable differences in number and arrangement of Open Reading Frames that look to be characteristic of each virus species. According to genetic variability, gene organization and the genome size, GLRaVs in the genus *Ampelovirus* are divided into two subgroups (Maliogka *et al*., 2009)**.** These subgroups are GLRaV-1 and -3 in subgroup I, and GLRaV-4 and it strains -5, -6, -9, -Pr, -De, and -Car in subgroup II (Maliogka *et al*., 2009). Different researches on genetic diversity of GLRaV-1, -2, and -3 have shown the presence of genetically diverse, and this genetic diverse are closely related variants in several grapevine-growing regions (Alabi *et al*., 2011). Based on phylogenetic analysis of full-length CP gene sequences, GLRaV-3 indicates the presence of seven possible variant

groups which have been reported in different cultivars and vineyards worldwide (Maree *et al*., 2013). Also, a pairwise comparison of the full-length genome of numerous GLRaV-3 isolates showed an uneven distribution of sequence variation along the virus genome (Maree *et al*., 2013). Because grapevines are clonally propagated and no resistance strain is known in *Vitis* spp, the variants of GLRaVs could be propagated without being subjected to severe purifying selection or bottleneck events (Oliver and Fuchs, 2011). The intrinsically errorprone nature of the viral RNA-dependent RNA polymerase further contributes to global genetic variability of GLRaVs and leading to the increase of genetically diverse, however closely related variants of each virus, often termed "quasi-species" (Domingo *et al*., 2006). But also, an individual grapevine may harbor a myriad of variants whose evolutionary dynamics can be influenced by host- and vector-imposed bottleneck events (Steinhauer *et al*., 1992). In additional, precisely the genetic diversity and fitness of genetic variants among GLRaV-1, -3, and -4 and its strains may be influenced to a greater extent by constraints imposed by horizontal vector-mediated transmissions. However, GLRaV-2 and GLRaV-7 are not yet known to be transmitted by vectors.

b) Impact of Grapevine Leafroll Diseases

Grapevine Leafroll Diseases can cause the reduction of plant vigor and longevity, as well as lead to significant losses in both fruit yield and quality. Between 14 and 40% of crop losses have been reported due to infection of Grapevine Leafroll Diseases (Basso *et al*., 2010). The magnitude of crop yield losses always depends on cultivar rootstock combinations, age of the vines at time of infection, causal viruses, single or mixed virus infection, and environmental conditions **(**Basso *et al*., 2010**)**. Under field conditions, the disease is also reported to affect photosynthesis in some red-berried cultivars (Cretazzo *et al*., 2010). Grapevine Leafroll Diseases lead to modulation of host genes involved in a widespectrum of biological functions (Espinoza *et al*., 2007). It leads to reduce cluster size, loose clusters, and cause the plant to bear small berries. The most effect of this disease in red-fruited *V. vinifera* cultivars is asynchronous fruit ripening and poor color development of berries (Alabi *et al*., 2012). It also alters fruit maturity indices (soluble solids or °Brix, titratable acidity or TA, and pH), and cause modifications of individual and total anthocyanins, total phenolics, as well as total tannins (Alabi *et al*., 2012).

c) Diagnosis

Physical or visual identification of Grapevine Leafroll Diseases symptoms is basically unreliable for consistent diagnosis of the disease under vineyard conditions. This is because of highly variable nature of grapevine leafroll disease symptoms. For example, foliar symptoms of the disease are obvious appear only during late summer and fall, but not in spring. The foliar symptoms also can be due to abiotic and biotic stresses, particularly in red-fruited *V. vinifera* cultivars. The lack of clear physical observable symptoms in white-fruited cultivars, interspecific hybrids, and rootstocks lead to complicated symptom-based diagnosis of the disease in vineyards. Currently, there is improved understanding of working with associated field-based diagnosis and the nature of Grape vine leafroll associated Viruses. There are methodologies and technologies for the specific and accurate detection of individual Grapevine Leafroll associated Viruses. These methodologies which evolved nowadays, they include highly sophisticated and sensitive detection techniques in the laboratory that enable to target individual Grapevine Leafroll associated Viruses and their molecular variants. The current used diagnostic methods in the laboratory, include serological assays (enzyme-linked immunosorbent assay (ELISA)), biological indexing using woody indicator hosts, molecular methods (reverse transcription-polymerase chain reaction (RT-PCR) and PCR) and recent approaches such as micro- and macro-arrays as well as next generation sequencing (NGS). These methods are described below.

d) Biological Indexing

Biological indexing is the method which has been used as an important and fundamental assay for the diagnosis of Grapevine Leafroll Disease. The method is used as a standard method for establishing graft transmissibility of Grapevine Leafrool associated Viruses and other graft-transmissible pathogens associated with a disease, and also further characterization of not yet known or uncharacterized agents. It is used routinely in clean plant programs. In this method, budwood from a vine which have suspected to be infected by the disease is grafted onto an indicator plant by chip-, bench-, or micro-grafting. The grafted plant will be observed for symptoms over 2 to 3 years in a field setting (Rowhani *et al*., 2005). The common diagnostic indicator for Grapevine Leafroll Disease is a *V. vinifera* 'Cabernet franc'. Apart from that indicator, there other indicators like cultivars Pinot noir, Mission, Cabernet Sauvignon, and Barbera which are used for diagnostic of the disease indexing. The choice of a specific cultivar to be used as an indicator host depends on personal preferences that is performing indexing and also on climatic conditions where indicator plants are grown. By using the Cabernet franc, symptoms are interveinal reddening of the leaf blade; they begin in late summer and increasing thereafter with conspicuously green primary veins and rolling downward of the leaf margins. It is impossible to identify a specific virus which is present in a selected vine through biological indexing due to Grapevine Leafroll associated Viruses and their strains to produce similar symptoms on Cabernet franc. Additionally, the nature of asymptomatic of some strains in both GLRaV-2 and GLRaV-7 limits the reliability of biological indexing (Alkowni et al., 20211 and Al Rwahnih *et al*., 2012). However biological indexing is also influenced by various factors which include the efficient spread of viruses from the budwood piece to the recipient indicator host and climatic conditions where the field indexing is performed (Constable *et al*., 2013). Therefore, biological indexing is not always reliable, it is labor-intensive, also requires large field or greenhouse space to grow grafted vines, and it takes 2 to 3 years for vines to grow and show disease symptoms in a field setting.

e) Serological Essays

This is the most common method used to detect the disease. In this method, ELISA is the most common serological method used for rapid detection of Grapevine Leafroll associated

Viruses. The technique is based on the recognition of virus antigens with immunoglobulins or monoclonal antibodies which are produced against purified virions or the virus Coat Protein expressed in *Escherichia coli* cells. There are serological reagents which are commercially available and routinely used in ELISA for the detection of GLRaV-1, -2, -3, -4, and -7 for grapevine tissues (Besse *et al*., 2009). ELISA is one of the methods that are sensitive, reliable, and adapted in high amount applications for testing large numbers of sample. But also, the method has some limitations which can be influenced by various factors that include sensitivity (if a virus is present at extremely low concentrations), specificity (the presence of variants of GLRaVs that may not be recognized by available antibodies) and quality of antibodies (Weber *et al*., 2002). However, ELISA is the method which remains a consistent diagnostic tool in large scale surveys for Grapevine Leafroll Disease and for research purposes (Besse *et al*., 2009).

