ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

MSc THESIS

Fidaa ARAFAH

DETECTION AND MOLECULAR DIVERSITY OF GRAPEVINE LEAF ROLL- ASSOCIATED VIRUS 2 AND 4 IN PALESTINE

DEPARTMENT OF PLANT PROTECTION

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ABSTRACT

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Grapevine (*Vitis vinifera*), a member of the Vitaceae family, is the world's oldest crop, while it is Palestine's second most important crop after olives. Grapevine can be infected by up to 60 viruses because to the widespread use of vegetative propagation. The Grapevine leafroll virus, which belongs to the genus *Ampelovirus* and is mostly transferred to the vine by mealybugs, is the most serious. Due to a lack of current data on grapevine prevalence, the goal of this study was to investigate the hygienic state of grapevines in Palestine.

To identify GLRV, field surveys were done in the principal vineyards of Palestine's West Bank. Samples were collected in the Hebron area from Al-Reheia (Al-Fawwar region), Farsh Al-Hawa north of Hebron, Namra in the city center, and Baqa'a in the east. The infection ratio was confirmed to be 16.90 %when the collected samples were examined with ELISA. Positives for GLRV-4 were coded. By employing RT-PCR, none of the examined samples (12) was able to amplify any of GLRV-4 using virus-specific primers, however, RT-PCR was able to identify four positive samples from various parts of Hebron using degenerated *Ampelovirus* primers. These findings show that *Ampelovirus* was discovered in Palestine grapevine areas, but GLRV-2 was not. The findings of this study can be used as a documented report on the health of grapevines. The discovery of *Ampelovirus* would pave the way for more investigation into their identification.

Key Words: Grapevine, GLRV, ELISA, Survey, RT- PCR

ÖZ

YÜKSEK LİSANS TEZİ

FİLİSTİN'DE GRAPEVINE LEAF ROLL- ASSOCIATED VIRUS2 VE 4'ÜN SAPTANMASI VE MOLEKÜLER ÇEŞİTLİLİĞİ

Fidaa ARAFAH

ÇUKUROVA ÜNİVERSİTESİ
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Vitaceae familyasına ait olan asma (*Vitis vinifera*) dünyanın en eski ürünü iken, Filistin'de zeytinden sonra ikinci mahsul olarak kabul edilmektedir. Vejetatif olarak çoğaltma halen yaygın olarak kullanıldığından, 60 kadar virüs asmayı infekteleyebilmektedir. *Ampelovirus* cinsine ait olan ve asmaya esas olarak unlu bitler tarafından bulaşan Grapevine yaprak kıvırcıklığı virüsü en şiddetli olanıdır. Bu çalışma, yaygınlık durumu hakkında mevcut güncel verilerin eksikliğinden dolayı Filistin'deki asmaların sağlık durumunu değerlendirmeyi amaçlamıştır.

Survey çalışmaları, Filistin'deki Batı Şeria'da bulunan üzüm bağlarında yürütülmüştür. Örnekler, 2019 yaz sezonunun sonunda Hebron bölgesinde Al-Reheia (Al-Fawwar bölgesi), Hebron'un kuzeyindeki Farsh Al-Hawa, şehir merkezinde Namra ve doğuda Baqa'dan toplanmıştır.Toplanan numuneler ilk olarak ELISA yöntemi ile testlenmiş ve enfeksiyon oranı %16.90 olarak hesaplanmıştır. GLRV-4 pozitif izolatlar kodlanmıştır. Test edilen numunelerden (12) hiçbiri GLRV-4'e özgü primerleri kullanarak RT-PCR'da amplifiye edilememiştir. Ancak, *Ampelovirus'ün* dejenere primerleri ile yapılan RT-PCR çalışmalarında, Hebron bölgelerinden alınan 4 örnek pozitif olarak tespit edilmiştir. Bu sonuçlar, *Ampelovirüs'ün* Filistin'de asma yetiştirilen alanlarda bulunduğunu, GLRV-2'nin ise bulunmadığını doğrulamaktadır. Bu çalışmanın sonuçları, asmaların sağlık durumunu belgeleyen bir rapor niteliğindedir. Ampelovirüs'ün varlığının ortaya konulması, bundan sonra benzer konularada yapılacak daha fazla araştırma için yol gösterecek niteliktedir.

Anahtar Kelimeler: Grapevine, GLRV, ELISA, Survey, RT-PCR

EXTENDED SUMMARY

Grapevine (*Vitis vinifera*) is one of the oldest fruit trees, and the most cultivated in the world due to its economic importance. Currently in Palestine, it ranks second in the cultivated fruit crops in terms of area and economic return. Due to its unique geographical and ecological location, grape cultivation is The southern in half of the West Bank, particularly Hebron and Bethlehem. There are more than 13 varieties of cultivated rain-fed agriculture. It is used as a table fruit or a manufactured food product Dibis (molasses), raisins, fruit rolls (malban), vinegar, and juice (Basheer-Salimia, 2014).

The area planted with grapes worldwide is about seven million hectares. The total production in the world is about seventy-eight tones. It is grown in Europe, Asia, America, Africa and Oceania (42.7%, 28.8%, 20%, 5.8%, and 2.7%). The most common grape producing countries are Italy, China, France, Argentina, Iran, Chile, and South Africa, as well as the United States of America, Spain, and Turkey. The productivity of Palestinian grapes in 2019 reached about thirty-five thousand tones, and the area under cultivation is 3184 hectares (FAO, 2019).

According to the data provided by the Palestinian Center for statistics at the end of 2020, the cultivated area of grape varieties is estimated to be more than 80 thousand da with rainfed land accounting for 74 thousand da. While the irrigated area is about 6 thousand da of these. Due to its unique geographical and ecological location, grape cultivation is concentrated in the southern part of the West Bank, especially in the Hebron and Bethlehem regions. About 45 thousands are in the governorate of Hebron area and 15 thousands are in the Bethlehem area. Accounting for 2.8% of the total cultivated area. Grape is second of olives in terms of volume of production, and contribute about 12% of total agricultural production (PCBS, 2010).

There are more than 13 varieties of cultivated rainfed agriculture, the main cultivated species include; white grape species: such as Dabogi (Al-Baladi), Al-Jandali, Al-Zaini, Beiruti (Al-Romani), Al-Hamadani and Al-Marrawi. Red grape species: such as Al-Halawani, Black Grape: Beituni (Baloti), Shami, Al-Fahaissi (Al-Motatrash), and Al-Shoyoukhi (Darawishi). Other seedless white grapes, such as Berlait and Superior, have recently been introduced to Jericho and are mostly cultivated under irrigation (Sarah, 2016).

The health status of vineyards in Palestine is not entirely clear because of the similarities of the industries to Jordan and the Israeli occupation (Tanner et al., 1996). More than 60 infectious agents affect grapes (viruses, viroids, phytoplasmas, bacteria and fungi). (ICVG, 2003). Most of them cause disorders that reduce the vitality and longevity of plants and affect the quality and quantity of the crop (Tomazic et al., 2008).

Grape leafroll disease (GLRD), the most commercially significant grape disease, is responsible for during the grapevine's productive life, it might lose up to 30-70% of its productivity (Walter and Martelli, 1997). It's a rapidly spreading illness that's been found in grape-growing areas (Atallah et al., 2012) In both red and white grape varietals, GLRD impacts fruit ripening and lowers soluble solids (Martinson et al., 2008).

Grapevine leafroll (GLR) is a viral illness that has been suspected for a long time but has to be verified (Bovey and Martelli, 1992). A family of viruses known as the Closteroviridae causes it.

The severity of GLD symptoms varies widely depending on the season, grape cultivar, and environmental circumstances. Furthermore, some kinds, such as rootstocks and some white *V. vinifera* cultivars, might be symptomless, serving as a reservoir from which GLD can be transferred to cultivars with a variety of symptoms. In GLD-affected vines, bud break and shoot growth are frequently delayed in the spring. This is generally a transient occurrence that lasts only a few

weeks. Early to mid-summer is when leaf symptoms begin, however they might appear sooner on vines that are water strained. Until late fall, the quantity and intensity of these symptoms increases. GLD induces reddening of the interveinal zones in most red cultivars, although the principal and secondary veins remain green. Some red cultivars' leaves acquire a uniform crimson hue without green veins, especially those with intensely coloured berries. The interveinal region of white cultivars can develop chlorotic. This symptom is frequently modest and may go unnoticed. The leaf edges roll downward in late fall, but the extent of this rolling varies greatly across affected cultivars.

To identify GLRV, field surveys were done in the principal vineyards of Palestine's West Bank. Samples were collected in the Hebron area from Al-Reheia (Al-Fawwar region), Farsh Al-Hawa north of Hebron, Namra in the city center, and Baqa'a in the east. The infection ratio was confirmed to be 16.90 %when the collected samples were examined with ELISA. Serological techniques have a basic flaw in that they rely on the antigenic properties of viral structural proteins. As a result, immunological approaches are developed without taking the rest of the viral genome into consideration.

The PCR findings revealed that the Ampelovirus genus had been successfully recovered. Ampelovirus-induced illnesses, particularly leafroll, are uncommon in Palestinian vineyards. Positives for GLRV-4 were coded. By employing RT-PCR, none of the examined samples (12) was able to amplify any of GLRV-4 using virus-specific primers, however, RT-PCR was able to identify four positive samples from various parts of Hebron using degenerated Ampelovirus primers.

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LIST OF ABBREVIATIONS

µl	: Microliter
^{0}C	: Celsius degree
A	: Adenin
Bp	:Base pair
C	:Cytosine
cDNA	: Complementary DNA
CP	: Coat Protein
CTAB	: Cetrimonium bromide
d.H_2O	: distilled water
dATP	: Deoxyadenosine triphosphate
dCTP	: Deoxycytidine triphosphate
dGTP	: Deoxyguanosine triphosphate
DNA	:Deoxyribonucleic Acid
dNTPs	: Deoxyribonucleotide triphosphates
EDTA	: Ethylenediaminetetraacetic acid
ELISA	: Enzyme-linked immunosorbent assay
EtOH	: Ethanol
FAO	: Food and Agriculture Organization
G	: Guanin
GLRV	: Grapevine leafroll virus
gr	: Gram
HCL	:Hydrochloric acid
LB	: Lysogeny broth
M	:Molarite
mg	: Milligram
ml	: Milliliter

mm : Millimeter

mM : Millimole

M-MLV : Moloney murine leukemia virus

N : normality

NaOH : Sodium hydroxide solution

Nm : Nanometre

No. : Number

PCR :Polymerase Chain Reaction

pH :potential of hydrogen

PVP : Polyvinylpyrrolidone

RNA : Ribonucleic acid

Rpm : Revolutions per minute

RT-PCR : Reverse transcription – Polymerase Chain Reaction

spp : species

T :Thymine

TAE : Tris-acetate-EDTA

Taq : Thermus aquaticus

Tm : Temperature

TNA : Total Nucleic Acid

U/cm : Unit/ centimetre

1. INTRODUCTION

The cultivation and domestication of grapes began seven millennia B.Cin the region Black Sea (Romania, Bulgaria, Ukraine, Russia, Turkey and Georgia) and Iran (Terral et al., 2010). Then the grape varieties ofthese regions were transferred and cultivated in the Middle Eastern regions, the Middle East and Central Europe (Arroyo-Garcia et al., 2006).

Cultivation sites in tropical and subtropical regions (Asia, North America and Europe) and their varieties are derived from wild forms. *V. vinifera* subsp. *sylvestris* (Crespan, 2004; Rosseto, McNally, and Henry, 2002; Sefc et al., 2003) The genus of North America is used as rootstock because it is very resistant to phylloxera (*Daktulosphaira vitifoliae*) and powdery mildew (Jackson 1994).

The area planted with grapes worldwide is about seven million hectares. The total production in the world is about seventy-eight tonnes. It is grown in Europe, Asia, America, Africa and Oceania (42.7%, 28.8%, 20%, 5.8%, and 2.7%respectively). The most common grape producing countries are Italy, China, France, Argentina, Iran, Chile, and South Africa, as well as the United States of America, Spain, and Turkey. The productivity of Palestinian grapes in 2019 reached to thirty-five thousand tonnes3184 hectares cultivation area (FAO, 2019).

Grapevine (*Vitis vinifera*) is one of the oldest fruit tree and one of the most cultivated in the world due to its economic importance. Currently in Palestine, it ranks second in the cultivated fruit crops in terms of area and economic return. Due to its unique geographical and ecological location, grape cultivation is concentrated in the Southern part of the West Bank, mainly in the Hebron and Bethlehem regions. There are more than 13 varieties of cultivated rain-fed agriculture. It is used as a table fruit or a manufactured food product (Dibis (molasses), raisins, fruit rolls (malban), vinegarand juice) (Basheer-Salimia 2014).

