T.R.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

RESEARCH TOWARDS ELUCIDATION OF THE COMPLETE GENOME OF GRAPEVINE DEFORMATION VIRUS (GDefV) ISOLATES IN TURKEY

SABINA MAMEDOVA

February 2022

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Master Thesis

Supervisor

Prof. Dr. Çiğdem ULUBAŞ SERÇE

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The study titled "**Research Towards Elucidation of the Complete Genome of Grapvine Deformation Virus (GDefV) Isolates in Turkey**" are presented by **Sabina Mamedova** under supervision of **Prof. Dr. Çiğdem ULUBAŞ SERÇE** has been accepted as Master Thesis by juries, at the Department of **Plant Production and Technologies**, Niğde Ömer Halisdemir University Graduate School of Natural and Applied Sciences.

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THESIS CERTIFICATION

It is certified to “Research towards elucidation of the complete genome of grapvine deformation virus (GDefV) isolates in Turkey” thesis by Sabina MAMEDOVA were written based on principals of Niğde Ömer Halisdemir University - Graduate School of Natural and Applied Sciences, Department of Plant Production and Technologies. It is also certified that, all information and sources used in this thesis were cited and referenced definitively, by author.

Sabina MAMEDOVA

SUMMARY

RESEARCH TOWARDS ELUCIDATION OF THE COMPLETE GENOME OF GRAPEVINE DEFORMATION VIRUS (GDefV) ISOLATES IN TURKEY

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In this research, the main objective consists of determination the prevalence of *Grapevine deformation virus* (GDefV) in Nevşehir and Niğde vineyards, and elucidation of the genomic variation of the virus isolates. Totally 150 symptomatic grapevine samples was collected from Nevşehir and Niğde vineyards in June-July 2020. The overal GDefV infection rate was 14% among the tested grapevine samples, and the infection rate was 18% in Niğde and 12% in Nevşehir. Two grapevine samples (sample 105 and 107) sequenced previously with illumina sequencing technology were investigated for providing the complete RNA1 and RNA2 genomes of the GDefV by bioinformatics tools CLC Genomic Workbench 11 and Geneious Prime software. The 105 and 107 GDefV isolate RNA2 complete sequences were identical 96% to each other while sharing the identity of 91% and 90% with the reference isolate GDefV, respectively. We provided Nepovirus subgroup A polyprotein, Nepovirus coat protein N-terminal domain, central domain and C-terminal domain in sample 105 and 107 RNA2 consensus sequences validating the GDefV consensus sequences. This information is the exclusive in that, it contributes both to virus genomic information and to the addition of more GDefV isolates complete genome information to the literature of viral agents infecting vine.

Keywords: Nepoviruses, complete genome of GDefV, GDefV RNA1, GDefV RNA2, bioinformatics

ÖZET

TÜRKİYE'DE ÜZÜM DEFORMASYON VİRÜSÜ (GDefV) İZOLATLARININ TÜM GENOMUNUN AYDINLATILMASINA YÖNELİK ARAŞTIRMA

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Bu araştırmada, Nevşehir ve Niğde bağlarında Grapevine deformasyon virüsünün (GDefV) yaygınlığının belirlenmesi ve virüs izolatlarının tüm genom varyasyonunun araştırılması amaçlanmıştır. Nevşehir ve Niğde bağlarından, Haziran-Temmuz 2020'de, toplam 150 adet simptomatik asma örneği toplanmıştır. Test edilen asma örnekleri arasında GDefV genel enfeksiyon oranı %14 olarak belirlenirken, enfeksiyon oranı Niğde'de %18 ve Nevşehir'de %12 olmuştur. Daha önce illumina sekanslama teknolojisi ile sekanslanmış iki asma verisi (örnek 105 ve 107), GDefV'nin tam RNA1 ve RNA2 genomlarını sağlamak için biyoinformatik araçlar olan CLC Genomic Workbench 11 ve Geneious Prime yazılımı ile araştırılmıştır. 105 ve 107 GDefV izolatı RNA2 tam dizileri, sırasıyla referans izolatı GDefV ile %91 ve %90 benzerliği paylaşırken birbiriyle %96 benzerlik göstermiştir. Örnek 105 ve 107 RNA2 sonuç dizilerinde Nepovirüs alt grup A poliproteini, Nepovirüs kılıf proteini domainleri olan N-terminal domain, merkezi domain ve C-terminal domain belirlenerek GDefV RNA2 sonuç dizileri doğrulanmıştır. Elde edilen bilgiler, hem virüs genomik bilgisine hem de asmayı enfekte eden viral ajanlar literatürüne daha fazla GDefV izolatının tam genom bilgisinin eklenmesine katkıda bulunmuştur.

Anahtar sözcükler: Nepoviruses, tam genom GDefV, GDefV RNA1, GDefV RNA2, biyoinformatik

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SYMBOLS AND ABBREVIATIONS

Symbols	Descriptions
%	Percent
®	Registered trademark
°C	Degree centigrade
AAP	Acquisition access period
cDNA	Complementary DNA
Cm	Centimeter
CP	Coat protein
CTAB	Cetyl trimethylammonium bromide
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
EDTA	Ethylenediamine tetraacetic acid
IAP	Inoculation access period
mg	Milligram
Ml	Milliliter
mM	Milimolar
MP	Movement protein
MT	Metric ton
NaCl	Sodium chloride
NGS	Next-generation sequencing
nt	Nucleotide
ORF	Open reading frame
PCR	Polymerase chain reaction
PTGS	Post-transcriptional gene silencing
RNaseH	Ribonuclease H
RT	Reverse transcriptase
siRNA	Short-interfering RNA
TNA	Total nucleic acid
µl	Microliter

Abreviations	Descriptions
ArMV	*Arabis mosaic virus*
Blast	Basic Local Alignment Search Tool
EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization
GDefV	*Grapevine deformation virus*
GFLV	*Grapevine fanleaf virus*
GLRaV	*Grapevine leafroll-associated virus*
GLRaV-3	*Grapevine leafroll-associated virus-3*
NCBI	National Center for Biotechnology Information

CHAPTER I

INTRODUCTION

Grapes, *Vitis vinifera* L. (Vitaceae family), is among the species which has gained prominence regarding economic importance (Terral et al. 2010) due to its nutritional value, wine production (Gerrath et al. 2004), perfume manufacturing (Simonetti et al. 2019), and medicinal utilisation (Yadav et al. 2009; Fernandez-Marin et al. 2012; Doshi et al. 2015; Cui et al. 2018). The fruit is grown on over 7.6 million hectares, with a global yield of 74 million metric tonnes (OIV 2015). Furthermore, one fruit produces around 270 million hectolitres of wine. However, the fruit is also grown in different purpose such production of juice, seed oils, vinegar, also use in the form of fresh fruit, raisins, and other goods. In many Europenian coutries like Spain, China, Italy, France and Turkey currently account for 50 percent of global vineyards. However, the biggest production of wine are produced in France, Italy, Spain, the United States, and Argentina. The elegant species such as *V. vinifera* ssp. *sativa* and wild *V. vinifera* ssp. *sylvestris* were long contributed to meeting the needs of the people (Brașoveanu et al. 2020; De Rosa et al. 2021; Sargolzaei et al. 2021).

Turkey, particularly Anatolia, is a center for wild and cultivated grapevine production and growth (Ustaoglu et al. 2021). As a result, the site is appealing to breeders because genes of interest may be discovered and extracted from this valuable gene pool (Kupe et al. 2021; Ozer et al. 2021). Furthermore, due to its geographic importance, Southeast Anatolia has the potential to be a hotspot for wild grapevine populations and indigenous cultivars (Karatas et al. 2007).

Howsoever, certain factors such as, diseases and pest outbreaks can influence the quality and quantity of the fruits. Different diseases of fungi including; downy mildew, grey mold and powdery mildew negativelly effecting the fruit yield play important role to deacrese the quantity and quality of the grapes. (Urbez-Torres et al. 2014; Wilcox et al., 2015; Masi et al. 2018; Molitor et al., 2018). The presence of these fungal diseases is highly dependent on the environmental factors like relative humidity, temperature and various others (Hofstetter et al. 2012; Songy et al. 2019). Moreover, while talking about viral diseases, plant material selection, breeding, transportation and sanitary control are

important factors in addition to environmental factors. In viral disease, vector of the each disease changes thus it is difficult to analyze the climatic factors in this regard. Furthermore, viral diseases are arduous to pin point through field observation that is the reason of their underrated severity (Afechtal et al. 2021; Porotikova et al. 2021).

In grapevines, until now, more than 86 different species of viruses have been reported, 31 of them are play important role in major disease complexes. These complex disease currently occur as infectious degeneration, leafroll, decline, fleck, and rugose wood. Symptoms of viral diseases include stunt growth, discoloration, line pattern, ringspots and bright yellow mottling. The viruses have devastating effect on grapevines as they results in small fruit production and qualitative and quantitative effects on vineyard (Martelli 2014; Minafra et al. 2017; Fuchs 2020).

In the family Secoviridae, highest number of species (55) have been recorded in the genus Nepovirus including 40 recognized members and 15 unrecognized members. In this genus Nepovirus, 57 species are further categorized in 3 subgroups viz. A, B and C (Sanfaçon, 2015). Among them, 58% comprise of sequences predominantly genome and RNA organization, while, 59% among them are cleavage site specific proteases (Fuchs et al. 2017). Where, genus Comovirus has 15 viruses, genus Fabavirus contains 7 viruses, genus Cheravirus contains 5 viruses, genus Sadwavirus consists of 5 viruses, Sequivirus has 3 members, genus Torradovirus comprises of 6 viruses, and the genus Waikavirus has 4 virus species (Mann et al. 2019). Another member of this family viz. strawberry latent ringspot virus (SLRSV) has been documented but it is not yet assigned to any genera. Variable in sizes, multiple capsid proteins (CP) are the characteristic feature of all members of the family Secoviridae, except for nepoviruses which encode a larger CP of 52-60 kDa (Fuchs et al. 2017). Monocistronic features in the RNA1 and RNA2 of all the viruses can be observed except for comoviruses and torradoviruses, which have polycistronic RNA2. Once expressed, 3C-like proteinases cleave each of the segment-specific large polyprotein in mature peptides which code for different functions (Mann et al. 2019).