f) Molecular Fssays

The diagnosis of GLRaVs using molecular assays has rapidly advanced in the past two decades, such that a wide range of techniques are available for more reliable detection. For example, RT-PCR-based technologies have better sensitivity compared to ELISA (Rowhani *et al*., 2000). Its sensitivity is estimated to be 100 to 1,000 times more than ELISA (Rowhani *et al*., 2000). Currently, real-time PCR, by means of detection chemistries such as TaqMan, is increasingly being used for the detection and quantification of GLRaVs in plant tissue and insect vectors compared to conventional RT-PCR assays (Osman *et al*., 2007). The low-density PCR arrays (LDA) using the real-time TaqMan PCR primers or probes complexes in 384-well plates were established for the simultaneous detection and quantification of different GLRaVs (Osman *et al*., 2008). Generally, TaqMan-based real time RT-PCR and LDA detection are the methods which rapid and quantitative provide the required robustness for processing a large number of samples in the detection of GLRaVs (Osman *et al*., 2012). For monitoring the incidence of GLRaVs and their variants, the RT-PCR in combination with high-resolution melting curve analysis has been used in vineyards (Bester *et al*., 2012). Lately, the RT loop-mediated isothermal amplification assay was established for the rapid detection of GLRaV-3 (Walsh *et al*., 2013). Also, Microarray- and macroarray- based detection methods have been developed for the multiplex detection of GLRaVs (Thompson *et al*., 2012). However, these techniques are useful because of their relative simplicity and robustness. Although there are limitations that include: time, expertise, and costs in running the assay. In recent years, diagnostic developments of the application of Next Generation Sequencing (NGS) for mining sequences of viruses and virus strains in grapevines in an unbiased manner has been increasing (Al Rwahnih *et al*., 2013). Even if Next Generation Sequencing (NGS) is relatively expensive for use as a routine tool in virus diagnostics, but the information generated from this emerging technology are more effectively used in molecular diagnostics for reliable and sensitive identification of viruses.

Generally, ELISA and RT-PCR are regularly used method in a corresponding manner for the detection of GLRaVs. Regardless of the method used, molecular assays are more sensitive than serological assays (Tsai *et al*., 2012). It should be noted that consistent or best detection by any of the methods depends on sampling strategy and proper controls (Tsai *et al*., 2012). So, sampling the proper tissues at the right time, is the most critical factors which influencing reliable detection of GLRaVs because of their localization in phloem-associated tissue, low concentration, and uneven distribution in an infected vines. Furthermore, petiole samples collected during late summer and fall, and cambial scrapings obtained from dormant woody canes in winter, are used for GLRaVs testing by ELISA or RT-PCR (Weber *et al*., 2002).

g) Dissemination by Propagation Materials

In order to maintain clonal integrity and trueness-to-type, vegetative propagation of Grapevines is very crucial. This method is used to plant the vine as either own-rooted or grafted vines. Due to propagation practice, Grapevine Leafroll associated Viruses can spread easily from one area to another once cuttings are derived from infected vines and are used for propagation (Martelli, 2000). The disease can be disseminated along with scion or rootstock materials which are used for propagation, grafting, or planting new vineyards. Dissemination of GLRaVs by these practices can occur in all kinds of *V. vinifera* cultivars. Therefore, the use of infected cuttings or budwood for propagation, bench grafting, chip budding, and top working provide many avenues for the introduction of GLRaVs into vineyards (Martelli, 2000). Since GLRaVs are not transmitted mechanically between grapevines, their spread in vineyards through pruning shears, trimmers, thinners, harvesters, or saws does not occur (Martelli, 2000).

h) Dissemination by Insect Vectors

Spreading of GLRaVs in plant-to-plant, generally occur through mealybug (Pseudococcidae) and scale insect (Coccidae) vectors. There are numerous species of mealybugs belonging to the genera *Heliococcus, Phenacoccus, Planococcus,* and *Pseudococcus,* and also scale insects belonging to the genera *Pulvinaria*, *Neopulvinaria,* and *Parthenolecanium* which have been identified as vectors of GLRaV-1, -3, -4 and its strains (Karasev, 2000). There are no known insect vectors for GLRaV-2 and -7, despite the fact that several members of the genus *Closterovirus* are transmitted by different species of aphids (Martelli *et al*., 2002). Currently, there is no proof so far for transmission of GLRaV-2 by aphids under experimental or natural conditions (Martelli *et al*., 2002). Aphids are not common pests of grapevine, although the grapevine aphid (*Aphis illinoisensis*) has been reported in many grapevine-growing areas (Havelka *et al*., 2011)**.** But currently evidence shows that *A. illinoisensis* is not a vector for GLRaV-2, however, GLRaV-2 can be mechanically transmitted with some difficulty from grapevine tissue to *Nicotiana benthamiana* (Goszczynski *et al*., 1996). Recently, GLRaV-7 was proved to be transmitted experimentally by the parasitic dodder *Cuscuta reflexa* to *Tetragonia expansa* and *Cuscuta europea* to *Nicotiana occidentalis* (Mikona and Jelkmann, 2010.). Therefore, several informations on transmission of ampeloviruses are found in mealybugs rather than scale insects. Always Mealybugs show gender specific distinction in their

ability to transmit ampeloviruses. Male mealybugs are winged and capable of flight, but they have only vestigial mouthparts which are not suitable for feeding and acquiring virus, while female mealybugs have functional mouthparts allowing acquisition of virus by ingesting plant sap from phloem, and subsequent transmission (Daane *et al*., 2012). In general, the females are wingless and are largely sedentary, which limit the movement and spread of virus to short distances covered by crawling between immediately adjacent vines within a row and between next rows (Grasswitz and James, 2008). The limited mobility of female mealybugs, lead to the slow spread of GLD within a vineyard, except if the insect is dispersed by other means, such as human activities, or being blown by the wind or transported by foraging birds. Therefore, relatively long distances mobility of mealybugs can be facilitated or dispersed by wind-blown infested leaves, carried on vineyard workers' clothing, or spread by harvesting equipment carrying leaf or bark material infested with mealybugs (Tsitsipis *et al*., 2005).

B. Grapevine Fleck Virus (GFkV)

Grapevine fleck disease (GFkD), is the virus like disease which was reported from all viticulture countries in the world (Martelli, 2014). The causative agent of this disease is the associated virus of Grapevine fleck virus (GFkV) which belongs to the Tymoviridae family, and is a non-mechanically transmissible virus associated with fleck symptoms (Sabanadzovic *et al*., 2000 and 2001). Several GFkV-infected *V. vinifera* and rootstock cultivars are symptomless. It is very often found in mixed infection with other more harmful viruses (for example, GFLV, GLRaVs, GVA), making it very difficult to discriminate its specific impact. GFkV can cause latent infections in *V. vinifera* cultivars and lead to the induction of typical foliar symptoms of vein clearing, leaf deformation and reduction of the vegetative growth in the sensitive indicator V. rupestris St. George (Fajardo *et al*., 2012 and Martelli, 2014). Currently there is no any known or reported insect vector for GFkV. Fleck (alone or in mixed infection with VM and VN) was reported as having a negligible influence on the growth, yield, and juice composition of V. vinifera cvs. For example, GFkD has been reported in Brazilian vineyards, but its importance and damage were not specifically evaluated (Basso *et al*., 2010 and Fajardo *et al*., 2012)). However, a synergistic effect is possible when GFkV is in coinfection with other viruses (Spring *et al*., 2012).