Figure 1.1. Vitis vinifera tree (2020)

According to the data provided by the Palestinian Center for statistics at the end of 2020, the cultivated area of grape varieties is estimated to be more than 80 thousand da with rainfed land accounting for 74 thousand da. While the irrigated area is about 6 thousand da of these. Due to its unique geographical and ecological location, grape cultivation is concentrated in the southern part of the West Bank, especially in the Hebron and Bethlehem regions. About 45 thousands are in the governorate of Hebron area and 15 thousands are in the Bethlehem area. Accounting for 2.8% of the total cultivated area. Grape is second of olives in terms of volume of production, and contribute about 12% of total agricultural production (PCBS, 2010).

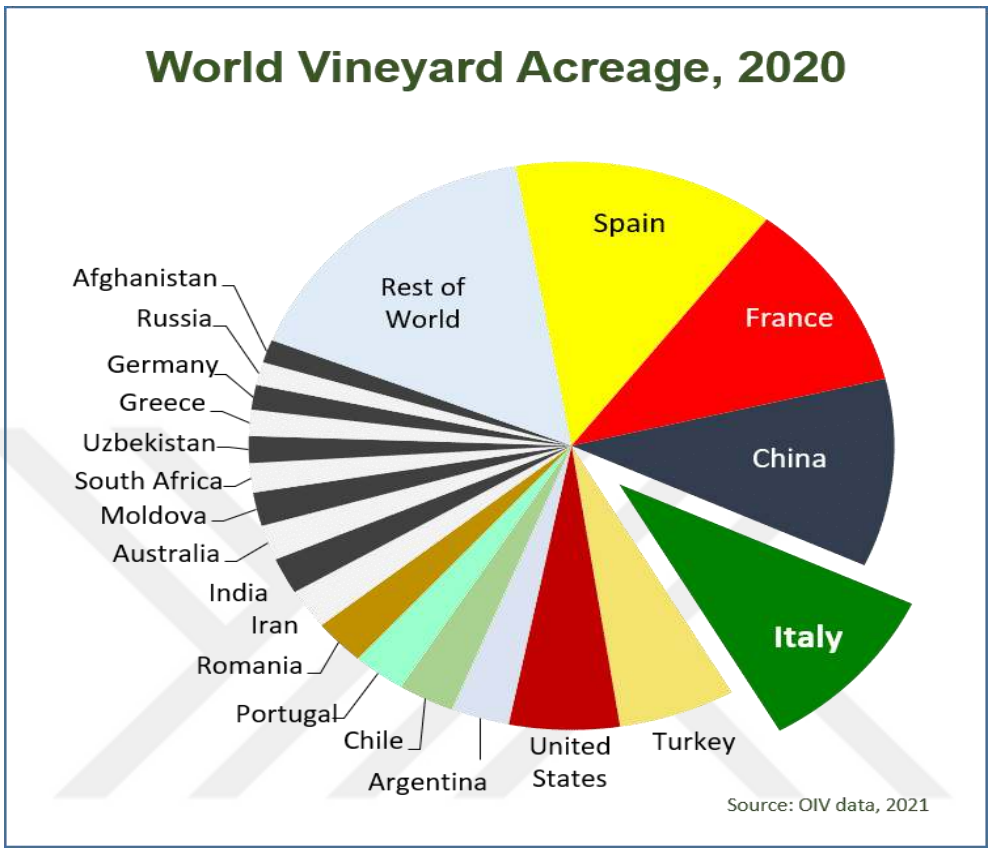


Figure 1.2. The top Twenty Grape-Growing Countries in 2020 for the production of grape (OIV, data, 2021).

Palestinians have been interested in grapes since the time of the CanaanitesThe grape is a cultural and heritage symbol of the Palestinian people. In addition, the first point to ensuring the steadfastness of farms against land confiscation by the Israeli occupation, as most varieties depend on rainwater. It is used as a table fruit or a manufactured food product such as Dibis (molasses), raisins, fruit rolls (malban), vinegar and juice) (Basheer-Salimia2014).

There are more than 13 varieties of cultivated rainfed agriculture, the main cultivated species include; white grape species: such as Dabogi (Al-Baladi), Al-Jandali, Al-Zaini, Beiruti (Al-Romani), Al-Hamadani and Al-Marrawi. Red grape

species:such as Al-Halawani, Black Grape: Beituni (Baloti), Shami, Al-Fahaissi (Al-Motatrash) and Al-Shoyoukhi (Darawishi). Recently, other types of seedless white grapes have been introduced to Jericho and are mainly grown under irrigation include Berlait and Superior (Sarah.2016).

The health status of vineyards in Palestine is not entirely clear because of the similarities of the industries to Jordan and the Israeli occupation (Tanner et al., 1996)The vine is a plant that has cultural and commercial importance in the world (Camargo et al., 2011). It is exposed to a variety of variables that are passed through the graft and produce a variety of illnesses with uncertain etiologies (Maliogka et al., 2015).Vegetative propagation of this crop contributed to the spread of diseases in vineyards, leading to the appearance of complex diseases that were difficult to control (Basso et al., 2014). There are more than60infectious agents that affect grapes (viruses, viroids, phytoplasmas, bacteria and fungi) (ICVG, 2003). Most of them cause disorders that reduce the vitality and longevity of plants and affect the quality and quantity of the crop (Tomazic et al., 2008).

Plant diseases are a worldwide danger to food security, environmental sustainability and economic stability of the country.

Grape leafroll disease (GLRD), the most commercially significant grape disease, is responsible for during the grapevine's productive life, it might lose up to 30-70% of its productivity(Walter and Martelli, 1997). It's a rapidly spreading illness that's been found in grape-growing areas (Atallah et al., 2012) In both red and white grape varietals, GLRD impacts fruit ripening and lowers soluble solids (Martinson et al., 2008).

Grapevine leafroll (GLR) is a viral illness that has been suspected for a long time but has to be verified (Bovey and Martelli, 1992). A family of viruses known as the Closteroviridae causes it (Each virus is given the name Grapevine leaf roll and a number).

The *Closteroviridae* is a plant virus family with large positive, uniform genomes. There are two or more genes, of *Closterovirus* and *Crinivirus*, whose members are important diseases spread by an insect, which are responsible for considerable harm to crops of sugar beet, citrus, grape other plants (Martelli et al, 2001).

A member of the *Closterovirus* genus within *Closteroviridae*, grapevine virus-associated 2 is associated with many serious grapevine diseases across the world, "Leafroll," "rapid decline", and “graft incompatibility," are some of the terms used. GLRaV-2 isolates have been detected in a variety of grapevines. However, the genomes of these isolates have either not been sequenced or have only been partially sequenced, and the genetic link between these viral isolates remains unknown.A study (Meng et al, 2005) looked at four strains of distinct GLRaV-2 isolates based on their coat protein sequences.

Environmental variables, grape species, rootstock, viral isolation or strain, agricultural circumstances, and the illness's connection with pathogens and other viruses are all variables that determine how the disease manifests (Maliogka et al., 2015a). All of these infections are propagated through vegetative multiplication over large distances (Martelli, 2014), with local transmission by insects, mites, and nematodes from plant to plant (Maliogka et al., 2015a).

Control of viruses that infect grapevines, as well as other plant species, is inextricably linked to assessing the vines' sanitary state. As a result, in addition to having expertise in detecting the symptoms produced by these viruses, having access to effective detection procedures is critical (Burger et al., 2015).

A lot of research had carried out in Palestine to identify and determine the incidence of the (GLRaV) virus on grapes (Mainly GLRaV-3). This study is one of the first studies conducted to detect strains 2 and 4 on grapes in Palestine.

Due to a lack of information about the prevalence of GLRaVs in Palestine, and in the interest of disseminating healthy propagating materials to farmers, the goal of this study was to assess the sanitary status of grapevines case of GLRaV- 2 and GLRaV- 4, using available serological and molecular diagnostic methods.

2. LITERATURE REVIEW

Bertazzon and Angelini (2004) has studied and compared some methods of detection of (GLRaV-2) variants. The GLRaV-2-RG strain and a new strain termed the GLRaV-2-BD strain was utilized in a combination of variables. When the samples examined contained a significant level of antigen, the test method recognized all GLRaV-2 isolates. The RT-PCR method was more sensitive than the ELISA method, although it did not detect all GLRaV-2 sequence variations. As a result, new primitive pairs were created, some of which allow simultaneous identification of all isolates and others which are exclusive to each variation. GLRaV-2 variants with sequence variants were quickly identified and characterized using pairs of GLRaV-2 universal primitives and regional front analysis.

Grapevine is a globally popular vegetatively propagated fruit crop with a high socioeconomic value. It is susceptible to graft-transmitted infections, which can cause a range of diseases and major crop losses, as well as lowering fruit quality and plant vitality, as well as shortening vine lifespan. Vegetative propagation and frequent international exchanges of propagative material aid in the spread of these illnesses, encouraging the development of complex diseases. Its inexhaustible life cycle speeds up the mixing and introduction of several viral agents into a single plant. About 65 viruses from various families infect grapevines, however not all of them cause economically significant illnesses. According to a study by Basso et al., (2017)the four major diseases that affect grapevines are grapevine leafroll, rugose wood complex, leaf degeneration, and fleck infections.

Türkmen et al. (2019) identified the locations of Turkey most affected by grape leaf diseases. More than 200 infected samples were gathered from vineyards in Turkey's commercial grape-growing regions of Manisa, Denizli, Nevsehir, Elazig, Izmir, Ankara, Anakkale, Tekirdag, Edirne, and Krklareli in 2009 and

2010.All samples were evaluated for of grape leaf-associated viruses (GLRaVs) using DAS-ELISA utilizing various kits,single or multiple infections were found in 143 of the total (67.14 %). In the examined region, GLRaV4-9, -7, -3, -1, -2 and -6 were found in 53.52%,36.15%, 34.74%, 32.86%, 32.39%, and 3.28%, respectively.The Eastern Anatolia Region has the highest infection rate (100%), followed by the Aegean, Central Anatolia and Marmara Regions (75%, 26.47%, and 69,74%, respectively). GLRaV-1, -2, and -3 infection rates were highest in the Marmara Region (43.42%, 44.74% and 50%t, respectively), whereas GLRaV4-9 infection rates were highest in the Eastern Anatolia Region (43.42%, 44.74% and 50%, respectively) (100%). In the Marmara Region, GLRaV-6 infection rates were reported to be as high as 3.95 %. With a frequency of 36.17 %t, GLRaV-1,2,3, 4,9 and 7 were determined to be the most common multiple infections.The Eastern Anatolia Region has the highest infection rate (100%), followed by the Marmara, Aegean, and Central Anatolia Regions (69.74 %, 75 %, 62 % and 26.47 %, respectively). GLRaV-1, -2, and -3 infection rates were highest in the Marmara Region (43.42 % 44.74 %, and 50 %, respectively), whereas GLRaV4-9 infection rates were highest in the Eastern Anatolia Region (43.42%, 44.74%, and 50%, respectively) (100%). In the Marmara Region, GLRaV-6 infection rates were reported to be as high as 3.95%. With a frequency of 36.17 %, GLRaV-1,-2, -3, 4-9, and -7 were determined to be the most common multiple infections. Sequence analysis of the CP and p23 genes in GLRaV-4 isolates revealed intra- and interspecies recombination events. The Indian isolates GRP-G (KT759152) and GDR-I (KT759153) differed from other GLRaV-4 isolates in their p23 gene. GLRaV-4 has been discovered in Indian vineyards for the first time, as well as recombination in the GLRaV-4 genome.

In order to identify Grapevine Leafroll-associated viruses1,2,3,4,5 and 9 especially, Real-Time RT-PCR (TaqMan®) assays were developed. A broad variety of regionally scattered isolates were assessed for testing. The

geographically varied isolates were packaged from the most preserved areas and TaqMan® samples were tailored to target 100 % sequence identity regions. It is shown to be possible to create improved diagnostic tests through the improvement of the RNA extraction process and test for the quality of the RNA by means of the ribosomal 18S TaqMan® Test as a particular internal RNA control to confirm RNA quality. The TaqMan® RT-PCR assays were compared to typical RT-PCR procedures that employed full purified RNA and crude extracts to detect viruses in real time. For usage in a real-time cocktail of TaqMan® RT-PCR, the ideal amount of crude oil extracted in GES was determined to be 1 millimeter per reaction. This study also found that TaqMan® RT-PCR was more sensitive than standard one-step RT-PCR when evaluating diverse isolates of these viruses using either RNA or crude tissue extract(Osman et al.,2007).