The causal organism of infectious degeneration (fanleaf) viz. *Grapevine fanleaf virus* (GFLV), mainly attack on European grapevines (Krebelj et al. 2015). Belonging to the genus *Nepovirus* other viral species viz. *Arabis mosaic virus* (ArMV), *Tomato*

black ring virus (TBRV), *Grapevine deformation virus* (GDefV), *Grapevine Anatolian ringspot virus* (GARSV), *Artichoke Italian latent virus* (AILV), *Grapevine chrome mosaic virus* (GCMV), *Grapevine Tunisian ringspot virus* (GTRSV), *Raspberry ringspot virus* (RpRSV) and *Grapevine Bulgarian latent virus* (GBLV) are renown to infest grapevines of European and Mediterranean states. GDefV, ArMV and GFLV are subgroup A members of the genus Nepovirus in the family Secoviridae. The members of subgroup A are closely related and GDefV may result from recombination between GFLV and ArMV (Elbeaino et al. 2012)

Nepoviruses, including some ArMV and GFLV isolates, contain large and small satellite RNAs (satRNAs). SatRNAs are apparently molecular parasites and absolutely depending on a helper genome for their biological survival such replication and encapsidation, and encode a nonstructural protein. They encode according of the size of the RNA and the type of protein. Pinck et al. (1988) pointed that four types of satellite RNA have been found, which one of them the B-type, consists of relatively large RNA molecules which tend to be greater than 1 kb and have an open reading frame (ORF), and composed of messenger RNA for non-structural proteins.

The symptoms of the genus nepovirus are quite similar to the fanleaf virus (Fuchs et al. 2017). In Cappadocia, Turkey, a virus which symptoms were similar to fanleaf virus, with isometric particles ca. 30 nm in measurement and angular form was recognized through mechanical transmission of grapevine and distinguished as GDefV (Cigsar et al. 2002). Recently, the virus has been surveyed in Turkey and the presence was determined as 1.2% (Ulubaş Serçe et al., 2020).

1.1 Objective of Thesis

The main objective of the thesis comprises of as following:

- Investigation of the prevalence of GDefV in Nevşehir and Niğde vineyards.
- Research towards elucidation of the complete genome of grapvine deformation virus (GDefV) isolates in turkey

CHAPTER II

GENERAL INFORMATION

According to archaeological evidence (McGovern 2003), the cultivation of *Vitis vinifera* ssp. *vinifera* goes back to 6000–8000 years ago in the Near East, from its wild progenitor, *V. vinifera* ssp. *sylvestris*. A multilocality of genetic selection has been discovered in the process of grapevine domestication, according to current genomic research (Samorini 2019). The chlorotypes of most Iberian Peninsula cultivars are viable with wild plant populaces from the western Mediterranean locale (Arroyo-Garca et al. 2006). The archaeological sites of Ohalo II, Israel (found two seeds of *V. vinifera* ssp. *sylvestris* (Kislev et al. 1999), Jiahu, and China have the oldest records of *Vitis* species. The presence of grape beans, as well as a fermented beverage consisting of rice, grapes, hawthorn, and honey, was verified by samples dating back to 7000 BC from Georgia's Shulaveris Gora and Gadachrili Gora. Vine pollen was detected (McGovern et al. 2017) at Hajji Firuz Tepe, Iran (the analisys showed that there was a product manufactured out of grapes and resin of turpentine tree – 5400–5000 BC, McGovern et al. 1997) and Areni, Armenia (a grape-pressing rig was unearthed – 4223–3790 BC) (Barnard et al. 2011).

2.1 Nepovirus Diseases of Grapevine

As grapevine propogated vegetativly, the possibility include many viruses are high, that is why most virus-induced grapevine diseases could be classified as complicated diseases, because they show the variety of symptoms and usually caused by different viral species. These multiple diseases show symptoms such as malformation of leaf, leaf yellowing, both caused by a number of species of the genus Nepovirus; "leafroll," caused by viruses of the family Closteroviridae; and "rugose wood," caused by viruses of the genera Vitivirus and Foveavirus (Martelli, 2014).

The impacts of grapevine viral infections on quality and quantity are regarded serious, despite the fact that assessing them is difficult and the findings are frequently inconsistent, as evidenced by the literature. Although the viral-host link (i.e., environmental circumstances, virus coinfection, vineyard management, virus strain,

grape cultivar and/or clone, and rootstock) is justified by the various variable factors, many early analyses were based on incorrect methodologies. The confusion of the disease was evaluated by indexing the performance of symptomatic vs. with out symptom vines or, at best, by comparing the performance of symptomatic or vines which no have any symptoms (Walter and Martelli, 1997). This technique, however, is not certain since environmental and cropping circumstances may amplify or dampen apparent symptoms, or it may be caused by a mix of viruses. Furthermore, because the studies frequently comprised genetically non-uniform vine populations, the degree of heterogeneity in vine performance increased. In many research, the study were focused on comparing of single clones in the diggerent period elimination of virus. These were by meristem tip culture and heat treatment. Also could be both by infecting resistant vines via mechanical inoculation. Currently, the relationship of the etiological agents of grapevine diseases has became very common topics, and studies have addressed the involvement of certain viral agents and/or their strains in various diseases.

Infectious degeneration (fanleaf) is quite possibly the most obliterating diseases of European grapevines, and it is mostly due to the *Grapevine fanleaf virus* (GFLV) member of the Nepovirus genus (Table 2.1). *Arabis mosaic virus* (ArMV), *Raspberry ringspot virus* (RpRSV), *Tobacco ringspot virus* (TRSV), *Tomato black ring virus* (TBRV), *Artichoke Italian latent virus* (AILV), *Grapevine chrome mosaic virus* (GCMV), *Bluberry leaf mottle virus* (BBLMV), *Grapevine Tunisian ringspot virus* (GTRSV), *Tomato ringspot virus* (ToRSV), *Peach rosette mosaic virus* (PRMV), *Cherry leaf roll vırus* (CLRV), *Grapevine Bulgarian latent virus* (GBLV), *Grapevine Anatolian ringspot virus* (GARSV), *Grapevine deformation virus* (GDefV), *Strawberry latent ringspot virus* (SLRV) are some other species in the genus Nepovirus.

Table 2.1. Nepoviruses infecting grapevine (*Vitis spp.)* worldwide and their vector

Family	Subfamily	Genus	Viral species	Vector
Secoviridae	*Comovirinae*	*Nepovirus* (SubgroupA)	*Arabis mosaic virus* (ArMV)	Nemotod
			Grapevine fanleaf virus (GFLV)	Nemotod
			Raspberry ringspot virus (RpRSV)	Nemotod
			Grapevine deformation virus (GDefV)	Unknown
			Tobacco ringspot virus (TRSV)	Nemotod
		Nepovirus (SubgroupB)	*Tomato black ring virus* (TBRV)	Nemotod
			Artichoke Italian latent virus (AILV)	Unknown
			Grapevine chrome mosaic virus(GCMV)	Unknown
			Grapevine Anatolian ringspot virus (GARSV)	Unknown
		Nepovirus (SubgroupC)	*Bluberry leaf mottle virus* (BBLMV)	Unknown
			Grapevine Tunisian ringspot virus (GTRSV)	Unknown
			Tomato ringspot virus (ToRSV)	Nemotod
			Peach rosette mosaic virus (PRMV)	Nemotod
			Cherry leaf roll virus (CLRV)	Unknown
			Grapevine Bulgarian latent virus (GBLV)	Unknown
	Unassigned		*Strawberry latent ringspot virus* (SLRV)	Nemotod

Nepoviruses are generally disseminated in warm localities. The host range of nepoviruses changes from wide to confined, contingent upon the infection. General characteristics are ringspot symptoms, however mottling and spotting are similarly regular. Infections of 12 species are acquired and transmitted by longidorid nematodes (*Xiphinema, Longidorus or Paralongidorus spp*), transmission among trees can occur by pollen, and mites (blackcurrant inversion infection) can transmit just viruses of one species, while the others have no information about biological vector (Brown et al. 1995). The common transmission is by seed and pollen. In herbaceous plants, the indications initiated by nepoviruses are regularly transient, with recently arising leaves seeming symptomless half a month after contamination (the supposed "recuperation" peculiarity). Side effect recovery is related with enlistment of RNA silencing, an antiviral protection, and is in some cases (yet not consistently) accompanied with reduced concentration of viral RNAs (Ghoshal and Sanfacon 2015).

The GFLV and some other European nepoviruses that infect grapevine are distorting and chromogenic strains that can coexist in more than one infections. Depending on virulence of the viral strains, cultivar sensitivity, rootstock type, and environmental variables play an important role in economic impact on a different varies of crops greatly (Mannini and Didiaro 2017).

GFLV resistance is uncommon in American *Vitis* species and hybrids. In a sense, GFLV susceptibility is passed to *V. vinifera* from rootstocks, which, when self-rooted, exhibits universal resistance to GFLV owing to coevolution with the virus. Although many genotypes from coutries like Iran and Afghanistan were extremely resistant (Aradhya et al. 2003), nearly all *V. vinifera* cultivars have varying degrees of sensitivity. The *V. labrusca*, an Amerian grapevine, can be influenced by infection but exhibits minimal symptoms, whereas *Muscadinia rotundifolia* and *V. munsoniana* are extremely resistant to *X. index* feeding and are participating in genetic development projects for rootstocks (Staudt and Weischer 1992; Walker et al. 1994).

Seriouse decline, low productivity of yield and low quality of fruit, an decrised productive life period, reduced taking of graft, decline ability in rooting, and less resistance to unsuitable climatic factors all have a negative impact on sensitive cultivars, whereas tolerant cultivars produce more crops (Martelli 2014).

Degenerative grapevine disease is also known as "Decline" is not resistance species or hybrids in North America usually occur in Nepovirus members. Depending on different host species (*V. vinifera*, *V. labrusca*, and interspecific hybrids) infecting virus, and the climatic circumstances, symptoms would chanched. For example, the TRSV and ToRSV cause more severe decline in European cultivars, especially if self-rooted, with more decline observed in colder climates compered with warmer climates. As a result, variouse virus symptoms such as stunted growth, mottled, ring spots, deformed in leaves and canes, poor fruit laying,straggly and shelled clusters occurred. Winter inter damage, on the other hand, frequently results in the mortality of weaker vines. In addition, warm clime decreace yield but not vigour. The ToRSV and TRSV susceptible *V. vinifera* cultivars and interspecific hybrids are easily infected by such viruses, whereas American *Vitis* species produce immunity or hypersensitivity to both of these viruses (Uyemoto et al. 1977). Infection with various strains of ToRSV

in cv. De Chaunac, on the other hand, caused in yield losses ranging from 75% to 95% (Rowhani et al. 2017). These viruses have a significant influence on propagation in nurseries.

TRSV was found in Chardonnay grapes in Missouri (USA), together with GFLV and *Grapevine rupestris stem pitting-associated virus* (GRSPaV). It was afflicted with a serious viruses known as "vein-clearing complicated". The symptoms of this viruses were vigour loss, hindered, crinkled, and mottled leaves, and minuscule bunches with not many berries together (Rowhani et al. 2017).