C. Grapevine Fanleaf

This is the oldest known virus disease of grapevines as an infectious degeneration disease. It is believed that this disease originated from ancient Persia and spread to other grape-growing regions through transport of vegetative propagative materials. Therefore, the disease has developed and established as one of the most serious and devastating grapevine virus diseases worldwide (Andret-Link *et al*., 2004 and Maliogka *et al*., 2015a). It can able to cripple infected grapevine with misshapen leaves, short internodes, zigzag growth of canes, and poor berry set. Grapevine fanleaf disease has detrimental effect on fruit yield, quality and longevity of grapevines. All cultivars of *Vitis vinifera* are susceptible to the disease and severe economic damage with yield losses up to 80 percent have been documented in several sensitive cultivars (Andret-Link *et al*., 2004 and Maliogka *et al*., 2015a). Its main etiological agent is Grapevine fanleaf virus of the genus Nepovirus and belongs to the Secoviridae family (Martelli, 2014). This disease is endemic to areas where soil-borne nematode vector is present (Villate *et al*., 2008). The typical symptoms of this disease are leaf distortion, yellow mosaic close to primary veins, bright yellow vein banding on leaves, widely open petiole sinuses, double nodes, short and malformed internodes. However, foliar symptoms appear at the beginning of the growing season. Grapevine fanleaf Viruses are semi-persistently vectored by both juvenile stages and adults stages of the ecto-parasitic nematodes *Xiphinema index* and *X. italiae* (Demangeat *et al*., 2010).

D. Management Strategies of Grapevine Virus Diseases

Generally, virus diseases can be controlled through the use of good health status of propagative material (cuttings, grafts, buds, rooted cuttings and grafted plants) (Oliver and Fuchs, 2011). This is the main prophylactic measure to mitigate impact of virus diseases. Also, production and use of certified virus tested or virus free propagative material led to reduce the inoculum potential especially in the areas where vectors are present (Martelli, 2014). However, establishment of vineyards in vector-free (for example nematodes and scale insects) areas reduces local and long-distance dispersal of viruses (Laimer *et al*., 2009 and Villate *et al*., 2008). Other measures like rouging of symptomatic grapevines and possibly adjacent plants (removing any remaining roots or remove and destroy virus-infected vines), chemical or biological control or management of insect- or nematode-vector, and cross-protection and conventional or transgenic grapevines tolerant or resistant to viruses or nematode-vector are possible strategies for viral disease management (Almeida *et al*., 2013). However, the chemical control of nematode is often inefficient, environmentally improper and harmful to humans, while transgenic plants is proper and a possible good choices (Laimer *et al*., 2009 and Maliogka *et al*., 2015a). The siRNA-mediated engineered resistance or expression of artificial microRNAs (amiRNA) has been a powerful tool, but it is still limited to experimental cultivars or model plant (Roumi *et al*., 2012). This is because the sources of genetic resistance to grapevine viruses are not available. But a major genetic resistance locus to vectors like *X. index* was recently reported from grapevine (Hwang *et al*., 2010). There are also sanitation methods which are used to eliminate grapevine viruses. The main sanitation techniques which are used to eliminate grapevine viruses are thermotherapy in vivo or in vitro, meristem and shoot tip culture, somatic embryogenesis, electrotherapy and cryotherapy (Skiada *et al*., 2013). However, there are limitations of the electro- and cryotherapy and the main limitation of these techniques is the low efficiency and possible induction of host genetic changes (Baranek *et al*., 2009). According to Maliogka *et al*., 2009, a higher efficiency in obtain virus-free grapevines was achieved by thermo- or

chemotherapy associated with meristem and shoot tip culture. Another practice is to maintain vineyard health and vigor through good nutrition, proper irrigation, and reduced crop load to reduce physical and environmental stresses. Also do not replant grapes in infested ground for 10 years unless special fallowing procedures and deeply fumigate must be adopted (Baranek *et al*., 2009).

3. Material and Methodology

A. Site Description

This study was conducted in a Grape vine plantation from the area of Central Transdanubia (Lesencefalu) (Figure 2). Lesencefalu is a village in Tapolca District, Veszprém County, Hungary. It is situated 8 km north of Lake Balaton between Lesencetomaj and Várvölgy with Latitudes 46° 50′ 39.48″ N and Longitudes 17° 20′ 36.24″ E (Figure 3). The village has an area of 718 hectares. This village takes its name from the Lesence stream that runs through the village towards Lake Balaton. Falu is the Hungarian word for "village". Until 1940 the village was known as Lesencenémetfalu, német being the Hungarian for "German", and before 1898 it was called Németfalu.

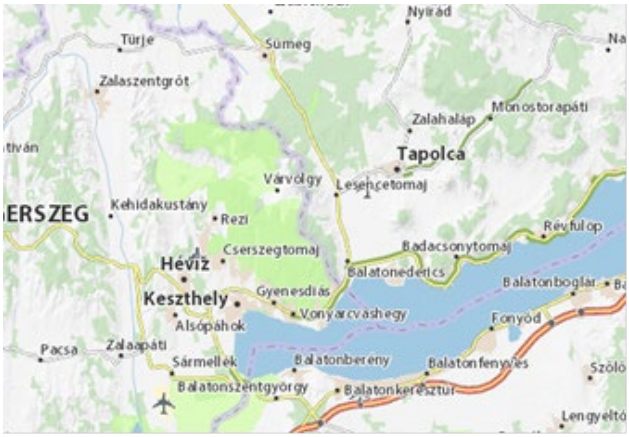

Fig. 2. A map showing study area

Fig. 3. A map showing the exact study area

B. Sample Collection

The samples were collected from Hungarian Grapevine variety called Othello. The number of samples collected was 60. After collection, the samples were individually wrapped in polyethylene bags and stored at -20 degrees until processed.

C. Routine Laboratory Methods

The samples were analysed or tested in the laboratory using the most commonly used serological test; Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) test. DAS-ELISA allows the reliable detection of the virus by using the available polyclonal antibodies prepared against the N protein (Kritzman *et al*., 2001). The reagents used were from the LOEWE Biochemical (Brome mosaic virus, Brome dwarf mosaic virus, Brome streak mosaic virus, Barley stripe mosaic virus, Barley yellow dwarf virus, Wheat dwarf mosaic virus and Wheat dwarf virus).

1) Composition of Required Buffers

Coating buffer: 1.59 g Na_2CO_3; 2.93 g $NaHCO_3$; 0.2 g NaN_3 1 liter distilled water (pH 9.6).

PBS–Tween wash buffer: 0.5 ml Tween-20 in 1 liter-PBS solution.

10 × PBS-solution (phosphate-buffer saline): 72 g NaCl; 4.3 g KH_2PO_4; 14,4 g $Na_2HPO_4 \times 2\ H_2O$ (vagy: 29 g $Na_2HPO_2 \times 12\ H_2O$); 1g Merthiolate (Thimerosal) in 1liter solution, with distilled water (pH 7.4).

Extraction buffer: IgG buffer + 2% polivynyl-pirrolidone (PVP).

IgG buffer: PBS–Tween-solution with 1% BSA, filtered with multi-layer filter paper.

Conjugate-buffer: same as the extraction buffer. *Alkaline phosphatase substrate buffer:* 97 ml diethanol-amin; 0.2 g $MgCl_2 \times 6\ H_2O$; 0.2 g NaN_3 with 1 liter distilled water (pH 9.8) (HORVÁTH–GÁBORJÁNYI, 1999).