Low-density arrays (LDA) were based on RT-PCR (TaqMan®) Real-Time tests to identify 13 viruses in grapevine in addition to the 18S rRNA housekeeping gene. Grapevine-leafroll viruses 1, 2, 3, 4 and 5, 9, Tomato ringspot and Grapevine-Fleck are all virus-related viruses, grapevine virus and GFkV. Three TaqMan® RT-PCR assays were examined, which have been created and incorporated with the RT-PCR and LDA detection for GLRaV-2RG, GFkv and ToRSV. The new tests have been included. A wide range of geographically dispersed isolates was tested for the LDAs. Africa, Europe, Australia, Asia, Latin America and America were part of the geographic locations. Compared with TaqMan® RT-PCR, and RT-PCR the high-performance detection of this virus was carried out using LDAs. For usage in low-density array detection, the effectiveness of various RNA extraction methods and buffers were measured. Additionally, improvements in the RNA extraction process and quality testing for RNA using the TaqMan® 18S ribosomal RNA test have been shown to provide superior diagnostic tests as a special RNA internal monitoring. This is Osman et al, first publication on the use of LDA to identify plant viruses in 2008.

The functions of the viral RNA genome and sub genome RNA production were mapped using a full-length cDNA clone and beet-yellow *Closterovirus* (BYV) clones. In the 10 open reading frames (ORF) discovered in BYV, ORFs 1a and 1b are adequate for RNA replication and transcription. Proteins encoded inside those ORFs are shared by all Sindbis viruses, but narrow viruses have a large interdomain region that is unique to them, including probable methyl transferase, RNA helicase, and RNA polymerase. A double role in genome amplification was detected in the Papain-like lead proteinase, L-Pro, encoded in ORF 1a in the 5 ′-proximal regions. For the first time, auto catalysts were necessary for reproducing RNA between L-Pro and the rest of the ORF 1aproducts. Second, for effective RNA accumulation, an extra L-Pro function was required which is separate from proteolytic activity. The removal of a big, 3″ terminal <~5,6 kb section for an HSP70 homologue and a 64kDa protein for a hydrophobic protein for a 6 kDa area for a 20 kDa protein for a small and large capsid protein for a 21 kDa protein for replication was a competent RNA. by Peremyslov et al. (1998) of mutants replacing beginning codons in each of these seven ORFs showed that p21 works as an enhancer of genome expansion. There are fascinating parallels between the structure of the genome and the replication needs of plant viruses and animal coronavirus viruses.

Symptoms of scion/rootstock incompatibility included yellow- (on white varieties) or red- (on red varieties) mottled leaves, rolling leaf edges, weak to moderate branch development, and scion overgrowth at the rootstock junction. Plants that have been weakened may live for a brief period, but they quickly deteriorate and die within two years. Up to five graft-transmissible agents (GTAs) were discovered based on varied responses to bud-chip inoculations onto a panel of 18 distinct grape rootstocks. Grapevine rootstock stem lesion associated virus was one of the GTAs discovered in the Red globe (GRSLaV). Uyemoto et al. (2003)

used RT-PCR tests on samples of struggling grape plants from various California wine grape locales, one out of every five samples tested positive for GRSLaV.

Four serologically unrelated *Closterovirus*-like particles (GLRV-1, GLRV-2, GLRV-3, and GLRV-4) were identified in the lab from leafroll grapevines by Zimmermann et al., (1990). Antibodies developed against these particles were effective for identifying them and detecting them in diseased plants. After denaturation, the coat proteins of these four serotypes were separated on SDS-PAGE, then transferred to nitrocellulose sheets and immunopurified with specific polyclonal antibodies. GLRV-1, GLRV-2, GLRV-3, and GLRV-4 each have a single protein species with molecular weights of 39 Kd, 26 Kd, 43 Kd, and 36 Kd, respectively, in their capsids. ELISA, immune electron microscopy, and immunoblotting studies revealed no serological relationship between these 4 filamentous particles. Serological study of numerous grapevines from the Middle East and Europe revealed a strong link between the presence of GLRV-1, GLRV-2, GLRV-3, and GLRV-4 antigens on *Vitis vinifera* Pinot Noir and leafroll symptoms. This link was established by analyzing symptomless and sick grapevines collected in the field, as well as a serological examination of heat-treated plants infected with GLRV-1 and GLRV-3, the most common antigens found in leafroll-infected grapevines. Fonseca et al.,(2016)studied genetic variants of grapevine-associated virus-associated disease 2 affecting Portuguese vineyards. Global phylogram results and genetic analyse revealed population growth, notably among the NP and 93/955 groupings. To complement the virus molecular detection coefficient, to detect a combination of variations from distinct groups of phylogram in certain isolates, a typological coefficient The method was created Reverse transcription was used, followed by polymerase chain reaction and analysis of fragment length polymorphisms. This methodology is adequate for regular GLRaV-2 testing and differentiates between variations of the phylloid discovered in this investigation. The complete capsid protein (CP) gene of 19 isolates of (GLRaV-2) from

Portuguese grapevine varieties was sequenced to describe the virus genetic diversity. GLRaV-2 variations from Portuguese isolates were divided into three main phylogroups (PN, 93/955, and H4), according to a global phylogenetic analysis of the CP gene that comprised nucleotide sequences acquired in this work and full homologous sequences from Gen Bank. The evidence of well-supported subdivision within H4 as well as within PN, with subgroup PN3 consisting only of variations from a Portuguese isolate, is the uniqueness of these phylogenetic studies.

Leafroll is a significant grapevine disease that affects viticulture all over the world. It gets its name from the distinctive foliar symptoms that appear on susceptible grapevine types in late summer and early fall. Grapevine leafroll associated viruses of all species are linked to the illness (GRLaV). Leafroll disease can impair the quality and quantity of fruit as well as the quality and production of wood for propagation in many grapevine varieties. Graft incompatibility has also been linked to GRLaV infection and illness. Infections can be asymptomatic, Infected grapevines can also act as a viral reservoir for other kinds that are susceptible (Constable et al., 2014).

Many mealybug species transfer grapevine leafroll-associated viruses (GLRaV), which cause the economically significant grapevine leafroll disease in grape-producing regions across the world. The mealybug *Ferrisia gilli* Gullan is a novel grapevine pest in El Dorado County, California's Sierra Foothill wine-growing region. GLRaV species 1, 2, 3, and 4LV have been discovered in vineyards with symptomatic vines in the Sierra Foothills.To conduct controlled viral acquisition and transmission testing, we employed source vine accessions infected with various combinations of GLRaV. According to Wistrom et al.(2016), *F. gilli* got GLRaV 1, 2, 3, and 4LV and transmitted GLRaV-3 and GLRaV-4LV to uninfected recipient vines. *F. gilli*, like many other mealybug species, is a direct

threat to plants, but it also poses a risk to the grape industry as a vector of economically destructive viruses.

Several types of phloem-limited grapevine leafroll associated viruses (GLRaV), some of which are transmitted by mealybugs and scale insects, are linked to grapevine leafroll disease. *Daktulosphaira vitifoliae* (Fitch) Biotype A (Hemiptera: *Phylloxeridae*), a common vineyard pest that feeds on the phloem of vine roots, is a frequent vineyard pest. These insects might spread one or more GLRaV species, notably GLRaV-2.In vineyards with a high frequency of grapevine leafroll disease, a field survey was conducted, and *D. vitifoliae* was tested for GLRaV acquisition. The capacity of *D. vitifoliae* to transfer GLRaV from infected root sections or vines to co-planted virus-free recipient vines was investigated in greenhouse studies. In the wild, there was no GLRaV-positive *Daktulosphaira vitifoliae* and *Daktulosphaira vitifoliae* did not transmit GLRaV in greenhouse transmission studies. After eating on infected source vines in the greenhouse, several insects tested positive for GLRaV, but there was no indication of viral transfer to healthy plants. These observations, together with the soil biotype of *Daktulosphaira vitifoliae* sedentary behavior, render it unlikely that *D. vitifoliae* is a GLRaV vector (Wistrom et al., 2017)

To assess the presence and incidence of virus and virus-like diseases in vineyards in Palestine's main grape-growing areas inspections were conducted in the main grape-growing areas (Hebron, Bethlehem, Gaza, Jerusalem, Ramallah, Jenin, Jericho, and Nablus). Leafroll symptoms were seen in local and imported cultivars in Bethlehem, Ramallah and Jerusalem, with greater incidence in the red-fruited Shami, Beituni, and Smari varieties. Rugose-wood signs were also seen in local and foreign varieties, particularly on grafted vines, which were found in significant numbers in Bethlehem. On the Biadi, Superior Seedless, and Beituni, fanleaf symptoms were infrequent, although phytoplasmas-induced symptoms were observedin Jenin, Jericho and Bethlehem. Alkowni et al.,(1998) did this

investigation, and they advised that more research be done to confirm or deny the virus's existence, as well as to ascertain the virus's distribution and strain.(1998) ELISA testing revealed that at least one virus had infected 463 of the 566 vines examined (82 %). GVA was the most common virus (66.1%), followed by GLRaV-1 (45.6%) and GLRaV-3 (33.6%). (21.7 %). GLRaV-2 (14.7%) and GFkV (15.7%) (8.3%). GVB and GFLV were discovered to a lesser extent, with rates ranging from 3.7 to 1.2%. GLRaV-7, on the other hand, was found in a single foreign-origin cv. Sultanina vine. Vineyards in the Bethlehem region were very hard hit (97.5%). and several native cultivars were either completely infected (Jandali, Marrawi, Shoyoukhi) or highly infested (Jandali, Marrawi, and Shoyoukhi) (Zaini, Biadi and Shami).

Palestine is home to a broad diversity of plants and has a wide range of agro-ecological issues. Grapes are an important component of the cultural history and a necessary dietary item. Because local cultivars have traditionally been identified based on physical features, geographical provenance, or the names of the vineyard owner, homonymy, synonymy, and misnaming greatly reduces their value.

To further understand the population's genetic diversity and relationships, DNA profiling utilizing 22 common SSR markers was used to identify 43 possible cultivars grown mostly for local table grape consumption in the West Bank's southern highlands. Consistent matching of SSR markers with grapevines cultivated in neighboring countries or maintained in European germplasm collections was found for 8 of the 21 non-redundant genotypes discovered, implying possible synonyms as well as the occurrence of breeding selections previously developed in the United States.SSR markers were utilized to link Palestinian cultivars to the ancestral population of *Proles orientalis* subpr. *antasiatica*, and they even found a relationship between local resources and cultivars generated through international table grape breeding. The research of

Basheer- Salimia et al., (2014) supports the need to harvest and preserve indigenous vines from a region with a long history.

Grapevine leafroll-associated virus 2

Grapevine leaf-associated virus 2 was identified in 1984 but not named until 1995. It is one of the five grape leaf viruses. Graft incompatibility diseases are also involved. GLRaV-2 is part of the genus *Closterovirus* of the family *Closteroviridae* due to its distinctive genetic structure and evolutionary history. The GLRaV-2 genome is a single-stranded positive-sense RNA of around 16,500 base pairs that is most likely capped at the 5″ end but lacks a 3″ poly (A) tail. The nine GLRaV-2 encoded ORFs include the Replication Modulus, the Quintuple Block Gene Module and the Modulus for RNA Silencer. In contrast to all members of the *Ampelovirus* genus that infect the grapevine, the translation product of ORF1a lacks an AlkB domain. With six lineages characteristic by up to 25% nucleotide differential and distinct biological activity in plant hosts, the genetic diversity of GLRaV-2 is quite great. All All grapevine part are susceptible to GLRaV-2. GLRaV-2 in Wild *Vitis* spp. The virus is confined to phloem tissue and the infected cells have a large presence of membrane vesicles typical of the *Closterovirus* genus infection. No GLRaV-2 vector is known, however the virus, unlike other GLRaVs, is transmissible to certain herbaceous hosts via graft and mechanically transmissible. Clean, virus-free propagation materials are used to control GLRaV-2 (Angelini et al., 2017)

Abdullahi et al., (2011) studied the identification of grapevine-infecting viruses were based on a monocoloured microarray hybridization technique. Total RNA (two separate techniques) (two distinct types of RNA) from the infected plant has also been transformed to cDNA. The 3 DNA approach, a post-hybridization detection process using highly fluorescent dendrimer agents, was needed to identify Oligonucleotides were tested and compared between 27 and 75 nucleotides

in length. Eight discovered viruses it *Nepoviruses*, two vitiviruses, one *Closterovirus*, fovea virus *ampeloviruses*, macula viruses and sadwaviruses. Results of this study showed that the microarray method may detect viral infections without amplifying the RNA template in sequence bias.