In "Concord" grapevine (*V. vinifera, V. labrusca*) in Michigan a common nepovirus known as *Peach rosette mosaic virus* (PRMV) causes delayed bud burst, poor fruit set, significant crop loss, leaf distortion and mottling (Dias and Cation 1976). Susceptibility to this virus varies widely in *Vitis* spp. and French-American hybrids, significantly decreasing productivity (up to 40%) and increase (up to 60%) in Concord. Grapevine from diseased vineyards had only one ripe seed and were tasteless (Ghafoor et al 2020).

Blueberry leaf mottle infection (BLMoV) has inactively contaminated European grapes, while Concord has postponed bud exploded, coming about in fanleaf-like indications on leaves and sticks, as well as poor fruit setting (Rumbos1989).

2.2 Genome Organisation of Nepoviruses

The genome organization of Nepoviruses is segmented, bipartite linear ssRNA(+) genome composed of RNA-1=7.5 kb and RNA-2=3.9 kb. Each genomic segment has a VPg linked to its 5' end and a 3' poly(A) tract (Figure 2.1).

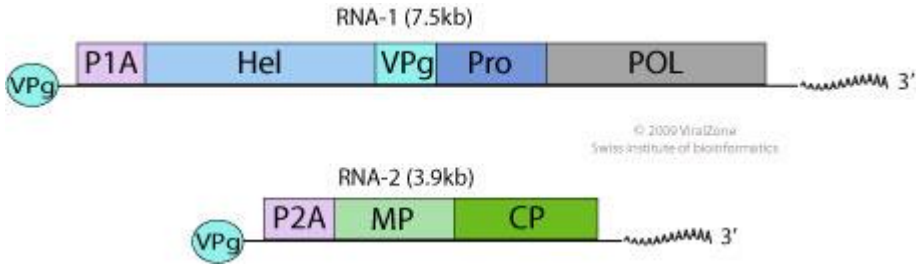


Figure 2.1. Genome structure of nepoviruses (ViralZone www.expasy.org/viralzone).

The genome of GDefV consists of two linear positive-sense ssRNA with differ lengths and contain a 3′-terminal poly(A) tract. RNA1 7.386 nt, and RNA2 3.753 nt length. A protein, designated VPg (2–4 kDa) is shown to be covalently bound at the 5′-end in RNA1 and RNA2. The N-terminal protein of the RNA-1-encoded polyprotein (P1A) is involved in RNA-1 replication. The replication block on the RNA-1-encoded polyprotein includes the 58K protein with sequence motifs characteristic of an NTP-binding helicase, the VPg, the Pro and the Pol. The enzymatic activity of Hel and proteins like VPg, Pro, and Pol is often referred to as the protease are essential for viral genome replication, transcription, and translation.

The N-terminal protein of the RNA-2-encoded polyprotein (P2A) leads to replication of RNA-2. The other two domains of proteins are the MP and CP and responsible for movement of the viral agent from cell to cell within the susceptible or host plant.

Nepoviruses, consisting of some GFLV and ArMV isolates, contain large and small satellite RNAs (satRNAs). The large satRNAs, which are greater than 1 kb in size and have an open reading frame (ORF), are known as type B satRNAs. The satRNAs are absolutely dependent on a helper genome for their replication and encapsidation, encoding a nonstructural protein. Howerver, there is little knowledge regarding the origin and function of nepovirus type B satRNAs or their encoded protein. GFLV strain F13 associated satRNA is 1,114 nucleotides (nts) long, encodeing a 37-kDa protein known as P3. It is the only large GFLV satRNA characterized so far although recently a new GFLV satRNA had been reported. SatRNAs of ArMV range from 1,092 to 1,139 nts in size, producing a protein of 39 kDa (Song et al. 2021).

Satellite RNAs for a surviving need a host cell. They depends on helper virus for mutliplication and replication but indepent of the satellite. On the basis of the size of the RNA and the type of protein they encode, totally four types of satellite RNA have been identified (Pinck et al. 1988). Which is one of them the B-type, consists of large RNA molecules and are composed of messenger RNA for non-structural proteins. Consisting of nepoviruses as helper viruses is a key characteristic of many of these satellites. Generally, nepovirus mRNA satellites' presence in a virus culture exhibit little or no

modification to the symptoms of infection by the helper virus as well as show little effect on its yield (Latvala-Kilby et al. 2000). Some satellites show high specificity to a strain of helper virus while, others can be helped by heterologous viruses. Moreover, nepovirus mRNA satellites encode proteins having a M_r of 38000 to 48000 and are relatively basic in nature, particularly, in the N-terminal and C-terminal parts of the molecules (Meulewaeter et al. 1998). However, proteins encoded by different satellites show little similarity in amino acid sequences among themselves. Also, there is no peptide motif found in all satellite proteins. The experiments of reverse genetics with satellites suggest that the satellite-encoded protein is vital for satellite RNA multiplication. This provides potential for studying the replication mechanistic processes in both the helper virus and satellite (Fritsch et al. 1993).

2.3 Prevalence and Genetic Variances of Nepoviruses

Secoviridae (Picornavirales order), a non-enveloped virus group, typically infects vertebrates, arthropods, plants, and algae. It has a significant effect by infecting many kinds of plants from grapvine to rice. Different ecdysozoan vectors such as nematodes, beetles, and aphids could be a seriouse factor in infecting substantially of plants. An assortment of computational strategies has been used to investigate the evolutionary characteristics of these viruses. Two species have shown proof of both codon specific and episodic diversity determination; however, strong purifying selection pressures were calculated for the coat protein (CP) sequences of nine species. Rates of CP nucleotide substitution for four species were estimated using Bayesian phylogenetic reconstruction methods, ranging from 9.29×10^3 to 2.74×10^3 (subs/site/year). These findings are comparable to short-term estimates for other plant- and animal-infecting virus species. A time-measured phylogeny of the subfamily Comovirinae was constructed using the data obtained, estimating divergence of ninety-four extant sequences that occurred less than 1,000 years ago. The current virus species evolved over a period of 50 to 250 years, coinciding with the intensity of agricultural practises in industrial societies. Significant and often unique protein domain commonalities between secovirids and other infectious microbe-infecting picorna-like viral agents suggest common ancestry, though recombination (modularity) was limited to closely related taxa. The resultant outcomes in a broader context of view and preliminary proofs suggest that some Secoviridae members may have evolved from insects colonising

plants in various founding events, leading to speciation. In a scenario where viruses infect species from different taxonomic kingdoms, there is a significant implication for virus emergence (Thomson et al. 2014).

Study conducted on the prevalence and genetic variation of GFLV in China included DAS-ELISA, RT-PCR, and nested RT-PCR on 142 grapevine samples from 13 provinces and regions. As a result, 38 percent of the samples tested positive for GFLV using DAS-ELISA, and 26.8 percent tested positive using RT-PCR and nested RT-PCR. Cloning and sequencing of PCR products from the movement protein (MP) and coat protein (CP) genes were also performed. The percentage of shared identities between MP or CP nucleotide and protein sequences ranged from 94.9 to 100 percent. Phylogenetic analysis revealed that the Chinese GFLV isolates were found distinct from the isolates reported in GenBank (Zhou et al. 2015).

Study was also conducted on the genetic variability of the coat protein (CP) gene on RNA2 and the presence of GFLV in 74 vineyards in grapevine (growing in Andalusia, southern Spain). The overall prevalence of GFLV (the percentage of vineyards infected with GFLV) was 24.30%. However, it was most common in the denominations of origin (DO) of JerezXérèsSherry and ManzanillaSanlcar de Barrameda (29.40%), Condado de Huelva (24.00%), and MontillaMoriles (13.30%). In GFLV infected plants, fanleaf and yellow mosaic symptoms were observed, as well as a decline in overall vine health. Except for one sample from the DOs of Jerez Xérès Sherry and Manzanilla Sanlcar de Barrameda and one from the DO of Condado de Huelva, almost all of the samples showed soil infestation by the virus vector nematode species *Xiphinema* index and/or *X. italiae*. Reverse transcriptionpolymerase chain reaction (RT-PCR) was used to successfully amplify a partial CP gene product of the expected size (555 bp) in leaves and nematode vectors. A total of 135 clones of the partial GFLV CP gene were analysed using singlestrand conformation polymorphism sequence variation, yielding 12 different haplotype patterns. Haplotypes were separated among the three different DOs. Except for Jerez de la Frontera429., the haplotype patterns MOB, MOC, MMA, MMC, and HB were detected in all leaf samples and nematode vectors. Sequence analysis of the GFLV haplotypes revealed sequence variability within the haplotypes. It also revealed that some variants were more abundant than others in the same sequenced haplotype pattern.

As a result, as in other grapevinegrowing areas around the world, GFLV exists as a 'quasispecies' in southern Spain (Rius et al. 2012).

During the 2011-2012 growing season leaf samples were collected to determine the distribution of GFLV in Khorasan-Razavi, Iran. The GFLV was detected in leaf samples using enzyme-linked immunosorbent assay (ELISA) and specific antibodies raised against an Iranian isolate of the virus (Zakiaghl and Izadpanah 2003). *Chenopodium quinoa* plants were used as systemic herbaceous hosts for GFLV propagation. Extracts from ELISA positive samples were used to inoculate carborundum dusted seedlings in phosphate buffer. Silicon dioxide was used for extraction of total RNA plants from fresh leaves (Boom et al. 1990). In an indirect ELISA, GFLV was found in 187 of 280 samples. The infection rate of GFLV in Khorasan-Razavi ranges from 32% to 63% were found in the result of ELISA. In Kashmar, 90 percent of the samples had the serious infection in grapevines and were infected with virus. GFLV was found to cause symptoms like vein banding and yellow mosaic in infected leaves. Infected grapevines also had shorter internodes, zigzag stem growth, and double nudes, but the majority of the GFLV vines were symptomless. Mechanical induction of chlorotic local lesions followed by vein clearing in *Chenopodium quinoa* systemic leaves was observed two weeks after inoculation, mechanically with sap extracts from GFLV positive leaf samples. RT-PCR amplifies a 1000 bp fragment corresponding to the GFLV coat protein gene using specific primers. There was no fragment found in the healthy control group. In comparison to previously published GFLV isolates, the coat protein gene of four Iranian isolates showed 89–97% nucleotide sequence identity and 90–92% amino acid identity using the pairwise method. On the basis of the phylogenetic tree, GFLV isolates were divided into two main clusters, with the Iranian isolates in one cluster and the other GFLV isolates in the other. The tree was built using 1000 bootstrap replicates and was based on the coat protein gene of four Iranian isolates and 15 other isolates. The Iranian cluster, which was subdivided into two sub-clades, corresponded to two distinct evolutionary lineages, indicating their geographical separation. Grapevine fanleaf virus is the primary cause of grapevine degeneration disease. It has been proposed that GFLV originated in old Persia, specifically the region between the Caspian Sea and the Black Sea. This theory is supported by the high levels of divergence and distinct phylogenetic position of Iranian isolates. According to sequencing data, geographical separation is an important

determinant factor in the phylogenetic divergence of GFLV isolates (Gholampour et al. 2014).