2) Procedures Carried Out in the Laboratory

The detection of viruses requires the ELISA kit containing the antiserum and the antiserum enzyme conjugate, together with the plant samples. In the experiment, microtiter plates were made of polystyrene. A solution of 200-200 µl virus-specific immunoglobulin in 500 µl of the microtiter plates diluted to 500 µl was pipetted. At 4 hours incubation at 35 ° C, the IgG molecules (alkaline phosphatase) were bound to the cells. The cells were washed twice, then twice more, but then the buffer was left in cells for 3 to 3 minutes. After the unbound IgG molecules were removed with the wash buffer, the vegetable pressurized pipetted into the wells. The microtiter plates were incubated at 4 ° C overnight, washed twice with washing buffer twice and then three times with incubating the wash buffer for three minutes in cells. If the sample contained the virus (antigen) sought, it was bound to the IgG previously bound to the plate, other viruses or proteins with the buffer. After washing, the virus-specific immunoglobulin (conjugate) was pipetted onto the samples after diluting it 500 times in conjugate buffer. If the virus was present in the cell, the conjugate was bound to the other half of the virus, creating the "immunosuppressive". After incubation at 35 ° C for 4 hours, the cells were washed twice with buffer and then twice more and then the buffer was left in cells for 3 to 3 minutes, removing the unbound conjugate. As a next step, the substrate of the enzyme was transferred to the cells. The substrate was dissolved in a 1: 1 ratio of substrate buffer under continuous stirring, and the para-nitrophenylphosphate was dissolved in substrate buffer. 150-150 µl was pipetted into the cells, then at

about 35 ° C. Incubated for 30 minutes. Para-nitrophenyl phosphate is a colorless compound. The alkaline phosphatase enzyme cleaves the phosphate groups from the substrate molecules and produces a yellow para-nitrophenol. The strength of the color change depends on the concentration of the virus. The enzyme works until the chemical environment and the substrate are present. The reaction was quenched with 50 µl of 3N NaOH. Positive reactions are clearly visible, but we can get reliable results using an ELISA photometer (ELISA reader). The degree of color change was evaluated with a Labsystems Multiscan RC ELISA reader at 405 nm wavelength. We considered positive samples whose extinction values exceeded three times the negative control extinction value.

4. Results and Discussion

A. Single Virus Infections

During this study 60 samples of Othello grapevine (Hungarian grape vine variety/cultivar) were collected and sent to the laboratory for analysis or detection the presence of viruses' infection using DAS ELISA and the result is shown on (Appendix 1). Among 60 samples, 27 (45%) samples were found to be positive/infected with at least one virus from 6 viruses (*Grapevine Leafroll Associated Virus* 1 (GLRaV1), GLRaV2, GLRaV3, GLRaV6, GLRaV7 and *Grapevine fleck virus* (GFkV), while 33 (55%) samples were not infected with any virus (showed negative virus infection) (Appendix 1). The magnitude of infection was different for each virus. Among 60 samples, 27 were positive/infected with GLRaV1, 12 were positive/infected with GLRaV2, 1 were positive/infected with GLRaV3, 5 were positive/infected with GLRaV6, 6 were positive/infected with GLRaV7 and 11 were positive/infected with GFkV (Table 1 and Figure 4).

However, between those 27 samples which were found to be infected with viruses, only 14 samples were found to be infected with only one virus (GLRaV1), while 13 samples were found to be infected with more than one virus among the six viruses (Appendix 1).

B. Multiple Viruses Infections

According to the results obtained from the laboratory, 13 samples were found to be infected with more than one virus. Some samples were detected to be infected with 2 or 3, 4, 5 viruses and other sample were found to be infected with all the 6 viruses (Table 2 and Figure 5). From table 2 below and figure 5 illustrated below, the result show that 3 samples were infected with 2 viruses (1 sample was detected to be infected both with GLRaV1 and GFkV, while 2 samples were found to be infected both with GLRaV1 and GLRaV2). Also 3 samples were diagnosed to be infected with triple (3) viruses (GLRaV1, GLRaV2 and GFkV) (Table 2 and Figure 5). Likewise, the results show that 3 samples were infected with 4 viruses (2 samples were infected with GLRaV1, GLRaV2, GLRaV7 and GFkV while 1 sample was infected with GLRaV1, GLRaV2, GLRaV6 and GFkV) (Table 2 and Figure 5). Similarly, 3 samples were diagnosed to be infected with 5 viruses (GLRaV1, GLRaV2, GLRaV6, GLRaV7 and GFkV). However, only 1 sample was detected to be positive infected with all the six viruses (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 and GFkV) (Table 2 and Figure 5).

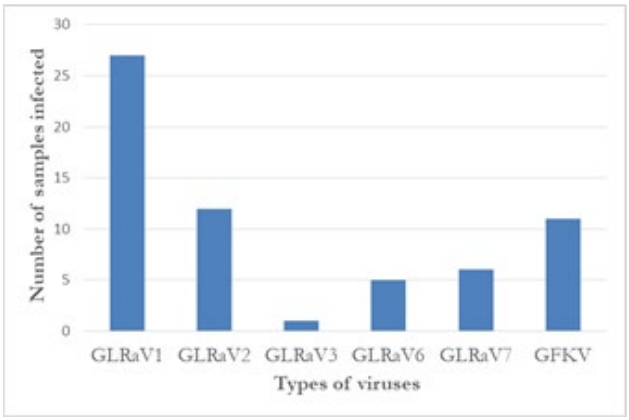

Fig. 4. The graph of virus single infections

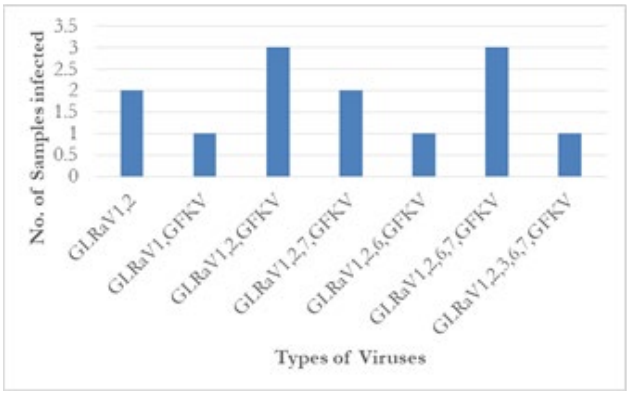

Fig. 5. The graph of viruses multiple infections

Therefore, Othello grapevine variety was found to be more susceptible to GLRaV1, the magnitude of infection was high 45% followed by GLRaV2 20%, GFkV 18.33%, GLRaV7 10%, GLRaV6 8.33% and GLRaV3 1.67% (Table 1). This implies that Othello grapevine variety is resistance/ not susceptible to GLRaV3, only 1 sample was found to be infected

Table 1
Virus single infections

Types of Viruses	**GLRaV1**	**GLRaV2**	**GLRaV3**	**GLRaV6**	**GLRaV7**	**GFKV**
Number of Infected Samples	27	12	1	5	6	11
Percentage (%)	45	20	1.67	8.33	10	18.33

Table 2
Viruses multiple infections

Types of Viruses	**GLRaV1,2**	**GLRaV1, GFKV**	**GLRaV1,2, GFKV**	**GLRaV1,2,7, GFKV**	**GLRaV1,2,6, GFKV**	**GLRaV1,2,6,7, GFKV**	**GLRaV1,2,3,6,7, GFKV**
Samples infected	2	1	3	2	1	3	1

with GLRaV3 among 60 samples. The magnitude of infection associated with GLRaV1 was found to be high, followed with GLRaV2 and GFkV, this is because GLRaV1 is the most common virus in Hungary infecting grape vine, followed by GLRaV2 and GFkV. These viruses are the most common in Hungary which infect grapevine and cause great losses of yield and quality.