Grapevine (*Vitis* spp.) is an important agricultural commodity and one of the most often grown forest crops in temperate climates. The vintage plant is vulnerable to a variety of pests and pathogens, including infectious intracellular substances (viruses, viroids, phloem- and xylem-limited prokaryotes) that play a major role in most vegetatively propagated plants, causing significant losses, reducing vine productivity, and jeopardizing vineyard survival. The importance of the Grapevine industry and the scope of these illnesses' difficulties have sparked a lot of interest, and intensity research, which has been particularly active in the global arena since the late 1950 (Martelli, 2014).

This study by Abou Ghanem et al., (2015) aimed to learn about viruses in the South Eastern United States that attack native grapes. These dsRNAs were reverse transcribed and PCR with taxon-specific primers for the HSP70h closterovirid gene. DNA ~600-bp bands have been cloned and sequenced, amplified by both samples. Pairly, the two viruses have 75% common nucleotides (nt) and 82% amino acids (aa) in the sequenced genome region. The results show that these viruses are different isolates of Grapevine leaf-related viruses2 when compared with existing sequences in NCBI/GenBank (GLRaV-2).The set of specific primers GLRaV-2 were employed in a one-step RT-PCR assay on total nucleic acid extracts, from an additional 65 samples (LR-2F: 5′-TCGGGCGTACCAACTTAC-3′ and LR-2R: 5′-CTGAGTGAACGCACTGATC-3′) (60 muscadines and five summer grapes). Four other samples of muscadines have detected GLRaV-2.

Abu Ghanem et al.,(1998) used the purification process of *Nicotiana benthamiana* to investigate the purity of the virus linked with grapevine leafroll

(GLRAV-2). The electrophysiological coat protein components of polyacrylamide have a molecular weight of around 21.5 kDa. An infected *N. benthamiana* was cloned and sequenced using the two-piece RNA retrieved. Through point hybridization and reverse transcription of polymerase chain reaction in infected grapes, molecular samples and primates were effectively employed during cloning to detect viruses. The genome's structure was remarkably identical to that of the beet yellow virus. the *Closterovirus* type, and also the Tristeza citrus virus. The comparative analysis of phylogenetically relevant proteins confirmed this similarity. GLRaV-2 is morphologically, physically and physically chemical, ultrastructure and molecularly qualified in the *Closterovirus* genus.

Different animal and plant viruses can translate their virions through intercellular connections between their adjacent cells. This study by Alzhanova et al.,(2001) analyzed a plant *Closterovirus* in the construction of virions and migration from cell to cell and showed a significant connection between both processes. Filamentous virions from a *Closterovirus* have a long body formed by the major capsid protein (CP) and a short queue made by the tiny protein capsid (CPm). In the serological and molecular analysis of several grapevine accidents of various grapevine types Angelini et al.(2004) used data from Europe wineries in their 2001-2002 study. Leafroll-associated virus 2 (LAV2) detection (GLRaV2). Among the grapevines examined were eight different GLRaV-2 variations.Three more Southern and Central Italian GLRaV-2 variations differed from all the reference strains. There have been two different GLRaV-2 mutations discovered with a Tuscan grapevine accession. Nucleotide homology assessed in the CP gene was sometimes <88% across 23 GLRaV-2 isolates.

Bertazzon et al. (2010) looked at the genetic diversity of Grapevine leafroll-associated virus 2 (GLRaV-2) isolates by looking at three genomic segments encoding the heat-shock protein homologue 70 kDa, the protein coat, and

the osteo-60 kDa protein from a variety of Grapevine accessions from different geographical origins.

Various techniques have been developed for the identification of GLRaV-2, including indexing, serological and molecular procedures. However, a technique with better sensitivity and capability to identify all of the known Because of the virus's low concentration in plants and the variety of GLRaV-2, variations are required. Such advancements are essential for grapevine root stalks, which are thought to Multiple GLRaV-2 infections are present, which are difficult to detect and hence aid in the virus's propagation.

The creation of new universal primers is detailed in the 3″ end of the viral genome with a target sequence. In combination with the SYBR Green real-time RT-PCR test in one step, these elements are used for quantitative detection. All 43 GLRaV-2 isolates examined in this investigation, irrespective of their geographical origin or grapevine type, were quickly and reproducibly identified. The sensitivity was 125 times higher than the standard single-tube RT-PCR using the method established in this study by Beuve et al. (2007). This approach brings up new prospects for hygienic selection of grapevine and for the monitoring of leafroll 2 diseases.

Ultra-structural observations were performed on cells of the grapevine leafroll-associated virus 2 (GLRaV-2-4H), the grapevine virus infected by the same isolate, *V. vinifera* cv. 'GLRaV-2 isolate Se' infected by the same mechanically transmissible isolate, and an undetermined Grapevine leaf roll-associated Virus *Vitis vinifera* 7 (GLRaV-7). The *Closterovirus* genus is GLRaV-2 and GLRaV-7.Notwithstanding the host, both viruses seemed to multiply in the phloem exclusively, damaging the cytology of the distinguishing sieves, parenchyma and accompanying cells. Induced both GLRaV-2 isolates in herbaceous host and Vitis in the same kind of ultra-structural changes, mainly of membrane proliferation, inclusion bodies, and aggregation of virus particles. The inclusion systems

consisted of clusters of fibrillary membranous vesicles, surrounded by a single mucosa, interspersed with loose particulate aggregates. In both GLRaV-2 and GLRaV-7 infections, these vesicles did not seem to arise from mitochondria; therefore they have already established a distinction between these viruses and two other closteroviruses (GLRaV-1 and GLRaV-3). GLRaV-2 and GLRaV 7 virus particles have been abundant and accumulated in both of the Vitis varieties and nuclei (Castellano et al., 2000).

Coetzee et al. (2010) analyzed the Double-specked RNA obtained 44 randomly picked grapes from a sick South African vineyard were pooled and utilized in a deep sequencing study to generate a viral population census. There have been four viral pathogens identified. Grapevine leafroll-associated virus 3 (GLRaV-3) accounted for 59 percent of the total data read, followed by Grapevine rupestris pitting-associated virus and Grapevine virus. A was revealed to be the most often reported species.

Australian certification systems providing high-health planting material rely on precise techniques of virus identification. In Australian circumstances, the reliability and reversibility of enzyme-linked immunosorbent testing (ELISAs) and RT-PCR testing was evaluated. Reproduction studies with Grapevine viruses A, Grapevine fleck virus, Grapevine leafroll-associated virus GLRaV-2 and GLRaV-3 were carried out in a hot and cool climate. For three years, Grapevines have been examined monthly. The reliability of both tests rose after 12 months and up to 3 years after inoculation in both climates. RT-PCR found viruses more frequently than the ELISA assays. According to Constable et al (2012), viruses cannot be identified on a regular basis until 12 months following an infection episode. For viral detection throughout spring and summer, The RT-PCR method is more reliable than the ELISA method. Virus detection was seldom 100 percent accurate, and re-testing is recommended to improve detection rates.

As stated by Dolja et al. (2003) the yellow beet virus is a *Closterovirus* type member of Closteroviridae family. One member of the positive-strand RNA viral alphavirus subgroup. Properties physical: Flexible filaments are around 1300 nm in length and about 12 nm in diameter, consisting of about 15.5 kb of RNA and five proteins. The main capsid protein creates a helical symmetry virus body, accounting for around 95% of the length of the vision. A small capsid protein, Hsp70 homologue, around 64-kDa protein and around 20-kDa protein construct the short virion tail. Protein from the virus: The leader proteinase and RNA replicas of 5'-most ORFs 1a and 1b encodes.

The other ORFs 2-8 are produced by sub genomic mRNA coding 6-kDa membrane protein, Hsp70 homologue, roughly 64-kDa protein, small and large capsid proteins, approximately 20-kDa and roughly 21-kDa protein. Hosts: Sugar beet (*Beta vulgaris*) and spinach (*Spinacia oleracea*) are the main agricultural plants infected by the beet yellows virus (BYV). Moreover, over 120 species in 15 groups were reportedly infected by BYV. *Nicotiana benthamiana*, *Tetragonia expansa* and *Claytonia perfoliata* are the most suited species for propagation.

Coronaviridae and *Closteroviridae* are two separate families of positive-beach RNA viruses discovered by Dolja et al. (2006). The largest surviving RNA genomes were found here. Comparative analyses of the latter family's genes reveal three levels of gene conservation. The most conserved gene module defines RNA replication and is shared by plant and animal viruses in the alphavirus-like superfamily. The *Closteroviridae* family has a symbol consisting of a five-gene module that works in particle assembly and transportation. This module contains an HSP70 molecular chaperone homologue as well as three distinct copies of the capsid protein gene. The remaining genes show significant differences in number, function, and origin across and among closteroviruses. RNA silencing suppressors, RNAse III, papain-like proteases, the AlkB-domain of RNA repair, RNA ribbon-containing protein, and a variety of proteins that have no detectable homologues

may all be identified in today's databases. The evolutionary techniques used to create the complex and fluid genomes of large RNA viruses may be similar to the genomic complexity evolution seen in other life forms.

Several GLRaVs are discovered often in grapevines with GLD behavior. GLRaVs are connected with the grapevine. The GLRaV-2 is the sole *Closterovirus* induction, in particular, for leafroll symptoms and incompatibility with the graft. Fan et al., (2015) studied to identify GLRaV-2 in 132 samples collected in 14 provinces and regions of China, new degenerated primers developed against the HSP70 gene were used in a polymerase chain reaction (PCR) and nested PCR (nPCR). Fiftyone-fifth of the samples had GLRaV-2 infections, and a majority had no GLD symptoms. Popular grape varieties such as Cabernet Sauvignon (92.3%), Chardonnay (80%), Red Globe (75%) and Italian Riesling have a high prevalence of GLRaV-2 infection (73.7%). The 'Beta' roots were likewise found to be severely infected with GLRaV-2 and had previously been classified as negative samples (50 %). The isolates of GLRaV-2 obtained in this investigation revealed identities at the nucleotide and amino acid levels of 68.9% to 100% and 76.47% to 100.0%, respectively. A phylogenetic study using the HSP70 gene indicated that three out of five reported phylogenetic groupings belong to all GLRaV-2 isolates in China. In a single isolate, there were several variations of PN and RG groups. The results revealed that more GLRaV-2 isolates could be detected in the new degenerate primers than those previously reported.

This is the first comprehensive research on GLRaV-2 prevalence and gene diversity in China and proposes an nPCR approach to enhance PCR sensitivity as an alternate way if there is no real-time PCR instrument available.

The visual monitoring of symptoms or laboratory testing is used to diagnose grapevine viral disorders and their related viruses. In the event of manifested pathologies, or by biological indexing with sensitive indicators in the event of latent conditions, the observation of signs can be carried out immediately

in the field. Different serological and bio molecular tests based on viral capsid and nuclear acid detection respectively are part of the laboratory testing. Because of the availability of commercial kits, serological testing is widely utilized. In recent years, bio molecular tests have seen tremendous changes, from dot-blot hybridization through several PCR-based methods and eventually microarrays, which have improved their sensitivity and reliability (Gambino et al., 2010).

Goszczynski et al. (1996) studied it. In two grapevine leafroll related viral strains, mechanical transmission of grapevines in *Nicotiana benthamiana* was studied (GLRaV-2). In terms of symptom development, there have been consistent variations between strains 94/970 and 93/955. The first created local chlorotic and sometimes white-necrotic lesions, whereas the second induced chlorotic lesions followed by severe metallic opalescent necrotic lesions. The strains could not be discriminated by their capsular proteins' molecular weight or serological weight. The pattern of the little dsRNA bands was constantly variable.

The genetic diversity of the Grapevine Leafroll associated virus 2 (GLRaV-2) field populations in Pacific Northwestern (PNW) vineyards was employed by Jarugula et al. (2010) to study a sequence of the complete coat protein (CP) and part of the heat-shake protein-70 homogeneous genes. Several alignments of CP amino acid sequences revealed lineage-specific modifications. According to data from an ELISA test, GLRaV-2 commercial source-specific antibodies are unable to properly detect RG-2 isolates, limiting the diagnosis of all GLRaV-2, which is currently prevalent in PNW vineyards, to antibodies alone. A method based on reverse-transcription chain-reaction polymerase and restriction-length fragment polymorphism analysis was created to differentiate GLRaV-2 isolates belonging to the three lines prevalent in the region. The taxonomic status of GLRaV-2 is discussed in light of current knowledge of the virus.