The characterization of sequences of the complete coding region of RNA2 was carried out which included genes 2BMP, 2AHP, and 2CCP, as well as a partial sequence from the RNA1-encoded gene 1EPol of 14 GFLV isolates from three naturally infected California vineyards, which helped gain insight into the evolutionary mechanisms of GFLV, genus Nepovirus, family Secoviridae. Phylogenetic analyses revealed no evidence of the isolates' vineyard origin or association with rootstock genotype or scion cultivar. Phylogenetic analyses, on the other hand, revealed two to three evolutionarily divergent lineages. Three RNA1 and 44 RNA2 coding sequences are available for analysis of the genetic variability of California isolates as well as isolates from other parts of the world, revealing similar patterns of molecular evolution for different regions of the GFLV genome. It does, however, show distinct selection constraints, with the strongest pressure applied to genes $2B^{MP}$ and $2C^{CP}$, an intermediate level of pressure applied to gene 1EPol, and the weakest pressure applied to gene $2A^{HP}$. Interspecies recombination events between GFLV and ArMV were observed in some Californian isolates, with crossover sites suspected in gene $1E^{Pol}$ and identified in genes $2A^{HP}$ and $2B^{MP}$; however, intraspecies recombination events were observed in all four target genes, but most frequently in gene 2CCP. According to the findings of this study, purifying selection and recombination are important evolutionary mechanisms in the genetic diversification of GFLV (Oliver et al. 2010).

Large satellite RNAs (type B satRNAs) of GFLV were identified in a naturally infected vineyard and collected grapevine germplasm. These GFLV satRNA variants had a higher nucleotide sequence identity with satRNAs of ArMV strains NW and J86 (93.8 to 94.6 percent) than with GFLV strain F13 and other ArMV strains (68.3 to 75.0 percent). Phylogenetic analyses revealed no distinction between GFLV and ArMVsatRNAs in terms of helper virus identity. Furthermore, seven stretches of 8-15 conserved nucleotides (I-VII) were identified in the 5′ region of subgroup A nepovirus genomic RNAs (GFLV, ArMV, and GDefV) and nepovirus type B satRNAs. This included the previously reported motif I, implying that the origin of large satRNAs could have resulted from recombination between an ancestral subgroup A nepovirus RNA and an unknown RNA sequence with the 5′ region acting as a putative cis-

replication element. The comparative analysis of two GFLV strains containing or not containing satRNAs found no discernible effect on virus accumulation and symptom expression in a systemic herbaceous host called Chenopodium quinoa. This study focused on the origin and biological effects of large satRNAs associated with A nepovirus subgroups (Gottula et al. 2013).

GFLV has been documented as one of the oldest viral diseases in vineyards in a few provinces of Iran, including Isfahan and East Azerbaijan. In fact, this virus was named after the symptoms observed in virus-infected grape vine leaves. In this series of experiments, the genetic variability of GFLV was calculated in East Azerbaijan province (which is located in Iran). A total of 310 samples were collected at random for this study between 2017 and 2018. Using GFLV specific antibodies prepared against the Iranian isolate, 197 infected samples were found by using indirect ELISA. Nucleotide sequence identities between previously reported GenBank sequences and isolates from this study ranged from 87 to 90 % (Razzaq 2020).

Multiple pathogens can cause a similar set of symptoms in a host plant in some diseases. Because of the pathogens' diverse biology, management of such diseases can be difficult due to control treatments. Grapevine leafroll disease is a widespread disease that has been linked to many viral species in the closteroviridae family. A few viruses are transmitted through infected grafts, while others are transmitted through insect vectors. Vineyards in various parts of California were surveyed for diseased of a grapevine plants, and plants which have showed disease symptoms were tested for all viral species related with grapevine leafroll disease. When compared to the graft-transmitted, the relative incidence of each viral species varied among the three locations, with species linked to insect-vectors having the highest relative incidence. In one region, diseased plants were discovered in the absence of any insect vector known to transmit the pathogen. Some virus species from other surveyed locations, on the other hand, were vector-borne. Despite the fact that disease symptoms were detected at some sites, this survey failed to detect grapevine leafroll viruses. It can be attributed to the unknown genetic diversity of this group of viruses, which could not be detected with the biotechnology tools available at the time of the survey, or to incorrect identification of the visible symptoms, which can also lead to virus detection failure. According to the differences in relative appareance of every virus species among regions and vineyards

within regions, it is suggested that, the area and location-specific disease diagnostic strategies be implemented (Sharma et al. 2015). Many researchers have indiceted that the ArMV, GFLV and GdefV are have a mosaic structure as a symptoms (Martelli 1993, Cigsar 2003)

CHAPTER III

MATERIALS AND METHODS

3.1 Sample Collection

In this research, total 150 samples of grapevine having typical symptoms of nepovirus infection including chlorotic or necrotic lesions, stripes or streaks, vein clearing, vein banding, yellowing, leaf rolling and curling were collected which are originated from provinces Nevşehir and Niğde, in June-July 2020 (Table 3.1). If there were no any symptoms on the leaves just randomly collection was performed. Sampling were collected as leaves and shoots together and immediately put in box including ice. After that, all samples were keept in the +4 °C for one week.

Table 3.1. The collection sites of plant samples obtained in the study

Province	District	Sample number
Niğde	Altunhisar	10
	Bor	15
	Merkez	5
	Ulukışla	7
	Çamardı	8
	Çiftçilik	5
	TOTAL	**50**
Nevşehir	Acıgöl	10
	Avanos	10
	Derinkuyu	10
	Gülşehir	7
	Hacibektaş	12
	Çökek	11
	Merkez	20
	Ürgüp	20
	TOTAL	**100**
General Total		**150**

3.2 Total Nucleic Acid (TNA) Extract from Plant Materials and PCR Reactions

The TNA extraction from the plant samples was performed using cetyl trimethylammonium bromide (CTAB) extraction method (Doyle and Doyle, 1987). For TNA extraction 200 mg of grapevine material including leaf pieces, leaf petioles including main veins were squashed by liquid nitrogen using morter and pestle and after tawing 2 ml of CTAB buffer (20 mM EDTA, %2 CTAB, 1,4 M NaCl, %0,2 β-mercapthoethanol, 500 mM Tris, pH 8.0) was added. After transferring 1 ml squashed tissues to a 2 ml tubes, incubation at 70 ° C for 30 min performed and then the tubes were centrifuged for a time period of 15 min at 13,000 rpm. There was a transfer of 850 µl supernatant to a new eppendorf tube and mixed gently by adding an equal volume chloroform: isoamyl alcohol (24: 1) followed by centrifugation at 13,000 rpm for 15 min. There was a transfer of 700 - 750 µl upper phase into newly autoclaved eppendorf tubes and mixed with 0.6 ml volume of cold isopropanol and stored overnight at -20°C. Following that, centrifugation for a time period of 15 minutes at 15,000 rpm was performed and after discarding the supernatant, the TNA pellet was washed with 70% cold ethanol, dired and diluted with 70 µl TE buffer (1 mM EDTA, pH 7.6, 10 mM TrisHCl). The TNAs were mantained at -20 ° C until use for further studies.

GDefV diagnostics was performed by RT-PCR. As GDefV is ssRNA virus it can easily degrade, so that is why for future study it is important to convert RNA to complementary DNA (cDNA). To obtain complementary DNA (cDNA) from TNA, two steps were performed. Step 1; 4 µl TNA mixed with 1 µl random primers, 1 µl oligo dT , and sterile water were used to complete final volume to 10 µl. The mixture was incubated at 65 °C for 5 min and immediately replaced into ice for 2 min. Step 2; the reaction mixture of reverse transcription of 5xreaction buffer, 0.1 µl RiboLock RNase inhibitor (Thermo Scientific), 0,5 µl DTT (0.1 M) (Invıtrogen), 0.5µl Reverse Transcriptase (Thermo Scientific), 1 µl dNTPs (10 mM) and sterile water to final volume 10 µl was added on the tubes (Table 3.2). The incubation was for 10 min at 25 °C, 45 min at 42 °C and 10 min at 70 °C to inactivate the enzymes.

For PCR, 6 µl FastStart Essential DNA Green Master Mix (Roche), 0.5 µl 10 mM forward and reverse primers (Table 3.3), 2 µl cDNA was mixed in final volume 12 µl adjusted with sterile water (Table 3.4). The amplification was performed on the

Sensequest thermal cycler (Qiagen) with the applicable primer annealing cycles. Initial denaturation was at 94°C for 5 min, followed by 40 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 58°C, extension for 45 sec at 72°C, and final extension for 10 min at 72°C (Table 3.5). The obtained fragments were confirmed with 1% agarose gel TAE Buffer and visualized under UV electrophoresis using redsafe DNA staining dye.

Table 3.2. The reactive conditions in synthesis of cDNA from TNA

STEP 1	
Reaction Buffers	**Total volume of buffers for 1 reaction**
d_2H_2O	5 µl
Random Hexamer Primer (Thermo Fisher Scientific)	1 µl
Oligo$(dT)_{18}$ Primer (Thermo Fisher Scientific)	1 µl
TNA	4 µl
65 C 5 minute	
In ice 2 minute	
STEP 2	
d_2H_2O	4 µl
5X Reverse Transcriptase Buffer	4 µl
dNTP (10 mM)	1 µl
RiboLock RNase Inhibitor (Thermo Fisher Scientific)	0.1 µl
RevertAid Reverse Transcriptase (Thermo Fisher Scientific)	0.5 µl
25 ^oC 10 minute	
42 ^oC 45 minute	
70 ^oC 10 minute	

Table 3.3. The primers were used for GDefV diagnosis

Primer name	**Genome**	**Sequence (5'-3')**	**Length (bp)**	**Literature**
GDef-2861F GDef-3368R	RNA2-CP	CGACAGTAGTGGAGAGTTTG TAGGGCTATAACCTCTGACC	508	Ulubaş-Serçe vd., 2018c

Table 3.4. The reaction mixture utilized in PCR amplification of GDefV

Reaction Buffers	Volume of reaction buffers for 1 reaction (µl)
d_2H_2O	4
FastStart Essential DNA Green Master mix (Roche)	6
Forward Primer (10 mM)	0.5
Reverse Primer (10 mM)	0.5
cDNA	2
Total	12

Table 3.5. Amplification conditions in PCR for GDefV

Temperature (^oC)	Time Duration	Cycle
95	5 minute	1
94	30 second	40
58	30 second	
72	1 minute	
72	10 minute	1
8	∞	1

3.3 Inoculation

The method of mechanical virus inoculation was performed for inoculation the *Nicotiana tabacum* var. *Xanthi*. Grapevine leaves of cuttings from the naturally GDefV infected were squashed with with inoculation buffer (0.1% of Na_2SO_3, 1.14 g/l of Na_2HPO_4, 0.199 g/l of KH_2PO_4, and 1% of PVP-40). The 3th real leaves of the tobacco plants were used for virus inoculation. The inoculation was performed to 14 tobacco plants. In order to achieve good result, before inoculation, plants were placed in dark place for a night. After carborundum pouring on the surface of tobacco leaves, GDefV infected grapevine squash was rubbed over the surface of leaf by using cotton-swab. Following the incubation for 2-5 mints the tobacco plants were carefully washed by a tap water. Afterwords, plants leaved for 15 days to grow up. The cDNA and PCR were performed as mentioned above.