5. Conclusion

For several thousand years, grapevine has been grown in the Carpathian basin. It has been operated for the purpose of propagation material, table grape and wine grape. However, it is facing adversity of multiple virus infections that influenced negatively the performance of the cultivars at a certain extent, which cause premature death of the stocks and generating great yield losses. In that context, several studies have been conducted to unravel the situation by intensifying the methods of control and detection of viral infection presence in the vineyard to sustain virus free vineyard and limit their dissemination by infected propagation and grafting materials as it is the most common way of virus spreading.

The study was set out to investigate the viral infection of Othello grapevine variety by using serological test; Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS- ELISA) test. A total of 60 samples were subjected to this test, 6 viruses category (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 and GFkV) were found to infect this variety and a total of 27 samples were infected while 33 samples were not infected. However, the highest infection was caused by GLRaV1 while the lowest infection was found to be associated with GLRaV3. The infection was found to be associated with one virus (single infection) on some samples, while other samples were found to have double infection (infected with more than one virus).

A. Recommendation

After testing the presence of infection by 6 viruses of Othello variety using DAS- ELISA. More studies should be conducted to develop the immune resistant of the variety against those viruses. Also analyzing the main factors responsible for the infections found in the studied samples, other techniques like RT-PCR method should be used to study the presence of virus infection detected to compare the results. It is not 100% to attest the efficiency of the technique and approve it as a reliable. So, we need generic application which is improved in association with more precise and innovative techniques. However, Next generation sequencing should also be applied to deepen the knowledge on the infection mechanism of the different viruses and the immune system response of the host. This will open new perspectives in the diagnostic field to detect the presence of viruses and identify other viruses which may have not been described yet in Hungary. Lastly, breeders should select Othello grapevine variety as a breeding cultivar against GLRaV3. But also it can be improved to be resistant/ tolerant against other detected viruses.

References

[1] Abou Ghanem-Sabanadzovic, N. A., Sabanadzovic, S., Gugerli, P., and Rowhani, A. 2012. Genome organization, serology and phylogeny of Grapevine leafroll-associated viruses 4 and 6: Taxonomic implications. Virus Res. 163:120-128.

[2] Abou Ghanem-Sabanadzovic, N., Sabanadzovic, S., Uyemoto, J. K., Golino, D., and Rowhani, A. 2010. A putative new ampelovirus associated with grapevine leafroll disease. Arch. Virol. 155:1871-1876.

[3] Al Rwahnih, M., Dave, A., Anderson, M., Rowhani, A., Uyemoto, J. K., and Sudarshana, M. R. 2013. Association of a DNA virus with grapevines affected by red blotch disease in California. Phytopathology 103:1069-1076.

[4] Al Rwahnih, M., Dolja, V. V., Daubert, S., Koonin, E. V., and Rowhani, A. 2012. Genomic and biological analysis of *Grapevine leafroll-associated virus 7* reveals a possible new genus within the family *Closteroviridae*. Virus Res. 163:302-309.

[5] Alabi, O. J., Al Rwahnih, M., Karthikeyan, G., Poojari, S., Fuchs, M., Rowhani, A., and Naidu, R. A. 2011. *Grapevine leafroll-associated virus 1*occurs as genetically diverse populations. Phytopathology 101:1446-1456.

[6] Alabi, O. J., Gutha, L. R., Casassa, L. F., Harbertson, J., Mirales, M., Beaver, C. W., and Naidu, R. A. 2012. Impacts of grapevine leafroll disease on own-rooted wine grape cultivar in cool climate viticulture. Pages 170-171 Plant Disease / September 2014 1181 in: Proc. 17th Congr. Int. Counc. Study Virus Virus-like Dis. Grapevine (ICVG.

[7] Alkowni, R., Zhang, Y. P., Rowhani, A., Uyemoto, J. K., and Minafra, A. 2011. Biological, molecular, and serological studies of a novel strain of grapevine leafroll-associated virus 2. Virus Genes 43:102-110.

[8] Almeida, R. P.P.; Daane, K. M.; Bell, V.A.; Blaisdell, G. K.; Cooper, M. L.; Herrbach, E.; Pietersen, G. 2013. Ecology and management of grapevine leafroll disease. Frontiers in Microbiology, New York, v.4, n.94.

[9] Andret-Link, P.; Laporte, C.; Valat, L.; Ritzenthaler, C.; Demangeat, G.; Vigne, E.; Laval, V.; Pfeiffer, P.; Stussi-Garaud, C.; Fuchs, M. 2004. Grapevine fanleaf virus: still a major threat to the grapevine industry. Journal of Plant Pathology, Bari, v.86, n.3, p.183-195.

[10] Atallah, S., Gomez, M., Fuchs, M., and Martinson, T. 2012. Economic impact of grapevine leafroll disease on *Vitis vinifera* cv. Cabernet franc in Finger Lakes vineyards of New York. Am. J. Enol. Vitic. 63:73-79.

[11] BARÁNEK, M.; RADDOVÁ, J.; KRIZAN, B.; PITRA, M. 2009.Genetic changes in grapevine genomes after stress induced by in vitro cultivation, thermotherapy and virus infection as revealed by AFLP.Genetics and Molecular Biology, Ribeirão Preto, v.32, n.4, p.834-839.

[12] Basso, M. F., Fajardo, T. V. M., Santos, H. P., Guerra, C. C., Ayub, R. A., and Nickel, O. 2010. Leaf physiology and enologic grape quality of virusinfected plants. Trop. Plant Pathol. 35:351-359.

[13] Besse, S., Bitterlin, W., and Gugerli, P. 2009. Development of an ELISA for the simultaneous detection of Grapevine leafroll associated virus 4, 5, 6, 7, and 9. Pages 296-298 in: Proc. XVI Int. Counc. Study Viruses Virus-Like Dis. Grapevine.

[14] Bester, R., Jooste, A. E. C., Maree, H. J., and Burger, J. T. 2012. Real-time RT-PCR high-resolution melting curve analysis and multiplex RT-PCR to detect and differentiate grapevine leafroll-associated virus 3 variant groups I, II, III and VI. Virol. J. 9:219.

[15] Buyukbay, E., Uzunoz, M. and Sibel, G. (2011). Post-harvest losses in tomato and fresh bean production in Turkey. *Scientific Research and Essays* 6 (7): 1656-1666.

[16] Cabaleiro, C., and Segura, A. 2003. Monitoring the field spread of *Grapevine leafroll associated virus* 3 for 12 years. Pages 216-217 in: Proc. XIV Int. Counc. Study Viruses Virus-Like Dis. Grapevine.

[17] Charles, J. G., Cohen, D., Walker, J. T. S., Forgie, S. A., Bell, V. A., and Breen, K. C. 2006. A review of the ecology of Grapevine leafroll associated virus type 3 (GLRaV-3). N.Z. Plant Prot. 59:330-337.