A national study of commercial winegrowers was carried out in Virginia, the USA, throughout the 2009-2011 seasons in order to evaluate the events of

Grapevine leafroll associated virus 2 (GLRaV-2). The RT-PCRs have been found in 8, 25 and 1% correspondingly of the 415 samples from 77 sites (vineyards), GLRaV-2, GLRaV-3 and GFkV, and 64 % of the vineyards were positive for one or more of the three viruses of 415 vineyards. The negative samples for the three viruses were tested in 100 wild *Vitis* sp. Two grapes were found in the vineyards of VA (*Pseudococcus maritimus*) and Gill's (*Ferrisia gilli*). While there was no significant geographical influence (P>0.05), evaluation of variance demonstrated a larger variability of the incidence of GLRaV-3 on a cultivar in a vineyard than in a less spatial scale (i.e., at sampling site). In the presence of mealybugs (P < 0.001) and greater vine age (P < 0.001), the likelihood of discovering GLRaV-3 infected grapes was higher. The findings also show that the research of GLRaV-3 movement in mealybugs conducted by Jones et al. (2015) in VA vineyards. The high incidence of viral wines underlines the significance of clean plants as well as mealybugs treatment. it is the first research that reports the presence in the VA vineyard of *Ferrisia gilli*, GLRaV-2, GLRaV-3 and GFkV.

The *Closteroviridae* family includes more than 30 plant viruses with flexible filamentous viruses, including those with mono or double-positive ssRNA genomes. Closteroviruses are semi-persistently transmitted by insects from three Homoptera groups and are connected to phloem tissue in infected plants, implying broad and continuous growth. *Closteroviruses* co-evolved with insect vectors, according to phylogenetic analyses of their replication genes and HSP70 preserves, leading in three main groups: virus transmitted by aphid, mealybug, and whitefly. *Closteroviruses* are a large and diverse family of viruses that pose a serious danger to agriculture and demand immediate attention (Karasevet al., 2000).

Grapevine leafroll-associated virus (GLRaV)-1 to –5 and -9, and other viruses, examined for *Vitis* and non-*Vitis* spp. around nine Napa Valley grapes (GVD). Also included were *Vitis* spp. from three riparian regions outside the vineyards. in 6 out of 66 V. California and 11 out of 19 V. California − V. vinifera

hybrids, single and mixed GLRaV-2, -3, GVA, or GVB infections were identified as conventional or quantitative chain reverse transcription-polymerase reactions. No detections were made of GLRaV-1, -4, -5, -9 and GVD. GLRaV-2 and -3 sequences of partial coat protein gene nucleotide phylogenetic revealed a strong relationship to vinifera isolate in *V. vinifera* and V. california (Klaassen et al., 2011).

Three years of test data from the FPS Imported Program assessed the diagnostic performance of three assays now utilized by Foundation Plant Services (FPS). ELISA, RT-PCR and RT-qPCR were the three assessments tested. Grapevine Virus 2 (GLRaV-2), Grapevine Virus 3 associated with leaf-roll (GLRaV-3), Grapevine Fleck virus (GFkV), and Grapevine Virus A were the viruses included in the evaluation (GVA). The diagnostic sensitivity and diagnostic specificity were the two metrics estimated. The findings revealed that RT-qPCR was the most sensitive method, followed by RT-PCR. ELISA has the lowest sensitivity, especially for GLRaV-2. In comparison, RT-qPCR exhibited the lowest test specificity (Klaassen et al., 2012).

Several *Closterovirus*-like viruses, notably A pair of prominent papain proteases are coded by Grapevine Leafroll-Associated Virus-2, which have stayed big uncharacterized in their functional profiles. We have produced a full range of GLRaV-2 clones tagged reporters, and shown they are systemically infectious with *Nicotiana benthamiana* agro infections.

The revisions in the genetic structure and variability of grapevine leafroll-associated virus (GLRaVs) gained by worldwide efforts over the last decade, as well as the production and use of new types of serological reagents, provided a solid foundation for Martelli et al., (2012) review of the taxonomic structure of the family *Closteroviridae* and the *Ampelovirus* family.

Grapevine leafroll-associated virus 4

A putative novel *Ampelovirus* with moderate leafroll symptoms was discovered and molecularly described in *Vitis vinifera* cv. Carnelian. The genome was 13,625 nucleotides long and exhibited a structure comparable to that of *Ampelovirus* subgroup I members (fam. *Closteroviridae*). Sabanadzovic et al. (2010) found that the viruscv. Carnelian is the most unique member of the GLRaV-4 family of *ampeloviruses*, which includes GLRaV-4, -5, -6, -9, and the newly described GLRaV-Pr and GLRaV-De. This virus appears to be a novel member of the Closteroviridae family, for which grapevine leafroll-associated Carnelian virus is proposed as a temporary name.

Grapevine leafroll-associated virus 4 and GLRaV-6 from cv Estellat (GLRaV-6Est) complete nucleotide sequences were produced and compared to each other as well as to related viruses. Both viruses have genomes that matched those of *Ampeloviruses* belonging to Subgroup I. (fam. *Closteroviridae*). The availability of these genomes, as well as previously available data on similar GLRaVs, allows for a thorough examination of these viruses' taxonomy and nomenclature. GLRaV-4 and -6Est are regularly clustered with GLRaV-5, -9, and -Pr in phylogenetic studies, producing a poorly defined sub-cluster (GLRaV-4 group) within the genus *Ampelovirus*. The genetic distances between these viruses do not exceed the intra-species diversity seen in other *Closteroviruses*, according to a detailed analysis. GLRaVs -4, -5, -6, and -9 partly purified preparations interacted exclusively with homologous monoclonal antibodies in Western blots, although polyclonal antisera to GLRaV-5 and GLRaV-9 identified them all. DAS-ELISA was used to validate the virus serological relationship.

GLRaV-6 particles looked evenly coated with homologous monoclonal antibodies in immuno-electron microscopy, but GLRaV-2, employed as a reference, and had a "bipolar" shape. The findings of Sabanadzovic et al. (2012) question the taxonomy and nomenclature of numerous GLRaVs, suggesting that

they are divergent isolates of Grapevine leafroll-associated virus-4 rather than different species (definitive and, or presumptive) in the genus *Ampelovirus*, as previously thought.

A virus linked with leafroll symptoms in *Vitis vinifera* was chosen for further examination since it was undetectable in all reverse-transcriptase polymerase chain reaction (RT-PCR) tests available for Grapevine leafroll related viruses (GLRaVs). Extracts from infected plant material were found to be negative in all enzyme-linked immunosorbent assays for GLRaVs. Despite this, the unknown virus showed conventional grapevine leafroll symptoms in the indicator host, *V. vinifera* cv Cabernet Franc.

Purified high molecular weight double-stranded RNA (dsRNA) from an infected vine looked comparable to dsRNA from Closteroviridae virus-infected plants. This purified dsRNA was employed in RT-PCR to amplify a section from any *closterovirus* heat shock protein 70 homologues using degenerate oligonucleotide primers (HSP70). The full-length HSP70 sequence of the unknown virus was discovered by comparing sequence data from RT-PCR products to sequence data from other GLRaVs. Alkowni et al. (2004) Using primers generated from this sequence, researchers performed an RT-PCR experiment and determined that all GLRaVs tested were negative. Except for the virus, which is yet unknown. The novel illness has been given the name Grapevine leafroll associated virus 9Grapevine leafroll-associated virus-5 was investigated in Turkey's main grape-growing region (Southeast Anatolia) (GLRaV-5). RT-PCR was used to identify the presence of GLRaV-5 in plant samples with characteristic leafroll symptoms and mealybugs, *Planococcus ficus* (Signoret). The virus's initial appearance in Turkish vineyards has been confirmed (Buzkan et al., 2010).

Grapevine leafroll is a viral disease that affects grapevines and is one of the most common and commercially significant. Grapevine leafroll-associated viruses (GLRaVs) have been linked to this illness in the grapevine by at least nine

different Closteroviridae viruses. Grapevine leafroll-associated virus 4 (GLRaV-4) was discovered in California and identified as a Closteroviridae member of the *Ampelovirus* genus. 35 dormant cane samples from 12 different cultivars were gathered from different places in Chile and reverse transcended to see if GLRaV-4 was present in Chilean grapevines, in addition to the previously known GLRaV-1, -2, -3, -7, and -9.Two of the 35 samples were chosen. Both positive samples had 98.4 percent nucleotide similarity and nearly 99 percent identification with a Californian GLRaV-4 isolate's matching segment.

Because commercial antibodies to detect GLRaV-4 are unavailable, the second set of primers, LR4CPINT-F: 5′- GAG AGT GAC AAG CAC CAG GTG C -3′ and LR4CPFIN-R: 5′- TCA CCT CCT GTT GCC CA -3′ (4), was used to amplify a 492-bp fragment of the coat protein gene. The 492-bp fragment sequences from both Chilean samples (GenBank Accession Numbers EU746620 and EU746621) had 99.6% nucleotide identity and 96.5 percent identity with an Israeli GLRaV-4 strain (GenBank Accession No. AM176759). Escobar et al., (2008) were the first to report GLRaV-4 in Chile.

After examining several sources of plant tissues, extraction buffers, pH values, and centrifugation procedures, a purification procedure for *Closterovirus*-like particles linked with grapevine leafroll disease (Zeeet al., 1987) was improved. Antiserum was made against the CA-4 isolate and utilized in an ELISA test. In crude plant preparations, the virus-like particles of the NY-1 isolate measured between 1,800 and 1,900 nm in length. In SDS-PAGE analysis, the coat protein of the NY-1 and NY-2 isolates had a molecular weight of around 43 103 daltons, and bands with this molecular weight interacted with particular polyclonal and monoclonal antibodies in Western blotting assays. Leafroll sick grapevines yielded a big dsRNA molecule (ca. 10 106 Mr) as well as smaller molecular weight species, while healthy grapevines did not. In a protein A-gold labelling immunosorbent electron microscopy experiment, seven leafroll isolates were

evaluated for serological relatedness. The findings revealed the existence of serologically diverse serotypes, as well as the common occurrence of combined infection of grapevines with various scrotypes. Hu et al., (1990) recommended classifying isolates into serotypes I, II, III, and IV.

Grapevine leafroll illness is caused by viruses linked with grapevine leafroll (GLRaVs). These viruses are ubiquitous in vineyards across the world, and they're frequently linked to vitiviruses that affect the rugose wood complex of grapevines. The apple mealybug *Phenacoccus aceris*, which is prevalent in Holarctic areas and capable of transmitting Grapevine leafroll-associated virus-1 and -3, is one of ten mealybug species known to be carriers of one or more of these grapevine viruses (GLRaV-1 and -3). The goal of Maguet et al. (2012) was to analyze the transmission characteristics of leafroll viruses by *Phenacoccus aceris*in order to better understand the mealybug's role in leafroll epidemics. *Phenacoccus aceris* may transfer GLRaV-1, -3, -4, -5, -6, and -9 to the grapevine, but not GLRaV-7, according to the findings. This is the first time GLRaV-6 has been transmitted by a mealybug. *Phenacoccus aceris* was also proven for the first time to be capable of vectoring (GVA) and (GVB). The most efficient stage for transmitting GLRaV-1, -3, and GVA was first instar nymphs. This study shed information on the biology of *Venacocus* Acres vineyard transmission and marks a step forward in the control.

According to ML phylogenetic topologies for both genes, GLRaV-4,-5,-6,-9,-Pr, and -De exhibit closer phylogenetic linkages than other ampeloviruses, suggesting particularly small inter-species evolutionary distances for this multitudinous branch. These small inter-species indicated a unique evolutionary trait for this lineage, which is most likely related to its unique biological niche, which involves efficient host adaptation, vegetative propagation, and a lack of high-efficiency vectors (Maliogka et al., 2008).

A nested RT-PCR was developed within the genus *Ampelovirus* to enable the general identification of a group of genetically-related viruses with a distinct evolutionary history. The 490-bp nested PCR amplicons, which belong to a phylogenetically significant region, maybe sequenced right away to provide preliminary genetic information for partial characterization and classification. Additional primers for GLRaV-4, detection in single or multiplex nested PCR testing were developed and successfully used. Because the nested PCR used a rising annealing temperature profile, all amplifications were allowed to proceed in parallel. Maliogka et al. (2008) proposed a method for enhancing sequencing information of known and uncharacterized ampeloviruses classified within this lineage, allowing for their selective amplification in mixed Closteroviridae viral infections.