3.4 Sequencing

GDefV plant amplicons were sequenced from both end using the GdefV forward and reverse primers. The sequencing of target DNAs through Sanger technology was carried out by Medsantek company (İstanbul/Türkiye). The quality of DNA sequences given was checked using their chromotogrames and assembled by Geneious Prime software. The sequences Basic local alignment search (BLAST) analysis was performed using Blast tool of National Center for Biotechnology Information (NCBI).

3.5 Bioinformatics Analyses

The aim of this work was try to complete the whole genome of GDefV RNA1 and RNA2. In order to achieve the reliable results, the CLC Genomic Workbench 11 and Geneious Prime softwares were used for DNA analysis and retrived consensus sequences were assembled to provide complete genom of RNA1 and RNA2 segments of GDefV. For this purpose, previously provided high throughput sequencing data of two grapevine samples having severe vein clearing and leaf deformation symtoms (sample id 107 (B36-Adana) and 105 (N34-Tokat)) were used. Total RNA was extracted and rRNA depletion was performed by treatment of Ribo-Zero rRNA Plant Removal Kit. NEBNext® Ultra™ RNA Library Prep Kit was used for library preparation. Deep sequencing was performed using Illumina Hiseq2000 RNAseq technology with 2x150 read length and 40 million depths for each reads. Deep sequencing yielded more than 131 M sequences in total for both grapevine samples.

Bioinformatic analysis was performed using Geneious Prime and CLC Genomic Workbench v.11 softwares. The trimmed and mapped to GDefV reference sequence consensus contigs (RNA1 and RNA2) and not trimmed and mapped to GDefV reference sequence consensus contigs (RNA1 and RNA2) from CLC and Geneious were extracted. Provided contigs were analyzed with Geneious Prime, BLAST tool of NCBI. Phylogenetic tree was constructed using available GDefV complete genomes deposited in Genbank using Mega X (Figure 3.1).

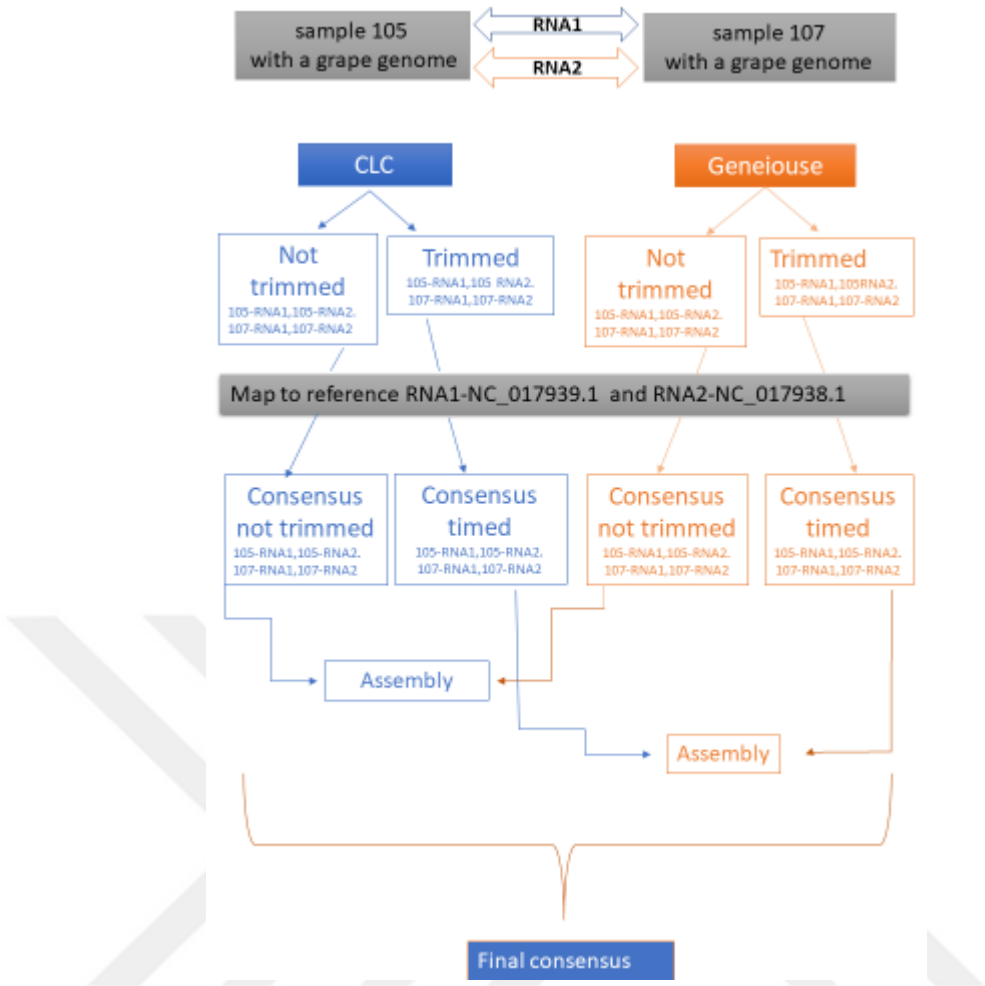


Figure 3.1. General schematic summarizing of bioinformatics analyses

3.5.1 Analyses in CLC genomic workbench 11 without trimming

The reads belong to sample 105 and 107 from illumina sequencing were imported as separate files then paired and merged using NCS Core Tools-Paired sequence option. Same operation was done with sample 107. The reference sequences were downloaded from National Center for Biotechnology Information (NCBI) as NC_017939.1-RNA1 and NC_017938.1-RNA2. The paired and merged reads were mapped to reference sequences (ref: RNA1 and RNA2) using Map Reads to Referens option of CLC software. After that the consensus from given results were extracted. Final results (consensus: 105-RNA1, 105-RNA2, 107-RNA1,107-RNA2) separately were exported and saved as Fastq format for a future work.

3.5.2 Analyses in CLC genomic workbench v.11 with trimming

All operations have done the same but with a quality control compared with a previous study. The reads belong to sample 105 and 107 from illumina sequencing were imported as separate files, then paired and merged using NCS Core Tools-Paired sequence option of CLC software. Same operation was done with sample 107. Following that the low quality sequences were trimmed using NCS Core Tools-Trim Reads option of CLC software. The paired, merged and trimmed reads were mapped to reference sequences (ref: RNA1 and RNA2) using Map Reads to Referens option of CLC software. After that the consensus from given results were extracted. Final results (consensus: 105-RNA1, 105-RNA2, 107-RNA1, 107-RNA2) separately were exported and saved as Fastq format for a future work.

3.5.3 Analyses in geneious prime without trimming

In Geneious Prime the similar work was performed. The reads belong to sample 105 and 107 from illumina sequencing were imported as separate files and then paired using Set Paired Reads option. After that, the reads were merged using Set Merge Reads option of Geneious software. At the end of operation, the "Merged Reads" and "Unmerged Reads" provided. We have chosen both merged and unmerged reads of each sample (105 and 107) in order to map to reference via Align/Assemble-Map to Reference option of Geneious software using the reference sequences NC_017939.1-RNA1 and NC_017938.1-RNA2. From the given results the consensus sequences were extracted via Consensus-Extract the Region.

3.5.4 Analyses in geneious prime with trimming

Similar work has done but in this time with a quality control. The reads belong to sample 105 and 107 from illumina sequencing were imported as separate files and then paired using Set Paired Reads option. By using paired reads, trimming were performed via Annotate/Predict-Trim using BBDuk without changing any parameters. After that, the reads were merged using Set Merge Reads option of Geneious software. At the end of operation, the "Merged Reads" and "Unmerged Reads" provided. This tool utilizes BBDuck Pluggin and is designed to merge two overlapping paired reads into a

single read. This tool is useful for generating a consensus from overlapping reads generated by amplicon sequencing. We have chosen both merged and unmerged reads of each sample (105 and 107) in order to map to reference via Align/Assemble-Map to Reference option of Geneious software using the reference sequences NC_017939.1-RNA1 and NC_017938.1-RNA2. From the given results the consensus sequences were extracted via Consensus-Extract the Region.

3.5.5 Assembly of consesus sequences retrived from CLC geneomic workbench and geneious prime

All trimmed and not trimmed consensus sequences from CLC were imported to the Geneious Prime software. Next, the consensus sequences of trimmed and not trimmed were assembled among themselves using Geneious Prime sofware. The provided consensus sequences (RNA1 and RNA2) were used for further genomic analysis of GDefV.

3.5.6 Phylogenetic tree

Evolutionary analyses were conducted in MEGA X software (Kumar et al., 2018). The consensus sequences of RNA2 belong to sample 105, 107 and the RNA2 available complete sequences of GDefV, ArMV and GFLV from Genbank (ArMV RNA2-AB279741.2, ArMV RNA2-EU617327.1, ArMV RNA2-MN599985.1, ArMV RNA2-NC_006056.1, GDefV RNA2-NC_017938.1, GFLV RNA2-GU972580.1, GFLV RNA2-GU972584.1, GFLV RNA2-MW380916.1, GFLV RNA2-MW380917.1) were used to construct phylogenetic tree.