[18] Constable, F. E., Connellan, J., Nicholas, P., and Rodoni, B. C. 2013. The reliability of woody indexing for detection of grapevine virus-associated diseases in three different climatic conditions in Australia. Aust. J. Grape Wine Res. 19:74-80.

[19] Cretazzo, E., Padilla, C., Carambula, C., Hita, I., Salmerón, E., and Cifre, J. 2010. Comparison of the effects of different virus infections on performance of three Majorcan grapevine cultivars in field conditions. Ann. Appl. Biol. 156:1-12.

[20] Cseh, E., Takács, A., Kocsis, L. and Gáborjányi, R. (2012): General properties of grapevine viruses occurring in Hungary; *Journal of Central European Agriculture,* 2012, 13(1), pp. 44-57.

[21] Daane, K. M., Almeida, R. P. P., Bell, V. A., Botton, M., Fallahzadeh, M., Mani, M., Miano, J. L., Sforza, R., Walton, V. M., and Zaviezo, T. 2012. Biology and management of mealybugs in vineyards. Pages 271-308 in: Arthropod Management in Vineyards. N. J. Bostanian, R. Isaacs, and C. Vincent, eds. Springer, Dordrecht, the Netherlands.

[22] Dawson, W. O., Garnsey, S. M., Tatineni, S., Folimonova, S. Y., Harper, S. J., and Gowda, S. 2013. Citrus tristeza virus-host interactions. Front. Microbiol. 4:88.

[23] Demangeat, G.; Komar, V.; Van-Ghelder, C.; Voisin, R.; Lemaire, O.; Esmenjaud, D.; Fuchs, M. 2010.Transmission competency of single female Xiphinema index lines for Grapevine fanleaf virus. Phytopathology, Saint Paul, v.100, n.4, p.384-389.

[24] Dolja, V. V., and Koonin, E. V. 2013. The closterovirus-derived gene expression and RNA interference vectors as tools for research and plant biotechnology. Front. Microbiol. 4:83.

[25] Dolja, V. V., Kreuze, J. F., and Valkonen, J. P. T. 2006. Comparative and functional genomics of closteroviruses. Virus Res. 117:38-51.

[26] Domingo, E., Martin, V., Perales, C., Grande-Perez, A., Garcia-Arriaza, J., and Arias, A. 2006. Viruses as quasispecies: Biological implications. Curr. Top Microbiol. Immunol. 299:51-82.

[27] Edit Hajdu. 2018. Viticulture of Hungary; Research Institute for Viticulture and Enology, Kecskemét,Hungary.

[28] Espinoza, C., Vega, A., Medina, C., Schlauch, K., Cramer, G., and Arce-Johnson, P. 2007. Gene expression associated with compatible viral diseases in grapevine cultivars. Funct. Integr. Genomics 7:95-110.

[29] Fajardo, T.V.M.; Eiras, M.; Nickel, O.; Dubiela, C.R.; Souto, E.R. de. 2012. Detection and partial molecular characterization of Grapevine fleck vir Grapevine virus D, Grapevine leafroll-associated virus 5 and 6 infecting grapevines in Brazil. Ciência Rural, Santa Maria, v.42, n.12, pp. 2127-2130.

[30] FAO (2012). Major Fruits and Vegetables producing Countries in the World (2010-2011). Rome, Italy.

[31] Faoro, F. 1997. Cytopathology of closteroviruses and trichoviruses infecting grapevines. Pages 29-47 in: Filamentous Viruses of Woody Plants. P. L. Monette, ed. Research Signpost, Trivandrum, India.

[32] Fei, F., Lyu, M. D., Li, J., Fan, Z. F., and Cheng, Y. Q. 2013. Complete nucleotide sequence of a Chinese isolate of *Grapevine leafroll-associated virus 3* reveals a 5′ UTR of 802 nucleotides. Virus Genes 46:182-185.

[33] Freeborough, M.-J., and Burger, J. 2006. Leaf roll: Economic implications. Wynboer.

[34] Fuchs, M., Martinson, T. E., Loeb, G. M., and Hoch, H. C. 2009. Survey for the three major leafroll disease-associated viruses in Finger Lakes vineyards in New York. Plant Dis. 93:395-401.

[35] Goszczynski, D. E., Kasdorf, G. G. F., Pietersen, G., and van Tonder, H. 1996. *Grapevine leafroll-associated virus 2* (GLRaV-2) – mechanical transmission, purification, production and properties of antisera, detection by ELISA. S. Afr. J. Enol. Vitic. 17:15-26.

[36] Grasswitz, T. R., and James, D. G. 2008. Movement of grape mealybug, *Pseudococcus maritimus*, on and between host plants. Entomol. Exp. Appl. 129:268-275.

[37] Havelka, J., Shukshuk, A. H., Ghaliow, M. E., Laamari, M., Kavallieratos, N. G., Tomanović, Z., Rakhshani, E., Pons, X., and Starý, P. 2011. Review of invasive grapevine aphid, *Aphis illinoisensis* Shimer, and native parasitoids in the Mediterranean (Hemiptera, Aphididae; Hymenoptera, Braconidae, Aphidiinae). Arch. Biol. Sci., Belgrade 63:269-274.

[38] Hellman, E. W. (2003): Grapevine Structure and Function. In: Oregon Viticulture, Corvallis, Oregon: Oregon State University Press.

[39] http://www.mercurynews.com/2015/04/07/wine-trivia-anatomy-lesson-from-rootstalk-to-tendrils/. Site visited on March 28th, 2020

[40] Hwang, C.F.; Xu, K.; Hu, R.; Zhou, R.; Riaz, S.; Walker, M. A. 2010. Cloning and characterization of XiR1, a locus responsible for dagger nematode resistance in grape. Theoretical and Applied Genetics, Berlin, v.121, n.4, p.789-799.

[41] Jarugula, S., Gowda, S., Dawson, W. O., and Naidu, R. A. 2010. 3′-coterminal subgenomic RNAs and putative *cis*-acting elements of *Grapevine leafroll-associated virus* 3 reveals 'unique' features of gene expression. Virol. J. 7:180.11.

[42] Karasev, A. V. 2000. Genetic diversity and evolution of Closteroviruses. Annu. Rev. Phytopathol. 38:293-324. Keller, M. (2010): The Science of Grapevines Anatomy and Physiology. Academic Press. 1-47.

[43] Krenz, B., Thompson, J. R., Fuchs, M., and Perry, K. L. 2012. Complete genome sequence of a new circular DNA virus from grapevine. J Virol. 86:7715.

[44] Kritzman, A., M. Lampel, B. Raccah, and A. Gera. 2001. Distribution and Transmission of Iris yellow spot virus. Plant Dis. 85: 838-842.

[45] Kumar, R. T. (2010). *Post-harvest profile of grapes*. Ministry of Agriculture, Department of Agriculture and Cooperation, Directorate of Marketing and Inspection, MPDC, Nagpur, India.72 pp.

[46] Laimer, M.; Lemaire, O.; Herrbach, E. H.; Goldschimidt, V.; Minafra, A.; Bianco, P.; Wetzel, T. 2009. Resistance to viruses, phytoplasmas and their vectors in the grapevine in Europe: A review. Journal of Plant Pathology, Bari, v.91, n.1, p.7-23.

[47] MAFS (2006). Strengthening the Viticulture Sub-sector for Economic Growth. Proceedings of the National Viticulture Stakeholders Workshop. Dodoma, Tanzania, 30 March, 2006.