In Greek grapevines, a new grapevine leafroll-associated viral isolate (GLRaV-Pr) was found. The GLRaV-4, -5, -6, and -9 viruses form a separate lineage within the *Ampelovirus* genus, together with the GLRaV-4, -5, -6, and -9 viruses. Maliogka et al. (2009) published the entire nucleotide sequence of GLRaV-Pr, making it the first virus of this lineage to be sequenced completely.

Grapevine leafroll disease (GLD) is a serious disease that affects grapevines all over the world. GLD is linked to nine *Closteroviridae* viruses that are serologically different. Grapevine leafroll-associated virus (GLRaV) -1, -2, -3, and -7 were found in grapevines in China in previous investigations, with GLRaV-1 and -3 being the most common viruses linked to GLD. (Y. Dong et al,2005). 36 dormant canes from individual vines of 29 cultivars that had GLD leaf symptoms throughout the growing season were gathered from the germplasm collection plot of the Research Institute of Pomology, Chinese Academy of Agricultural Sciences, to check if GLRaV-4 and -5 were also present in China. Total RNA was isolated from phloem-enriched bark of 36 samples using a silica capture procedure X. (Foissac et al., 2001) and tested for GLRaV-4 and -5 using virus-specific primers

by reverse transcription (RT)-PCR. To amplify a 321-bp fragment matching to a partial section of the HSP70 gene from GLRaV-4, the primers LR4F (5′-ACA TTC TCC ACC TTG TGC TTT T-3′) and LR4R (5′ CAT ACA AGC GAG TGC AAT TAC-3′) were used. GLRaV-4 was found in one sample from cv. Autumn Royal. The amplicon was cloned, and a single clone (GenBank Accession No. GQ246624) was sequenced, revealing 99 % nucleotide identity with a GLRaV-4 isolate from the United States (Accession No. AF039553). Due to the lack of antiserum specific to GLRaV-4, the second set of primers, LR4CP-F (5′-GGT GTC CAG CGC TTC CAA-3′) and LR4CP-R (5′-GCC AGA GAA GCA TCG TAA-3′), was designed based on the sequence of GLRaV-4 from Chile (Accession No. EU746620), which amplified a 300-bp fragment specific to the coat protein gene of G The amplicon was cloned, and a single sequence (Accession No. GQ479041) was compared to a GLRaV-4 nucleotide sequence from Chile (Accession No. EU746621), which showed 99 % identity. A selection from cv. Malaga Rose tested positive for GLRaV-5 using antibodies specific to the virus (Neogen Europe, Ltd. Scotland, UK), and this was confirmed by amplification of a 690-bp fragment corresponding to the GLRaV-5 coat protein gene using virus-specific primers LR5F (5′-CCC GTG ATA CAA GGT AGG ACA-3′) and LR5R (5′-CAG ACT TCA CCT CCT GTT AC-3′) (3). The amplicon was cloned, and a single clone (Accession No. GQ246625) was sequenced, which exhibited 95% nucleotide similarity with the GLRaV-5 CP gene sequence from Argentina (Accession No. EU815935).This is the first time GLRaV-4 and -5 have been found in grapevines in China. (Pei et al, 2010) Confirmation of these viruses in China is critical for creating virus-free plants, and this knowledge will also aid in the development of a multiplex RT-PCR test to identify numerous GLRaVs concurrently, as well as research on the viruses' genetic variability.

The complete genome sequence of a highly diversified strain of Grapevine leafroll-associated virus 4 (GLRaV-4) was found using 454 pyrosequencing

technology. This virus, known as GLRaV-4 Ob, was found in the grapevine viral collection of Agroscope in *Vitis vinifera* 'Otcha bala.' The GLRaV-4 Ob genome is similar in length and organization to those of *Ampelovirus* subgroup II members (family *Closteroviridae*).

A range of grapevine leafroll disease signs was identified during a study done in Slovenian vineyards. In 2010, DAS-ELISA revealed mixed infection with grapevine leafroll-associated viruses 3 and 4 (GLRaV-3, -4) in two grapevines from a vineyard in Slovenia's south-western region. The Slovenian GLRaV-4 isolate's 3'final 1769 nucleotides constructed from IC RT-PCR amplicons. Other GLRaV-4 sequences from GenBank were matched to the full coat protein (CP) and p23 gene sequences. The CP and p23 amino acid sequences of the Slovenian variation (055-SI) were found to be 88% and 85% similar to the corresponding genes of the reference sequence GLRaV-4 LR106, respectively (GenBank Acc. No. FJ467503). According to phylogenetic analysis, the Slovenian variation of GLRaV-4 clusters with other strains of GLRaV-4. The N-terminal region of the CP sequence has a lot of variabilities, indicating that this section of the genome isn't suited for molecular detection of the virus (Strukelj et al., 2016)

Grapevine leafroll ampeloviruses have recently been separated into two clades: GLRaV 1 and 3 and GLRaV-4 and its variants. To better understand biological factors mediating differential ampelovirus incidences in vineyards, researchers used quantitative real-time polymerase chain reactions to assess virus populations in three grapevine varieties with different infection status: GLRaV-3 + GLRaV-4, GLRaV-3 + GLRaV-4 strain 5, and GLRaV-4 alone. Specific primers based on the RNA-dependent RNA polymerase (RdRp) domains were used for GLRaV-3, GLRaV-4, and GLRaV-4 strain 5.

The complete genome sequence of one isolate of Grapevine leafroll-associated virus 4 strain 9 (GLRaV-4 strain 9) was found using a library constructed from short RNA population sequencing. The RNA genome has 13,858

nucleotides and seven open reading frames. The bulk of 21-nt reads identified in virus-derived siRNA populations corresponded to GLRaV-4 strain 9 genomes, indicating that DCL4 plays a role in viral silencing. The density profile of vsiRNAs along the viral genome was notably different from that of GLRaV-3, an *Ampelovirus* subgroup representative I discovered in the same plant (Velasco et al., 2015)

3. MATERIAL AND METHODS

3.1. Material

3.1.1. Information onSurvey Area

Survey studies were conducted in the Hebron area, where grapes are grown intensively, and this city occupies the first place in grape cultivation in Palestine.

3.1.2. Material Used in Serological Studies

Leaf and stem samples from grapes suspected of being infected with GLRV in the field, GLRV-specific ELISA kits commercially available from BIOREBA, buffer solutions, extraction bags, 96 well branded ELISA plates, automated pipettes and pipette tips. In the ELISA, testing, distilled water was utilized as a material.

The absorbance values of the samples were read on the Medispec branded ESR 200 model ELISA Plate Reader.

3.1.3. Material Used in DNA Extraction Studies

Field samples suspected of being infected with GLRV were subjected to DNA extraction tests. The research material was the plant sap collected from these plants. A sterile mortar and pestle, extraction bags,buffer solutions,Eppendorf tubes, pipette tips, a dry heater, and a 380R table-type chilled centrifuge were utilized in the DNA extraction procedure.

3.1.4. Material Used in PCR Studies

Total nucleic acid preparations from plants suspected of being infected with GLRV were utilized as PCR material in investigations to identify GLRV using molecular techniques.

PCR processes, Taq polymerase enzyme (Dream Taq polymerase enzyme, 5U/ul, Thermo Science) and DreamTaq 10x buffer solution, dNTP set (DATP, DGTP, dCTP, 'TTP, 100 mM, Amplicon), 1kbp DNA Ladder marker (NEB)), sterile water, pipette and sterile pipette tips, PCR tubes and GLRV 4 -specific primer pairs (10/pmol/µl) given in Table 4.1. The temperatures required for the amplification of the target regions of the virus genome were provided using the Applied Biosystem brand thermocycler.

Table 3.1. Primer pair and band size used in RT-PCR studies

Virus	Forward Primer (5'—3')	Reverse Primer (5'—3')	Size(bp)
GLRaV-4	GGT ATG AAC AAR TTC AAT GC	TAG ACA ACC ATG TAY TCT ATG	371bp
LR4CPINT	GAG AGT GAC AAG CAC CAG GTG C	TCA CCT CCT GTT GCC CA	492bp

3.1.5. Material Used in Agarose Gel Electrophoresis Studies

In agarose gel electrophoresis investigations, PCR products were employed as the material. Agarose, buffer solutions, BIORAD brand Mini Sub. DNA Cell Electrophoresis equipment and power supply, Ultraviolet (UV) Transilluminator brand UV light source, and gel imaging system were utilized in the agarose gel electrophoresis procedure.

3.1.6. Material Used in RNA Sequence Analysis

After the PCR studies, the PCR results of the tested samples was controlled by agarose gel electrophoresis. The PCR findings of the samples were based on the electrophoresis results. that gave ranges of the expected molecular size were sent to the commercial company (Molgentek) for sequencing.

3.2. Method

3.2.1. Plant sample collection and field surveys

During the late summer of 2019, surveys were conducted vineyards in the West Bank's key grape-growing region (Hebron) to determine the prevalence of any *Closteroviridae*-related viral infections. 80 leaf samples were randomly selected from sick and asymptomatic grapevines collected from 19 different vineyards in four areas to test for the prevalence of *Closterovirus* infections.

Samples were collected in the Hebron district from Al-Reheia (Al-Fawwar region), Farsh Al-Hawa north of Hebron, Namra in the city centre, and Baqa'a in the east.

Each sample comprised numerous leaves and petioles gathered from various tree locations, which were Sorted and labeled as needed. These samples were put in paper bags, placed in an ice-bag container, and sent to my residence the same day, where they were maintained at 4°C.

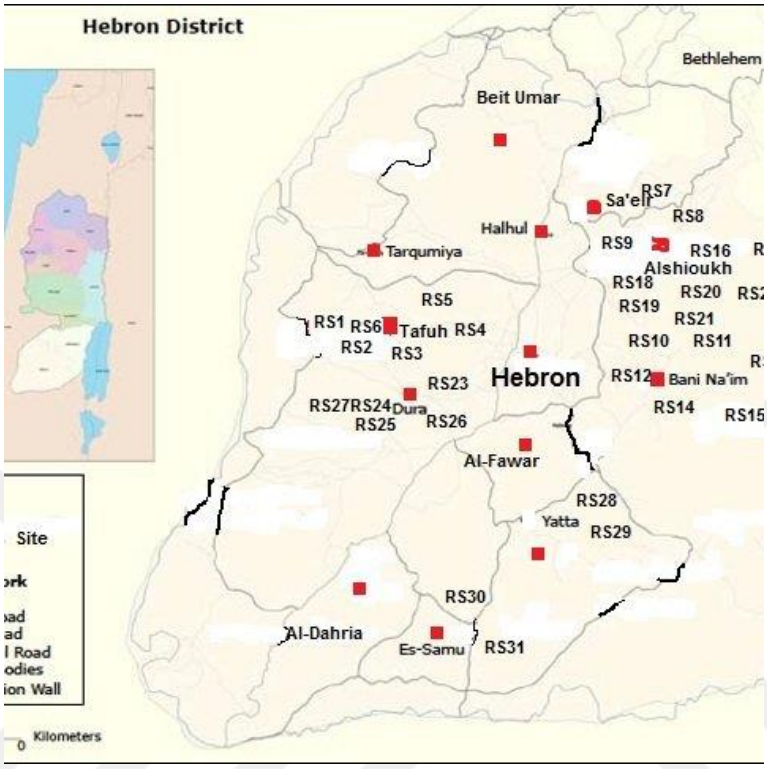


Figure 3.1. Location of the collected Grapevine sample in the Hebron-Palestine.

3.2.2. Transfer of samples from Palestine to Turkey

The Palestinian Ministry of Agriculture granted a certificate that enables us to dispatch samples beyond Palestine. Includes the sampling number, the purpose of sending the samples and the country name of the samples to whom they are sent.

3.2.3. Serological Studies

The presence of GLRV 2 and 4 strains was detected using the Double-Antibody Sandwich ELISA (DAS-ELISA) technique on grape plants obtained from the field suspected of being contaminated in serological investigations.

3.2.3.1. DAS-ELISA

The following DAS-ELISA tests were carried out according to the technique indicated by the business that supplied the ELISA kits:

1. GLRVs virus-specific ɣ-globulin was diluted in coating buffer in a specified ratio and 100 µL was put in each well of ELISA plates.
2. The plates were washed 3 times with washing buffer for 3 minutes each after incubation at 37°C for 2.5 hours.
3. 100 µl of the extract, which was prepared at a ratio of 1/10 in the extraction buffer solution, was placed in each well and the plates were left for incubation at +4°C overnight and washed with washing buffer as indicated in the 2nd step.
4. After incubation at 37°C for 2 hours, the plates were washed again with washing buffer as stated in step 2, and 100 l of label Y globulin (conjugate) with enzyme diluted in conjugate buffer solution was placed in each well.
5. Finally, 100 µl of the substrate prepared as 1mg/ml in the substrate buffer solution were added to each well of the plates and the plates were read in the ELISA reader after waiting for 30-60 minutes at room temperature under dark environment.