3.5.7 GDefV RNA1 and RNA2 gene motifs search

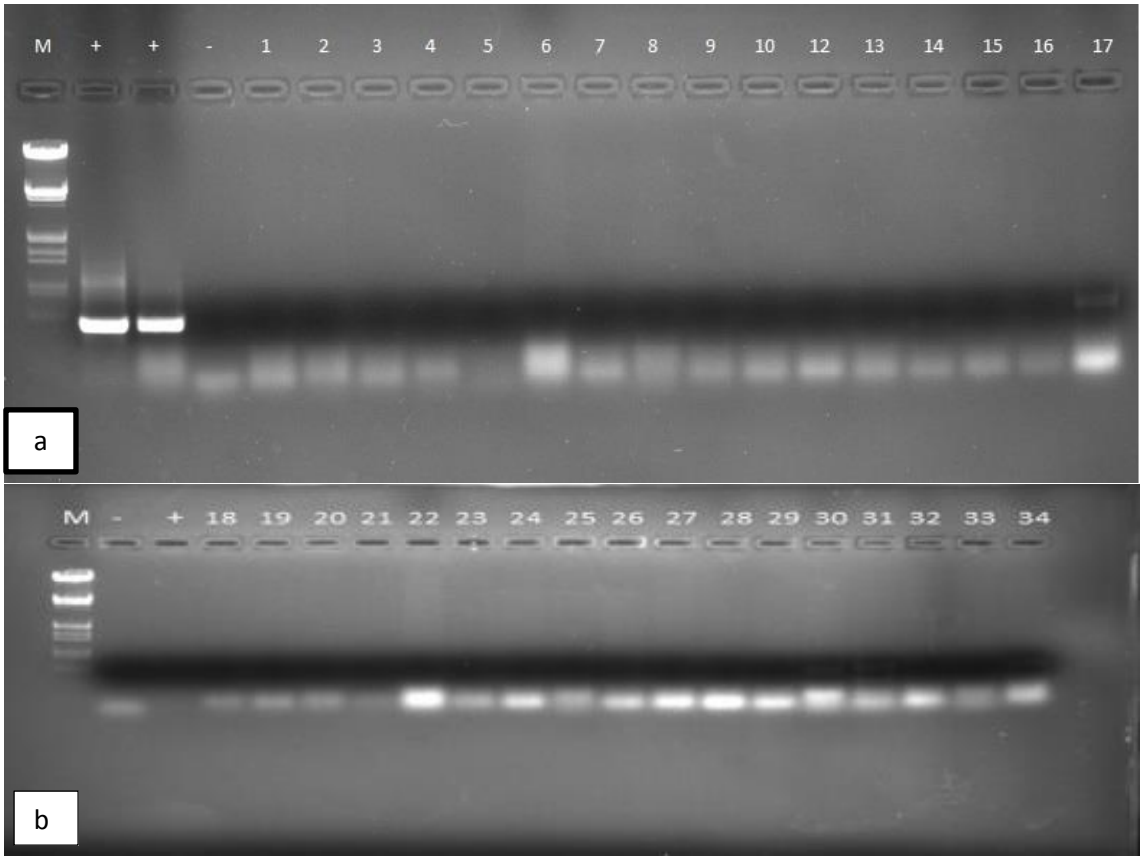
GDefV RNA-1 and RNA-2 nucleotide sequences gene motifs searchs were performed using Pfam database (http://pfam.xfam.org/) (Mistry et al., 2021). The Pfam database is a large collection of protein families; the DNA input was translated to proteins in six-frame translation and submits each of the six frames as a separate Pfam-A search. Finaly shows the results of searching input DNA sequence for matching Pfam-A families.

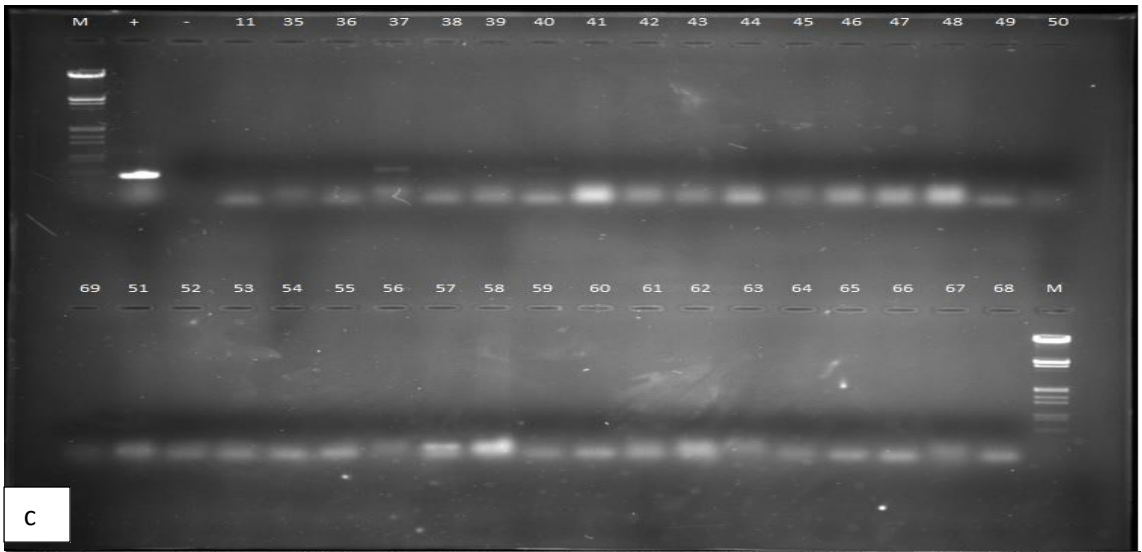
CHAPTER IV

RESULTS

4.1 Total Nucleic Acid (TNA) Extraction From Samples and PCR Reaction

In the survey carried out in vineyard of Central Anatolia, Turkey, in Nevşehir and Niğde, 21 GDefV isolates of 150 grapevine samples were detected (Figure 4.1). The overal infection rate was 14% among the tested grapevine samples. The infection rate was 18% in Niğde and 12% in Nevşehir (Table 4.1). The grapevine samples detected as infected with GDefV exhibited mainly chlorotic mottlling of leaves, and several of them had leaf veins swelling and leaf deformation symptoms related to virus infections (Table 4.2). All GDefV isolates were named according to number from 1 to 150. The jel image in Figure 4.1, (a) the 17 number, in (b) 30, 31, 34, in (c) 37, 38, 40, in (e) 92, 98, 100, 101, 102, 104, 106, 111, 112, 115, and in (j) 147, 150 isolates were showed the GDefV infection compared with our positive control. The 508 bp amplification DNA fragment was expected.

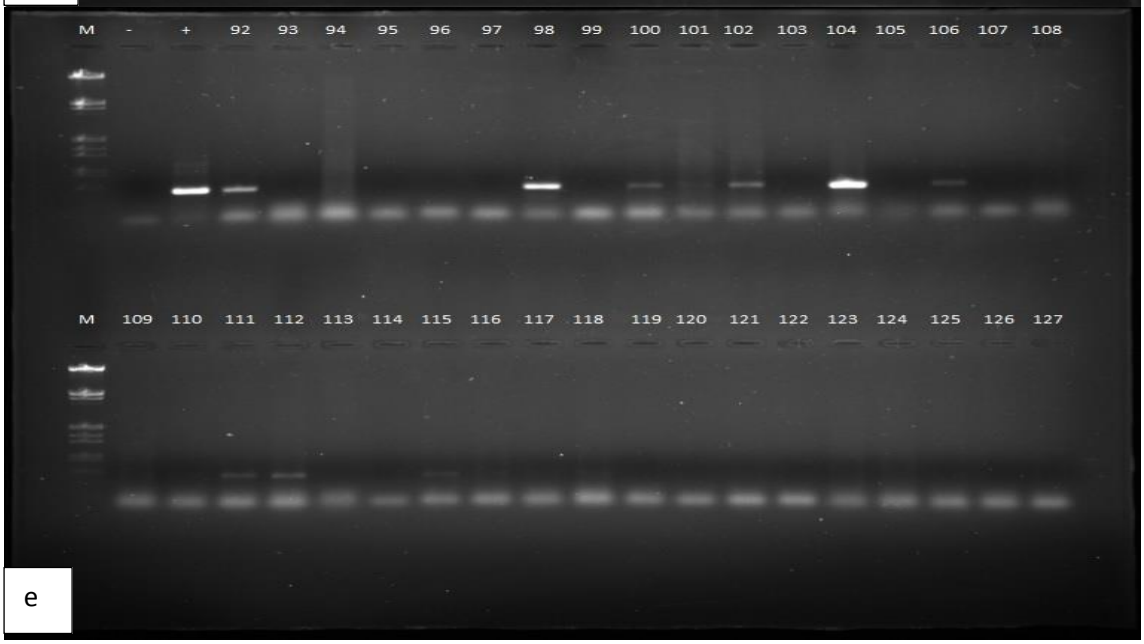



M + - 11 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
69 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 M

c


M + - 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91

d


M - + 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108
M 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127

e

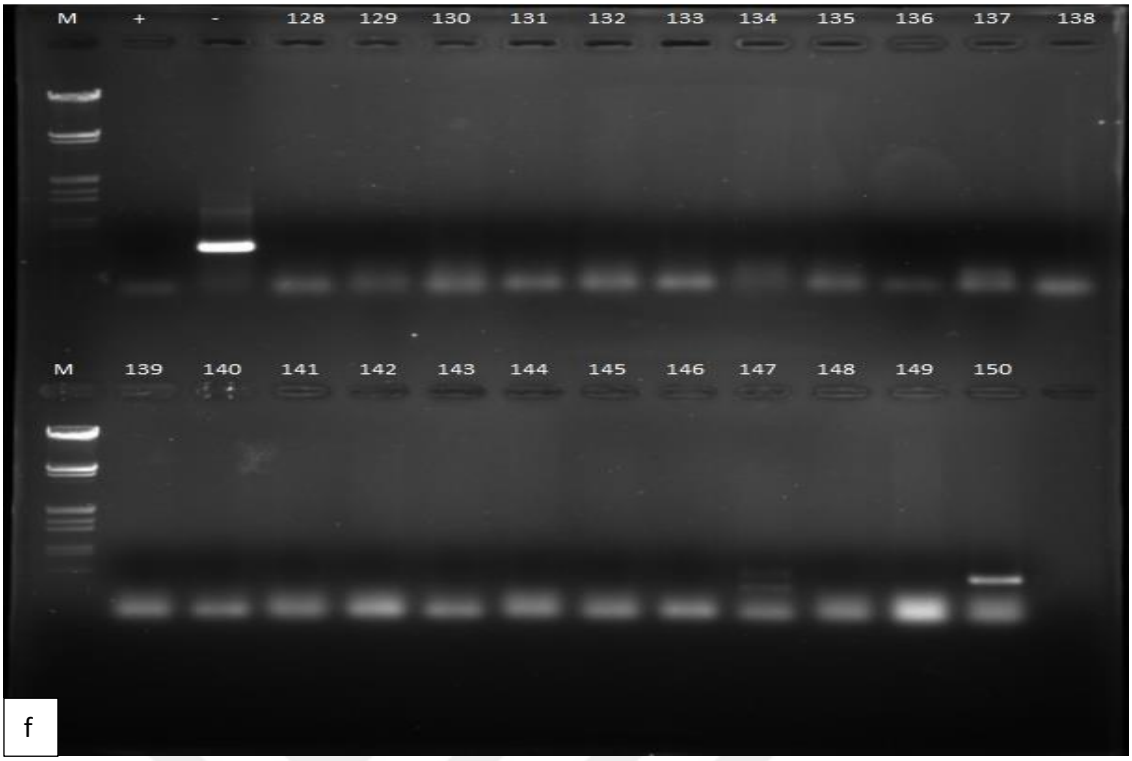


Figure 4.1. PCR agarose gel results of grapevine samples analysed using GDefV spesific primer pairs. M; *Eco RI/Hind* III DNA ladder. The '+' indicates positive control with 508 bp amplicon length. The '-' indicates negative water control