[48] Maliogka, V. I., Dovas, C. I., Lotos, L., Efthimiou, K., and Katis, N. I. Plant Disease/September 2014 1183 2009. Complete genome analysis and immunodetection of a member of a novel virus species belonging to the genus Ampelovirus. Arch. Virol. 154:209-218.

[49] Maliogka, V.I.; Martelli, G. P.; Fuchs, M.; Katis, N.I. 2015a. Control of viruses infecting grapevine. Advances in Virus Research, San Diego, v.91, and p.175-227.

[50] Maliogka, V.I.; Skiada, F. G.; Eleftheriou, E. P.; Katis, N.I. 2009. Elimination of a new Ampelovirus (GLRaV-Pr) and Grapevine rupestris stem pitting associated virus (GRSPaV) from two Vitis vinifera cultivars combining in vitro thermotherapy with shoot tip culture. Scientia Horticulturae, Amsterdam, v.123, n.2, p.280-282.

[51] Maree, H. J., Almeida, R. P. P., Bester, R., Chooi, K. M., Cohen, D., Dolja, V. V., Fuchs, M. F., Golino, D. A., Jooste, A. E. C., Martelli, G. P., Naidu, R. A., Rowhani, A., Saldarelli, P., and Burger, J. T. 2013. *Grapevine leafroll-associated virus 3.* Front. Microbiol. 4:82. doi:10.3389/fmicb. 2013.00082.

[52] Martelli, G. P. 2000. Major graft-transmissible diseases of grapevines: Nature, diagnosis, and sanitation. Pages 231-236 in: Proc. 50th Anniv. Annu. Meeting ASEV, Seattle.

[53] Martelli, G. P. 2012. Grape virology highlights: 2010-2012. Pages 13-31 in: Proc. 17th Congr. Int. Counc. Study Virus Virus-like Dis. Grapevine (ICVG), Davis, California, USA.

[54] Martelli, G. P. 2014. Directory of virus and virus-like diseases of the grapevine and their agents. J. Plant Pathol. 96(suppl. 1):1-136.

[55] Martelli, G. P., Abou Ghanem-Sabanadzovic, N., Agranowsky, A. A., Al Rawhanih, M., Dolja, V. V., Dovas, C. I., Fuchs, M., Gugerli, P., Hu, J. S., Jelkmann, W., Katis, N., Maliogka, V. I., Melzer, M. J., Menzel, W., Minafra, A., Rott, M. E., Rowhani, A., Sabanadzovic, S., and Saldarelli, P. 2012. Taxonomic revision of the family *Closteroviridae* with special reference to the grapevine leafroll-associated member of the genus *Ampelovirus* and the putative species unassigned to the family. J. Plant Pathol. 94:7-19.

[56] Martelli, G. P., Agranovsky, A. A., Bar-Joseph, M., Boscia, D., Candresse, T., Coutts, R. H., Dolja, V. V., Falk, B. W., Gonsalves, D., Jelkmann, W., Karasev, A. V., Minafra, A., Namba, S., Vetten, H. J., Wisler, G. C., and Yoshikawa, N. 2002. ICTV Study Group on closteroviruses. The family *Closteroviridae* revised. Arch. Virol. 147:2039-2044.

[57] Mikona, C., and Jelkmann, W. 2010. Replication of *Grapevine leafrollassociated virus 7* (GLRaV-7) by *Cuscuta* species and its transmission to herbaceous plants. Plant Dis. 94:471-476.

[58] Mpore, J.F. (2013). Nutrient Content, Microbial quality and Sensory acceptability of sun and solar dried raisins from Dodoma region. Unpublished Dissertation for Award of M.Sc. Degree at Sokoine University of Agriculture, Morogoro, Tanzania.

[59] Naidu, R. A. 2011. Virus update: The status of Washington vineyards. WSU Viticulture and Enology Extension News, Fall 2011. pp.6-7.

[60] Naidu, R. A., O'Neil, S., and Walsh, D. 2008. Grapevine leafroll disease. WSU Extension Bulletin EB2027E. Accessed on January 02, 2020.

[61] Nimmo-Bell. 2006. The economic effects and financial impact of GLRaV3. A Nimmo-Bell Publication, March 2006.

[62] Oliver, J. E., and Fuchs, M. 2011. Tolerance and resistance to viruses and their vectors in *Vitis* sp.: A virologist's perspective of the literature. Am. J. Enol. Vitic. 62:438-451.

[63] Osman, F., Leutenegger, C., Golino, D., and Rowhani, A. 2007. Real-time RT-PCR (TaqMan®) assays for the detection of grapevine leafroll associated viruses 1–5 and 9. J. Virol. Methods 141:22-29.

[64] Osman, F., Leutenegger, C., Golino, D., and Rowhani, A. 2008. Comparison of low-density arrays, RT-PCR and real-time TaqMan® RT-PCR in detection of grapevine viruses. J. Virol. Methods 149:292-299.

[65] Osman, F., Olineka, T., Hodzic, E., Golino, D., and Rowhani, A. 2012. Comparative procedures for sample processing and quantitative PCR detection of grapevine viruses. J. Virol. Methods 179:303-310.

[66] Peremyslov, V. V., Andreev, I. A., Prokhnevsky, A. I., Duncan, G. H., Taliansky, M. E., and Dolja, V. V. 2004. Complex molecular architecture of beet yellows virus particles. Proc. Natl. Acad. Sci. 101:5030-5035.

[67] Poojari, S., Alabi, O. J., Fofanov, V. Y., and Naidu, R. A. 2013. A leafhopper- transmissible DNA virus with novel evolutionary lineage in the Family *Geminiviridae* implicated in grapevine redleaf disease by next-generation sequencing. PLoS ONE 8(6):e64194.

[68] Prosser, S. W., Goszezynski, D. E., and Meng, B. 2007. Molecular analysis of double-stranded RNAs reveals complex infection of grapevines with multiple viruses. Virus Res. 124:151-159.

[69] Roumi, V.; Afsharifar, A.; Saldarelli, P.; Niazi, A.; Martelli, G.P.; Izadpanah, K. 2012. Transient expression of artificial microRNAs confers resistance to Grapevine virus A in Nicotiana benthamiana. Journal of Plant Pathology, Bari, v.94, n.3, p.643-649.

[70] Rowhani, A., Biardi, L., Johnson, R., Saldarelli, P., Zhang, Y. P., Chin, J., and Green, M. 2000. Simplified sample preparation method and one-tube RT-PCR for grapevine viruses. Pages 82-83 in: Proc. XIII Int. Counc. Study Viruses Virus-Like Dis. Grapevine, Adelaide 2000.

[71] Rowhani, A., Uyemoto, J. K., Golino, D. A., and Martelli, G. P. 2005. Pathogen testing and certification of *Vitis* and *Prunus* species. Annu. Rev. Phytopathol. 43:261-278.

[72] Sabanadzovic, S.; Abou-Ghanem, N.; Castellano, M.A.; Digiaro, M.; Martelli, G.P. 2000. Grapevine fleck virus-like viruses in Vitis. Archives of Virology, New York, v.145, n.3, p.553-565.

[73] Sabanadzovic, S.; Ghanem-Sabanadzovic, N.A.; Saldarelli, P.; Martelli, G.P. 2001. Complete nucleotide sequence and genome organization of Grapevine fleck virus. Journal of General Virology, London, v.82, n.8, p.2009-2015.