The ELISA test was conducted for both the GLRaV-2 strains and GLRaV-4 strains.

In ELISA tests performed, at the end of each ELISA plate, one positive (infected), negative (healthy), and buffer control ELISA tests.

Samples that provided at least twice the absorbance value measured at 405 nm for the negative control in the ELISA reader were judged positive at the end of the ELISA testing (Erkan ve ark., 1994).

3.2.4. Molecular Detection of *Ampelovirus*

3.2.4.1. Total Nucleic Extraction

DNA extraction studies were performed by Lima et al.,(2012). The technology of silica capture was employed. The steps in the DNA extraction technique are as follows:

1. 1 ml extraction buffer was used to pulverize 0.1 g of tissue stem (phloem) (100 mM Tris-HCl, pH 8.0, 50 mM EDTA, 500 mM sodium chloride and 0.1 percent 2-mercapthoethanol). In bags for extraction.
2. After extraction, it was centrifuged at 4.000 rpm for 3 minutes, and 500 µl was withdrawn from the inernatat part and transferred to the newly prepared Eppendorf.
3. After that, 100 L Sarkosyl 10% was added, vortexed, and the samples were incubated in a water bath at 70°C for 10 minutes. They were then incubated for 5 minutes before being centrifuged for 10 minutes at 14000 rpm.
4. 300 µL of the supernatant were collected, and 25 µL of silica, 150 µL of EtOH, and 300 µL of NaI were added and kept at room temperature for 10 minutes.
5. The pellets were washed twice with 500µL of washing buffer after being centrifuged for 1 minute at 6,000 rpm.
6. The pellets were then resuspended in 150 µL distilled water and heated at 70°C for 4 minutes before being stored on ice. The samples were centrifuged for three minutes at 14,000 rpm. Until it was time to use the tube, it was stored at -20°C.

3.2.4.2. Reverse transcription of RNA cDNA synthesis

To synthesize cDNA, random hexamer primers were employed, and 1 µl was added to 2 µl total extracted RNA. They were then denatured at 95 °C for 3 minutes and immediately refrigerated on ice before being added to a mixture containing 5 µl of M-MLV 10x buffer, 1µl of 10 mM dNTPs, 0.1 l of 200 unit/l reverse transcriptase (M-MLV enzyme), and 13.9 µl of distilled water. In a final volume of 25 µl, the reaction took place. After the reverse transcription process was completed, the tubes were incubated at 42°C for 1 hour, and the cDNA was stored at -20°C for subsequent use.

3.2.4.3. Polymerase Chain Reaction for Gene Amplification:

For molecular examination of Ampelovirus, Alkowni (2000) table 4.2 revealed that RT-PCR detection for the subgroup in infected grapevines was carried out utilizing degenerated and species-specific primers.

2 µl cDNA was added to the amplification mixture: 1 µlof 20 M sense and antisense of each degenerate primer, 0.5 µl of 10 mM dNTPs, 2.5 µlof 10x Taq DNA polymerase buffer, 1.5 $MgCL_2$, 0.2 µl Taq polymerase (5 unit/µl), 16.3µl distilled water (Macrogen Europe, Amsterdam) in a total volume of 25 µl.

The PCR procedure that was advised comprised 35 cycles. A first denaturation step was performed at 94°C for 30 seconds. Each cycle consists of 30 seconds of denaturation at 94°C 45 seconds of annealing at 55 °C. The temperature was 72°C for 30 seconds of extension and 72°C for 7 minutes of final extension. Before the amplification products were examined by gel electrophoresis, the amplified products were kept at -20 °C.

Table 3.2. Primers of Grapevine leafroll associated virus-2 and Grapevine leafroll associated virus-4

Virus	Forward Primer (5'—3')	Reverse Primer (5'—3')
Grapevine leafroll associated virus-2	ATAATTCGGCGTACATCCCCACTT	GCCCTCCGCGCAACTAATGACAG
LRaV-2	AGGCGGATCGACGAATAC	ATCCTGTCCGGCGCTGTG
Grapevine leafroll associated virus-4	GGTATGAACAARTTCAATGC	TAGACAACCATGTAYTCTATG

3.2.4.4. Agarose gel Electrophoresis

1. 200 mg of Agarose and 20 ml of 1 X TAE buffer solution (4.84 gr Tris, 2 ml 0.5 M Na2EDTA, 1.142 ml Glacial Acetic Acid/1lt) was put into the glass flask and kept in the microwave oven until the agarose melts completely.
2. The gel is poured into the prepared part of the electrophoresis apparatus, a comb was attached and the mixture is left to cool for a while.
3. After the gel is frozen, the comb was carefully pulled and this part was placed in the electrophoresis tank and 1 X TAE buffer solution was added to it, enough to pass the gel by 1-2 mm.
4. Carefully apply the samples to the wells in the gel once they have been prepared with 5 µl of the sample. In the first well, a marker (4 µl marker, 1 µl DNAse-free water, 1 µl loading buffer) was put.
5. The electrophoresis tank received a 55 U/ cm electric current until the orange hue in the 5th loading buffer reached the end of the gel. 55 U/

cm electric current was applied to the electrophoresis tank until the orange color in the 5th loading buffer reached the end of the gel.

6. After electrophoresis, the gel is incubated in ethidium bromide for 10 minutes at room temperature before being washed for 10 minutes to remove excess ethidium bromide.
7. The washed gel was placed on the UV Transilluminator, the bands appearing in the UV light were observed, their photographs were taken.

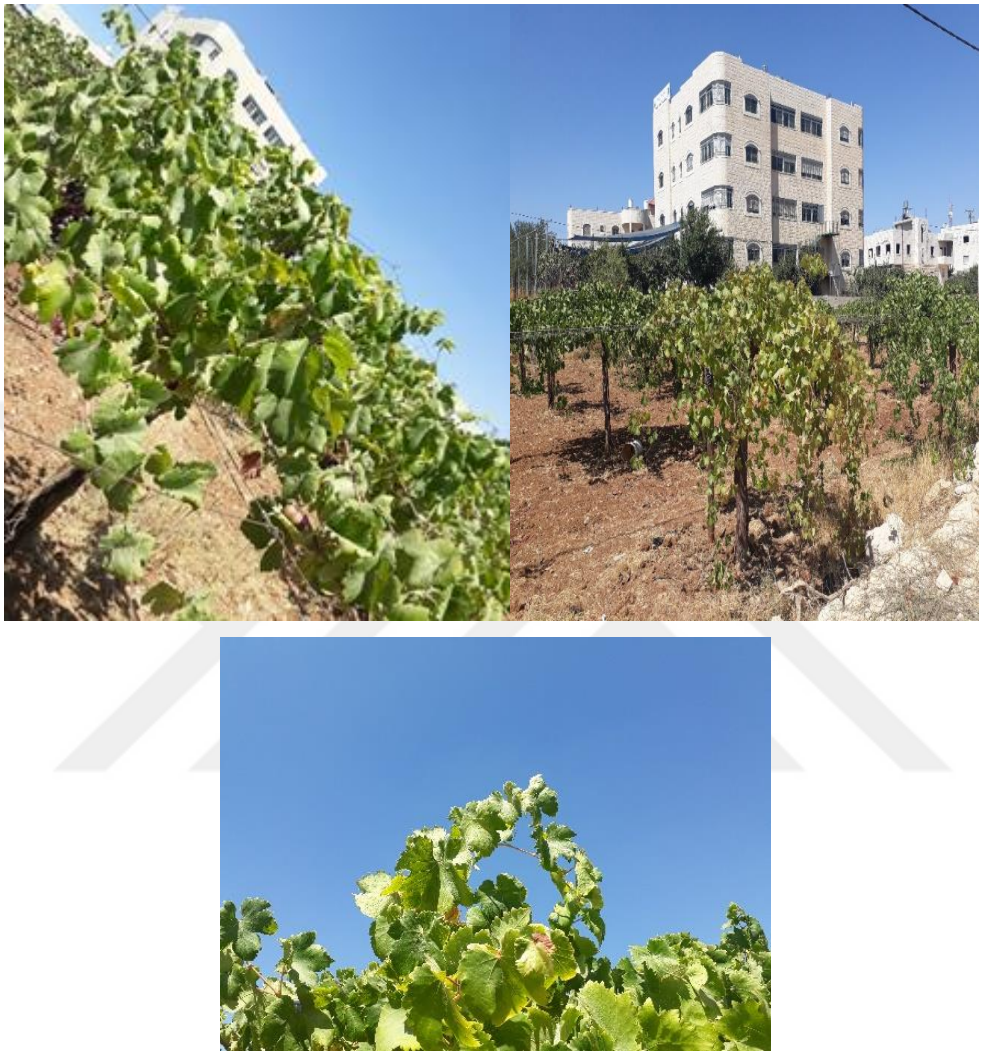
4. RESULTS AND DISCUSSION

4.1. Surveys in the field and Serological detection of virus-related symptoms in Palestinian land

During the 2019 growing season, surveys were done in vineyards in the West Bank's key grapevine-growing regions (Hebron) to assess the virus's existence and prevalence. Al-Reheia (Al-Fawar area) to Farsh Al-Hawa north of Hebron, Namra in the city center, and Baqa'a in the east, symptoms were noticed in the Hebron district. *Closterovirus* induced various symptoms in native and imported cultivars, including red-skinned *Vitis vinifera* types; the leaf tissue between the veins turns a deep red to purple color, and the leaf margins curl or cup inward. The leaf tissue of white types will become yellow, with the leaf edges curling or cupping. The veins will remain green in both symptom kinds. Rootstock, American native, and hybrid kinds, on the other hand, can be infected yet exhibit no symptoms. Moreover, symptoms were reported independently of the vine type.

Figure 4.1. *Yellowing with curling of the leaf in white*

Figure 4.2. Deep red to purple leaf edges and downward cupping of the leaf margins, in Red grape types.



(Figure 4.1 Contunie) Yellowing with curling of the leaf in white

4.2. DAS-ELISA tests

Of the 71 grapevine samples tested by DAS-ELISA against GLRaV 2and 4 viruses, 12 (16.90%) proved to be infected viruses. The present survey demonstrated the high levels of infections of some GLRaVs that were previously unreported in Hebron. Viruses detected by DAS-ELISA and their prevalence in

grapevine samples of commercially grown vineyards are shown. Based on our results, 12 (10 stems, 2 leaves) grapevine samples GLRaV 4 was discovered to be contaminated. None of the samples was reacted positively against GLRaV-2. (Table 4.3.)

Table 4.1. Viruses detected by DAS-ELISA in the investigated grapevine samples of Hebron Province

Districts	Tested NO	Infected %	Viruses-incidence %	
			GLRaV 2	GLRaV 4
Al-Reheia	8	0	0	0
Farsh Al-Hawa	14	37.5	0	3/37.5
Namra	47	14.89	0	7/14.89
Baqa'a	11	18.18	0	2/18.18
Total	80	-	-	12/16.90

4.3. The Molecular Detection

Degenerate primers developed for the specific detection of GLRV-4. Initially, they were examined in symptomatic plant tissues. The set of primers showed very effective for RT-PCR detection after adjustment of the testing settings. The primer set Primer LR4/5-UnI370 an amplicon of 371bp from vines infected. On the other hand, 4 LNV Grapevine Virus specific primers were utilized, and no particular sequence of the LR4CPINT virus was discovered after numerous trials of the analyzed samples.

4.4. Results of Gene Amplification

The results of molecular diagnostics of samples from the 2019 growing season were confined to *Ampelovirus* of GLRV-4, which was detected using a set

of degenerate primers. Table (4.4) shows the findings of all 12 samples, with 12 of them showing positive results.

Table 4.2. Results of gene amplification (RT-PCR) for the presence of Ampelovirus with the use of degenerate primers.