Table 4.1. Grapevine sample surveyed area and GDefV infected samples according to the collection sites

Province	District	Sample number	Infected Sample number	Infection rate (%)
Niğde	Altunhisar	10	2	20
	Bor	15	7	46.6
	Merkez	5	0	0
	Ulukışla	7	0	0
	Çamardı	8	0	0
	Çiftilik	5	0	0
	TOTAL	**50**	**9**	18
Nevşehir	Acıgöl	10	0	0
	Avanos	10	0	0
	Derinkuyu	10	0	0
	Gülşehir	7	0	0
	Hacibektaş	12	0	0
	Çökek	11	7	63.6
	Merkez	20	2	10
	Ürgüp	20	3	15
	TOTAL	**100**	**12**	**12**
General Total		**150**	**21**	**14**

Table 4.2. GDefV infected grapevine samples collection sites and symptoms

Collected province	Collected distict	Sample no	Observed symptom
Nevşehir	Center (Çat district)	**G17**	Leaf deformation
		G30	Mottlling of leaves
		G31	Mottlling of leaves
		G34	Mottlling of leaves
		G37	Mottlling of leaves
		G38	Mottlling of leaves
		G40	Mottlling of leaves
	Ürgüp (Çökek)	**G84**	Leaf deformation, chlorotic mottling, leaf veins swelling
		G86	Mottlling of leaves
	Ürgüp (Sarıhıdır)	**G92**	Chlorotic mottling
		G98	Leaf veins swelling
		G 100	Mottlling of leaves
Niğde	Center (Bahçeli)	**G 101**	Mottlling of leaves
		G 102	Mottlling of leaves
		G 104	Leaf deformation
		G 106	Mottlling of leaves
		G 111	Mottlling of leaves
		G 112	Mottlling of leaves
		G 115	Mottlling of leaves
	Bor	**G 147**	Mottlling of leaves
		G 150	Chlorotic mottling formation on the leaf

4.2 Inoculation

Mechanical inoculation is providing to us the information about infectivity of virus samples using local lesion host, and the information about if the virus transferred or propagate in plant or not. Four positive isolates of GDefV were used for 15 plants (isolates G92 for 3 tobacco plants, G104 for 3 tobacco plants, G17 for 3 tobacco plants, N176 for 3 tobacco plants-positive control) and for two plants were inoculated just buffer as mock (Figure 4.2). After inoculation, on 15th day we have checked our plants. After 15 days and later, there were no obvious symptoms on the plants, so no virus symptoms was observed on inoculated tobacco plants (Figure 4.3 a,b).

Figure 4.2. GDefV inoculated tobacco plants (*Nicotiana tabacum var. xanthi.)*

4.3 Sequence Analyses of Four Positive Isotates of GDefV

Totally four PCR GDefV positive amplicon 84, 86, 92,104 were sequenced. In the BLAST analyses, isolate-84 was paired with GDefV isolate deposited in NCBI with 90.93% identity and 92% query coverage (Fig 4.3 a). Isolate-86 was paired with NCBI GDefV isolate with 89.63% identity and 97% query coverage (Fig 4.3 b). Isolate-92 was paired with NCBI GDefV isolate with 95.95% identity and 96% query coverage (Fig 4.3 c). Isolate-104 was paired NCBI GDefV isolate with 92.27% identity and 90% query coverage (Fig 4.3 d).

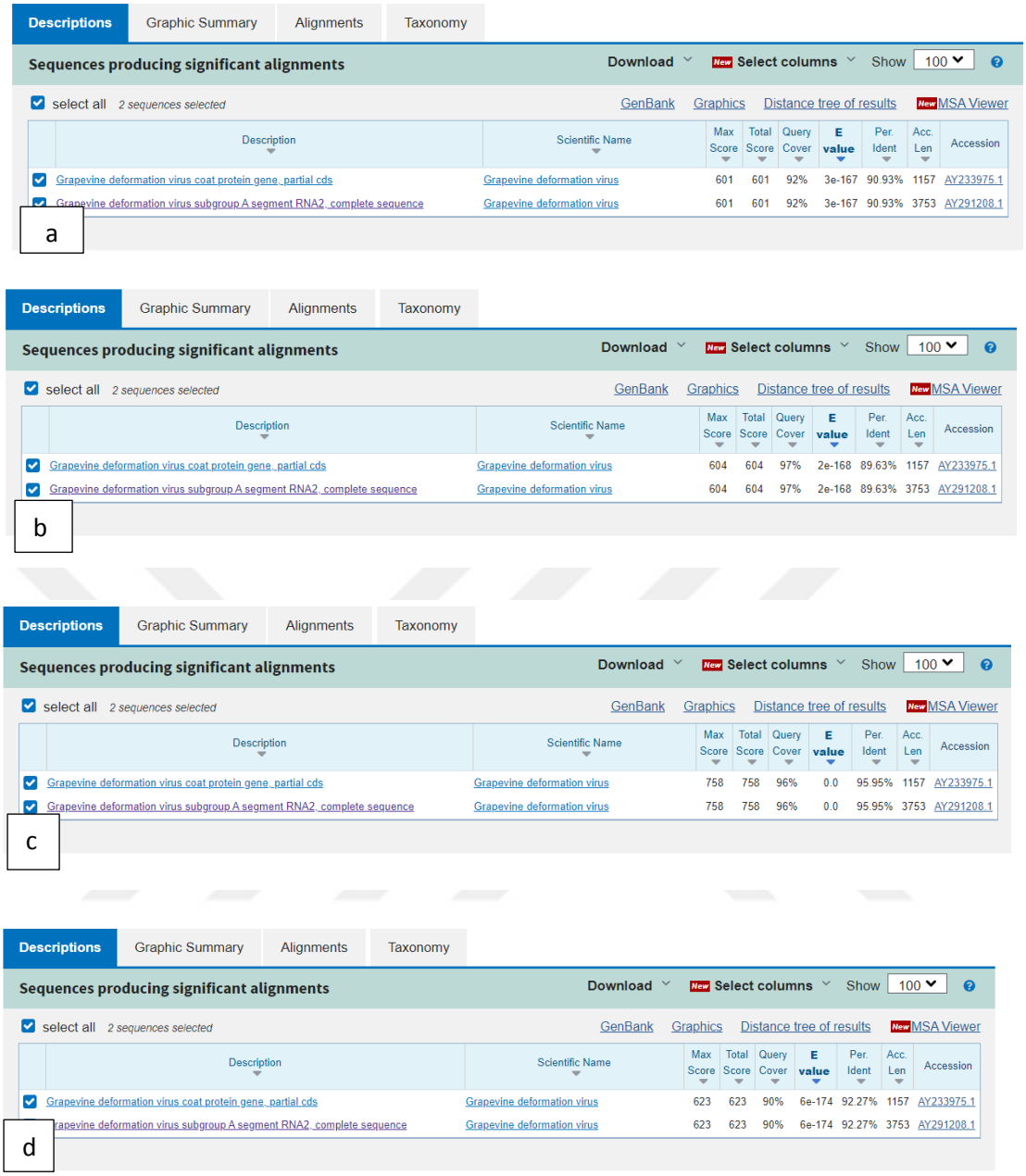


Figure 4.3. BLAST results of four GDefV isolate nucleotide sequences

4.4 Bioinformatic Analysis of High Throughput Sequencing Data

4.4.1 Analyses in CLC genomic workbench 11 without trimming

Before the sequence analysis using illumuna data, it is necessary to pair them for facilitating to eliminate repetitive sequence elements, as well as gene fusions and novel transcripts. Paired sequencing basically sequenced both ends of a fragment and generates alignable sequence with a high quality. So as a result of the sample105 paired

and merged reads, totally 131.024.652 reads were obtained. Totally 351 reads were mapped and 131.024.301 reads were not mapped in the analysis of sample105 paired and merged reads map to GDefV-RNA1 reference analysis. Totally 538 reads were mapped and 131.024.114 reads were not mapped in the analysis of sample105 paired and merged reads map to GDefV-RNA2 reference analysis.

As a result of the sample107 paired and merged reads, totally 131.640.142 reads were obtained. Totally 388 reads were mapped and 131.639.754 reads were not mapped in the analysis of sample107 paired and merged reads map to GDefV-RNA1 reference analysis. Totally 576 reads were mapped and 131.639.566 reads were not mapped in the analysis of sample107 paired and merged reads map to GDefV-RNA2 reference analysis.

The consensus sequence of sample 105 RNA1 included eigth fragments located on 16-1.570 nt, 1.593-3.031 nt, 3.041-3.576 nt, 3.585-4.120 nt, 4.138-5.132 nt, 5.141-5.480 nt, 5.494-6.842 nt, 7.054-7.413 nt of reference GDefV isolate RNA1 (NC-017939) (Table 4.3). The consensus seguence of sample 107 RNA1 included five fragments 14- 3.725 nt, 3.740-4.369 nt, 4.428-5.323 nt, 5.368-5.819 nt, 5.998-7.403 nt of reference GDefV isolate RNA1 (NC-017939), as well. The consensus seguence of sample 105 RNA2 included 16-3.749 nt and sample 107 RNA2 included 14-2.691 nt of reference GDefV isolate RNA2 (NC-017938) as one piece of fragment (Table 4.4).

4.4.2 Analyses in CLC genomic workbench 11 with trimming

As a result of the sample105 paired, merged and trimmed reads analysis, totally 129.296.158 reads were obtained. Totally 328 reads were mapped and 129.295.830 reads were not mapped in the analysis of sample105 paired, merged and trimmed reads map to GDefV-RNA1 reference analysis. Totally 520 reads were mapped and 129.295.638 reads were not mapped in the analysis of sample105 paired, merged and trimmed reads map to GDefV-RNA2 reference analysis.

As a result of the sample107 paired, merged and trimmed reads, totally 126.832.678 reads were obtained. Totally 363 reads were mapped and 129.832.315 reads were not mapped in the analysis of sample107 paired and merged reads map to GDefV-RNA1

reference analysis. Totally 558 reads were mapped and 126.832.120 reads were not mapped in the analysis of sample107 paired and merged reads map to GDefV-RNA2 reference analysis.