[74] Safari, J., Lwelamira, J. and Wambura, P. (2015). Grapevine Farming and its Contribution to Household income and Welfare among Smallholder Farmers in Dodoma Urban District, Tanzania. *American Journal of Agriculture and Forestry* 3(3): 73-79.

[75] Satyanarayana, T., Gowda, S., Ayllón, M. A., and Dawson, W. O. 2004. Closterovirus bipolar virion: Evidence for initiation of assembly by minor coat protein and its restriction to the genomic RNA 5′ region. Proc. Natl. Acad. Sci. 101:799-804.

[76] Senthil, R., Prabakar, K., Rajendran, L. and Karthikeyan, G. (2011). Efficacy of different biological control agents against major postharvest pathogens of grapes under room temperature storage conditions. *Phytopathol. Mediterr.* 50: 55−65.

[77] Skiada, F. G.; Maliogka, V.I.; Katis, N.I.; Eleftheriou, E. 2013. P.Elimination of Grapevine rupestris stem pitting-associated virus (GRSPaV) from two Vitis vinifera cultivars by in vitro chemotherapy. European Journal of Plant Pathology, Dordrecht, v.135, n.2, p.407-414.

[78] Smithsonian. 2013. Grapes and Viticulture in Hungary: Hungarian Heritage Roots to Revival. Folklife Festival- Washington D.C., June26-June 30 and July 3- July 7.

[79] SNV Tanzania (2005): Fruits Subsector a Study on fresh fruits: the case study in Morogoro and Dodoma (With Special emphasis on Pineapples); Final Report.

[80] Spring, J.L., J.S. Reynard, O. Viret, D. Maigre, P. Gugerli, and J.J. Brugger. 2012. Influence of the Grapevine Leafroll Associated Virus (GLRaV-1) and Grapevine Fleck Virus (GFkV) on the Grape and Wine Production of cv. Gamay. *Revue suisse de viticulture arboriculture horticulture* 31: 141–145 Google Scholar.

[81] Steinhauer, D. A., Domingo, E., and Holland, J. J. 1992. Lack of evidence for proofreading mechanisms associated with an RNA virus polymerase. Gene 122:281-288.

[82] Syller, J. 2012. Facilitative and antagonistic interactions between plant viruses in mixed infections. Mol. Plant Pathol. 13:204-221.

[83] Thompson, J. R., Fuchs, M., Fischer, K. F., and Perry, K. L. 2012. Macroarray detection of grapevine leafroll-associated viruses. J. Virol. Methods 183:161-169.

[84] Thomson, J. R., Fuchs, M., and Perry, K. L. 2012. Genomic analysis of *Grapevine leafroll associated virus-5* and related viruses. Virus Res. 163:19-27.

[85] Tsai, C. W., Daugherty, M. P., and Almeida, R. P. P. 2012. Seasonal dynamics and virus translocation of *Grapevine leafroll-associated virus 3* in grapevine cultivars. Plant Pathol. 61:977-985.

[86] Tsai, C. W., Daugherty, M. P., and Almeida, R. P. P. 2012. Seasonal dynamics and virus translocation of *Grapevine leafroll-associated virus 3* in grapevine cultivars. Plant Pathol. 61:977-985.

[87] Tsitsipis, J. A., Angelakis, E., Margaritopoulos, J. T., Tsamandani, K., and Zarpas, D. 2005. First record of the grapevine aphid *Aphis illinoisensis* in the island of Kriti, Greece. DEPP/EPPO Bull. 35:541-542.

[88] Villate, L.; Fievet, V.; Hanse, B.; Delemarre, F.; Plantard, O.; Esmenjaud, D.; Van Helden, M. 2008. Spatial distribution of the dagger nematode Xiphinema index and its associated Grapevine fanleaf virus in French vineyard. Phytopathology, Saint Paul, v.98, n.8, p.942-948.

[89] Walker, A. R., Lee, E., Bogs, J., McDavid, D. A. J., Thomas, M. R., and Robinson, S. P. 2007. White grapes arose through the mutation of two similar and adjacent regulatory genes. Plant J. 49:767-959.

[90] Walsh, H. A., and Pietersen, G. 2013. Rapid detection of Grapevine leafroll-associated virus type 3 using a reverse transcription loop-mediated amplification. J. Virol. Methods 194:308-316.

[91] Weber, E., Golino, D., and Rowhani, A. 2002. Laboratory testing for grapevine diseases. Practical Winery and Vineyards (PWV), January/ February. pp. 13-26.

[92] Zhang, Y., Singh, K., Kaur, R., and Qiu, W. 2011. Association of a novel DNA virus with the grapevine vein-clearing and vine decline syndrome. Phytopathology 101:1081-1090.

[93] Zhou, C. L. E., Ammar, E.-D., Sheta, H., Kelley, S., Polek, M. L., and Ullman, D. E. 2002. *Citrus tristeza virus* ultrastructure and associated cytopathology in *Citrus sinensis* and *Citrus aurantifolia*. Can. J. Bot. 80:512-525.

APPENDIX

GRAPEVINE (OTHELLO) VIROLOGICAL EXAMINATION USING DAS ELISA

	TYPES OF VIRUSES					
SAMPLES	**GLRaV1**	**GLRaV2**	**GLRaV3**	**GLRaV6**	**GLRaV7**	**GFKV**
1	0.04	0.055	0.053	0.056	0.057	0.053
2	1.186	1.013	0.343	0.145	0.403	0.543
3	1.091	0.82	0.355	0.149	0.376	0.612
4	0.805	0.565	0.346	0.142	0.336	0.438
5	1.16	0.822	0.375	0.144	0.345	0.505
6	1.222	0.553	0.442	0.145	0.356	0.574
7	1.067	0.85	0.449	0.141	0.353	0.621
8	1.354X	0.659	0.447	0.143	0.397	0.632
9	0.826	0.863	0.475	0.136	0.427	0.597
10	0.975	0.586	0.425	0.14	0.304	0.429
11	0.904	0.53	0.373	0.136	0.177	0.514
12	1.164	0.581	0.386	0.139	0.143	0.495
13	1.793X	1.08	0.534	0.154	0.452	0.303
14	0.959	0.618	0.489	0.148	0.388	0.542
15	2.024X	0.851	0.432	0.138	0.416	0.759
16	0.47	0.287	0.335	0.134	0.252	0.358
17	0.932	0.427	0.365	0.138	0.314	0.477
18	1.756X	0.52	0.366	0.133	0.302	0.636
19	0.454	0.304	0.378	0.127	0.233	0.413
20	0.649	0.347	0.423	0.134	0.234	0.464
21	1.121	0.403	0.414	0.135	0.316	0.583
22	2.984X	1.286X	0.39	0.168	0.445	0.425
23	2.728X	0.431	0.376	0.132	0.219	0.479
24	0.734	0.456	0.372	0.135	0.122	0.516
25	1.165	1.171	0.486	0.137	0.345	0.41
26	0.98	0.881	0.471	0.132	0.356	0.545
27	0.703	0.447	0.398	0.135	0.312	0.545
28	0.886	0.504	0.364	0.135	0.29	0.416
29	0.485	0.423	0.41	0.129	0.238	0.431
30	0.783	0.455	0.52	0.134	0.224	0.477

NB: The number contains x indicates the sample was infected with virus/virus positive