NO.	Place	Grape variety	plant tissues	PCR
3	Farsh Al-Hawa	Zeany	phloem	Positive
4	Farsh Al-Hawa	Zeany	phloem	Positive
6	Farsh Al-Hawa	Zeany	phloem	Positive
35	Namra	Shami	phloem	Positive
38	Namra	Shami	Leaf	Positive
48	Namra	Betyni	phloem	Positive
50	Namra	Betyni	phloem	Positive
59	Namra	Betyni	phloem	Positive
63	Namra	Shami	phloem	Positive
65	Namra	Shami	phloem	Positive
71	Baqa'a	Betyni	Leaf	Positive
80	Baqa'a	Betyni	phloem	Positive

4.5. Results of Agorose Gel Electrophoresis

At 1.2 percent agarose gel electrophoresis, the insert (target RNA) were examined, and bands were seen (Figure 4.4)

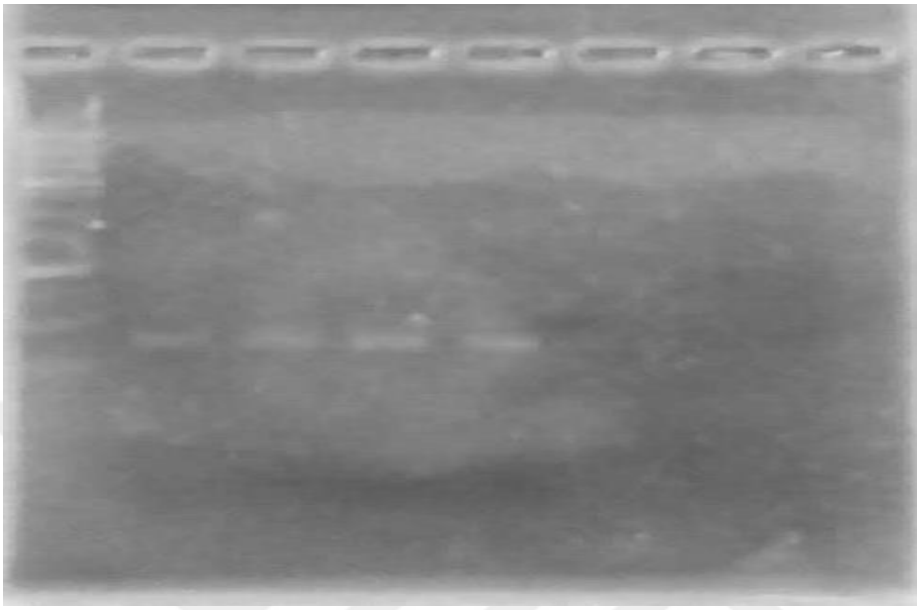


Figure 4.3. *Positive samples in Agarose Gel electrophoresis*

4.6. Results of Sequence analysis

GLRaV isolates obtained in this study with GLRaV 4- strain-specific primer pair (LR4/5-UnI370F/R) were found in USA (MN201920.1, FJ467503.1, KC113198.1, MF669483.1), China (KU971256.1, GQ849394.1, JN226663.1, KU971247.1, KU971253.1), Turkey (DQ325516.1, KP144370.1), Canada (KY381159.1), India (KT759151.1, KT598220.1), Pakistan (KY821095.1), Palestine Occupied (AM162280.1). It was revealed as a result of the examination of BLAST analyzes that it showed 89-95% similarity with the GLRaV 4 isolates previously studied (Table 4.5 and Figure 4.5).

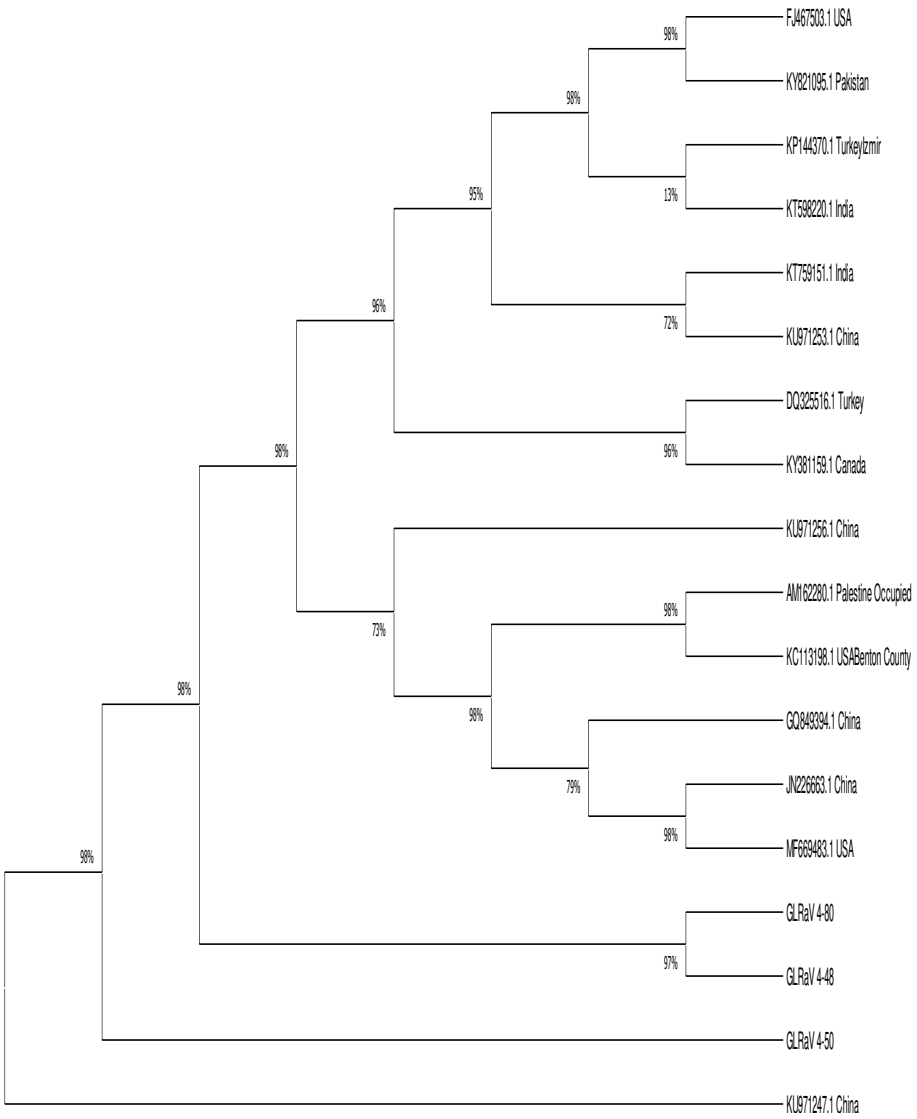


Figure 4.4. Phylogenetic analysis by neighbor joining method as a result of sequencing of 371 bp PCR products (55) obtained with ty primer pair.

Table 4.3. Origins, hosts and accession numbers of the isolates in the phylogenetic analysis.

Source of isolates	**Plant**	**Sequence ID**	**Similarity rate (%)**
USA	*Vitis vinifera*	MN201920.1	94.82%
Turkey	*Vitis vinifera*	DQ325516.1	95.43%
Canada	*Vitis vinifera*	KY381159.1	93.90%
India	*Vitis vinifera*	KT759151.1	90.21%
China	*Vitis vinifera*	KU971253.1	90.08%
India	*Vitis vinifera*	KT598220.1	90.55%
USA	*Vitis vinifera*	FJ467503.1	89.94%
Pakistan	*Vitis vinifera*	KY821095.1	90.94%
China	*Vitis vinifera*	KU971247.1	89.94%
Turkey, Izmir	*Vitis vinifera*	KP144370.1	92.08%
USA,Benton	*Vitis vinifera*	KC113198.1	94.82%
Palestine Occupied	*Vitis vinifera*	AM162280.1	94.21%
USA	*Vitis vinifera*	MF669483.1	89.94%
China	*Vitis vinifera*	JN226663.1	89.94%
China	*Vitis vinifera*	GQ849394.1	90.71%
China	*Vitis vinifera*	KU971256.1	90.84%

5. CONCLUSION

In Palestine, grapevine is the second most significant fruit crop after olives, spanning over 11,000 hectares. Due to the favorable environmental conditions, it is grown across Palestine. The bulk of commercial vineyards, on the other hand, are concentrated on the southern West Bank(Ruba Abu Amsha et al., 2014).

Grapevine leafroll diseases (GLD) causes leafroll degeneration, which is a major viticulture problem and one of the most serious viral diseases of grapevines globally. GLRV causes significant crop losses, lowers fruit quality, and accelerates grapevine aging in the vineyard. The ectoparasitic nematode *Xiphinema index* is responsible for the transmission of GLRV from grapevine to grapevine.

Environmental variables, grapevine cultivar, rootstock, viral isolate or strain, intrinsic agronomic circumstances, or connection with other infections or viruses can all impact disease manifestation (Malioka et al., 2015a). Vegetative propagation is used to spread these infections over large distances (Martelli, 2014), whereas insect, mite, or nematode vectors are used to spread them locally (Malioka et al., 2015a; Martelli, 2014).

The severity of GLD symptoms varies widely depending on the season, grape cultivar, and environmental circumstances. Furthermore, some kinds, such as rootstocks and some white V. vinifera cultivars might be symptomless, serving as a reservoir from which GLD can be transferred to cultivars with a variety of symptoms. In GLD-affected vines, bud break and shoot growth are frequently delayed in the spring. This is generally a transient occurrence that lasts only a few weeks. Early to mid-summer is when leaf symptoms begin, however they might appear sooner on vines that are water strained. Until late fall, the quantity and intensity of these symptoms increases. GLD induces reddening of the interveinal zones in most red cultivars, although the principal and secondary veins remain

green. Some red cultivars' leaves acquire a uniform crimson hue without green veins, especially those with intensely coloured berries. The interveinal region of white cultivars can develop chlorotic. This symptom is frequently modest and may go unnoticed. The leaf edges roll downward in late fall, but the extent of this rolling varies greatly across affected cultivars.

White cultivars like Chardonnay and Thompson Seedless exhibit obvious leaf rolling by harvest time, but Thompson Seedless and Sauvignon Blanc have little or no leaf rolling. In these white kinds, GLD is nearly impossible to spot visually. As the growing season progresses, GLD symptoms occur on more leaves, extending from the base of the shoot to the tip. Except for American rootstocks, these viruses are usually asymptomatic carriers(Weber et al., 1993; Martelli et al., 2012).

A particular collection of symptoms can be caused by a variety of factors. Red leaves, for example, may indicate a viral infection, a fungal root infection, a nutritional shortage, physical injury, or mite, insect, or rodent feeding. Leaf scorch, on the other hand, might be a sign of Pierce's disease, water stress, sulfur burn, or spray damage. Disease testing is frequently done to determine the real nature of the problem that has resulted in symptom expression.

Diagnostic signs for most grapevine viral illnesses only appear at particular seasons of the year. Leafroll, for example, causes red-fruited cultivars' leaves to become crimson in the late summer and fall. In the spring, a visual inspection of these vines would reveal nothing about their disease condition. During the dormant season, when many producers and nurseries remove wood for propagation, it is nearly hard to identify grape viral illnesses in the field. Furthermore, grapevines afflicted with some disease agents (particularly viruses) may not display any symptoms at all! The concentration of disease-causing substances might be so low that no illness symptoms appear, or the infection could be in a disease-tolerant grape variety. Only trustworthy disease testing procedures can detect such hidden

illnesses. When gathering wood for propagating new vines, this is an essential issue since viral illnesses that aren't visible on one rootstock or under specific growth circumstances can cause serious disease when infected wood is grafted to make new vines (Weber et al., 2002).

Serological techniques have a basic flaw in that they rely on the antigenic properties of viral structural proteins. As a result, immunological approaches are developed without taking the rest of the viral genome into consideration.

Serologically distant or unrelated viruses may have highly conserved sequences in genes other than the coat protein gene, whereas serologically related viruses may have minimal sequence similarity. In addition, in 2008, the Arabis mosaic virus, Tobacco ringspot virus, Peach rosette mosaic virus, Tomato ringspot virus (ToRSV), and Grapevine leafroll-associated virus 3 were all missed by ELISA (Lunden et al., 2008)

The PCR findings revealed that the Ampelovirus genus had been successfully recovered. Although 12 contaminated samples were found, Ampelovirus-induced illnesses, particularly leafroll, are uncommon in Palestinian vineyards, as evidenced by a field study.

Viruses and viroids are prevalent diseases in a wide range of economically important plants, including grapevine. Grapevine decline and stunted development are two of the disease's key symptoms; these viruses can cause considerable output losses, both in terms of quality and quantity. Because diseased plants are unable to recuperate, many farmers just tolerate or ignore viral infections until visible symptoms develop, at which point the diseased plants are removed. These asymptomatic host-pathogen pairings, on the other hand, may not be taken into account, and the problem emerges when the virus is transmitted to a more vulnerable host, causing severe symptoms or even death. As a result, asymptomatic hosts act as pathogen reservoirs (Matthews, 2010).

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