The consensus sequence of sample 105 RNA1 included ten fragments located on 16-1.535 nt, 1.580-3.554 nt, 3.563-4.098 nt, 4.116-4.369 nt, 4.473-4.851 nt, 5.006-5.101 nt, 5.110-5.448 nt, 5.463-5.852 nt, 5.901-6.809 nt, 7.081-7.383 nt of reference GDefV isolate RNA1 (NC-017939) (Table 4,3). The consensus seguence of sample 107 RNA1 included eight fragments 14-3.373 nt, 3.381-3.619 nt, 3.734-4.154 nt, 4.177-4.364 nt, 4.422-5.314 nt, 5.358-5.810 nt, 5.988-6.902 nt, 7.109-7.387 nt of reference GDefV isolate RNA1 (NC-017939), as well. The consensus sequence of sample 105 RNA2 included 16-3.749 nt and sample 107 RNA2 included 14-2.691 and 2.716-3.752 nt fragments of reference GDefV isolate RNA2 (NC-017938) (Table 4.4).

4.4.3 Analyses in geneious without trimming

The analysis of paired and merged reads of sample105 resulted 58.949.482 unmerged reads and 36.037.585 merged reads. The map to reference analysis performed using both merged and unmeged reads. Totally 588,945 reads were mapped and 94,987,067 reads were not mapped in the analysis of sample105 paired and merged reads map to GDefV-RNA1 reference analysis. Totally 589,115 reads were mapped and 94,987,067 reads were not mapped in the analysis of sample105 paired and merged reads map to GDefV-RNA2 reference analysis.

The analysis of paired and merged reads of sample107 resulted 30.713.892 unmerged reads and 50.963.125 merged reads. The map to reference analysis performed using both merged and unmerged reads. Totally 312 reads were mapped and 81.177.017 reads were not mapped in the analysis of sample107 paired and merged reads map to GDefV-RNA1 reference analysis. Totally 459 reads were mapped and 81,177,017 reads were not mapped in the analysis of sample107 paired and merged reads map to GDefV-RNA2 reference analysis.

The consensus sequence of sample 105 RNA1 included four fragments located on 16-1.573 nt, 1.596-4.125 nt, 4.132-6.847 nt, 7.043-7.418 nt of reference GDefV isolate

RNA1 (NC-017939) (Table 4.3). The consensus seguence of sample 107 RNA1 included five fragments 14-3.726 nt, 3.740-4.369 nt, 4.428-5.324 nt, 5.368-5.820 nt, 5.998-7.403 nt of reference GDefV isolate RNA1 (NC-017939), as well. The consensus sequence of sample 105 RNA2 included 16-3.758 nt and sample 107 RNA2 included 14-2.694 and 2.719-3759 nt fragments of reference GDefV isolate RNA2 (NC-017938) (Table 4.4).

4.4.4 Analyses in geneious with trimming

The analysis of paired, merged and trimmed reads of sample105 resulted 58.949.482 unmerged trimmed reads and 36.037.585 merged trimmed reads. The map to reference analysis performed using both merged and unmerged trimmed reads. Totally 588,895 reads were mapped and 94,952,138 reads were not mapped in the analysis of sample105 paired and merged reads map to GDefV-RNA1 reference analysis. Totally 589,066 reads were mapped and 94,952,138 reads were not mapped in the analysis of sample105 paired and merged reads map to GDefV-RNA2 reference analysis.

The analysis of paired, merged and trimmed reads of sample107 resulted 30.713.892 unmerged trimmed reads and 50.463.125 merged trimmed reads. The map to reference analysis performed using both paired and unpaired trimmed reads. Totally 291 reads were mapped and 81,115,704 reads were not mapped in the analysis of sample107 paired and merged reads map to GDefV-RNA1 reference analysis. Totally 460 reads were mapped and 81,115,995 reads were not mapped in the analysis of sample107 paired and merged reads map to GDefV-RNA2 reference analysis.

The consensus sequence of sample 105 RNA1 included eight fragments located on 16-1.547 nt, 1.567-2.997 nt, 3.008-3.543 nt, 3.552-4.087 nt, 4.105-5.091 nt, 5.099-5.437 nt, 5.452-6.799 nt, 7.012-7.373 nt of reference GDefV isolate RNA1 (NC-017939) (Table 4.3). The consensus seguence of sample 107 RNA1 included four fragments 14-5.321 nt, 5.365-5.817 nt, 5.995-6.909 nt, 7.079-7.399 nt of reference GDefV isolate RNA1 (NC-017939), as well. The consensus sequence of sample 105 RNA2 included 16-3.750 nt and sample 107 RNA2 included 14-2.681 and 2.706-3.746 nt fragments of reference GDefV isolate RNA2 (NC-017938) (Table 4.4).

Table 4.3. GDefV RNA1 consensus sequences with trimmed and untrimmed reads analyses

Sample number	**Sample 105 RNA1**			**Sample 107 RNA1**		
Total reads	131.024.652			131.640.142		
Software	**CLC**	**Geneious Prime**		**CLC**	**Geneious Prime**	
Number of Reads Without Trimming, Paired/Merged	351	Mapped	588.945	388	mapped	312
		Unmapped	94.987.067		unmapped	81.177.017
Number of Reads with Trimming, Paired/Merged	328	Mapped	588.895	363	mapped	291
		unmapped	94.952.138		unmapped	81.115.704
Consensus sequence lengths- Without Trimming (nt)	16-1.570 nt 1.593-3.031 nt 3.041-3.576 nt 3.585-4.120 nt 4.138-5.132 nt 5.141-5.480 nt 5.494-6.842 nt 7.054-7.413 nt	16-1.573 nt 1.596-4.125 nt 4.132-6.847 nt 7.043-7.418 nt		14- 3.725 nt 3.740-4.369 nt 4.428-5.323 nt 5.368-5.819 nt 5.998-7.403 nt	14-3.726 nt 3.740-4.369 nt 4.428-5.324 nt 5.368-5.820 nt 5.998-7.403 nt	
Consensus sequence lengths- Trimming (nt)	16-1.535 nt 1.580-3.554 nt 3.563-4.098 nt 4.116-4.369 nt 4.473-4.851 nt 5.006-5.101 nt 5.110-5.448 nt 5.463-5.852 nt 5.901-6.809 nt 7.081-7.383 nt	16-1.547 nt 1.567-2.997 nt 3.008-3.543 nt 3.552-4.087 nt 4.105-5.091 nt 5.099-5.437 nt 5.452-6.799 nt 7.012-7.373 nt		14-3.373 nt 3.381-3.619 nt 3.734-4.154 nt 4.177-4.364 nt 4.422-5.314 nt 5.358-5.810 nt 5.988-6.902 nt 7.109-7.387 nt	14-5.321nt 5.365-5.817 nt 5.995-6.909 nt 7.079-7.399 nt	

Table 4.4. GDefV RNA2 consensus sequences with trimmed and untrimmed reads analyses

Sample number	**Sample 105 RNA2**			**Sample 107 RNA2**		
Total reads	131.024.652			131.640.142		
Software	**CLC**	**Geneious Prime**		**CLC**	**Geneios Prime**	
Paired/Merged	538	Mapped	589.115	576	mapped	459
		unmapped	94.987.067		unmapped	81.177.017
Trimming Paired/Merged	520	Mapped	589.066	558	mapped	460
		Unmapped	94.952.138		unmapped	81.115.995
Consensus sequence lengths- Without Trimming (nt)	16-3.749nt	16-3.749 nt		14-2.691 nt	14-2.694 nt 2.719-3.759 nt	
Consensus sequence lengths- Trimming (nt)	16-3.749 nt	16-3.750 nt		14-2.691nt 2.716-3.752 nt	14-2.681 nt 2.719-3.759 nt	

4.4.5 Assembly of consesus sequences retrived from CLC geneomic workbench and geneious prime

All trimmed and not trimmed consensus sequences from CLC were imported to the Geneious Prime software. Next, the consensus sequences of trimmed and not trimmed were assembled among themselves using Geneious Prime sofware. Finaly trimmed and

not trimmed consensus sequences were also assembled to provide final RNA1 and RNA2 segments. The provided consensus sequences (RNA1 and RNA2) were used for further genomic analysis of GDefV.

The consensus sequence of sample 105 GDefV RNA1 included 7408 nt sequences located on 1-7413 nt of reference GDefV isolate RNA1 (NC-017939). The consensus seguence of sample 107 RNA1 included 7395 nt sequences located on 14-7413 nt of reference GDefV isolate RNA1 (NC-017939), as well. The consensus sequence of sample 105 GDefV RNA2 included 3749 nt and sample 107 GDefV RNA2 included 3743 nt sequences, covering completely the 3754 nt sequences of reference GDefV isolate RNA2 (NC-017938) (Table 4.5).

Table 4.5. GDefV RNA consensus sequences as a result of bioinformatics analyses

Sample number	**Sample 105**		**Sample 107**	
Total reads	131.024.652		131.640.142	
RNA no	RNA1	RNA2	RNA1	RNA2
GDefV Consensus sequence length (nt)	7408	3749	14-80 84-5336 5380-5832 610-7395	14-3695 3729-3743

4.5 Phytogenetic Analysis

The evolutionary history was inferred by utilizing the Maximum Likelihood technique premised on the General Time Reversible model. The Initial tree(s) explored in the heuristic search were automatically generated by employing BioNJ and Neighbor-Join algorithmic method. This gave an estimated matrix of pairwise distances using the Maximum Composite Likelihood (MCL) strategic approach, and then choosing the topology with superior log likelihood value. A discrete Gamma distribution was employed in evolutionary rate model to determine the differences among the sites (4 categories (+G, parameter = 1.1575) (Figure 4.4). In the phylogenetic tree our GDefV isolates were clustered with the GDefV isolates deposited in genbank (NC_017938). The 105 and 107 GDefV isolate sequences were identical 96% to each other while sharing the identity of 91% and 90% with the reference isolate GDefV (NC_017938),

respectively (Figure 4.5, Table 4.5). The isolates 105 and 107 identity varied 69-73% with GFLV and 68-71% with ArMV isolates nucleotide sequences.

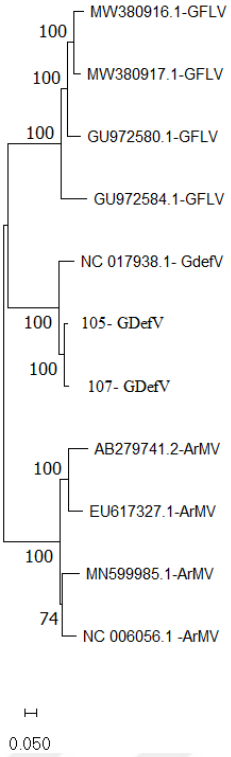


Figure 4.4. Phylogenetic tree of RNA-2 of GDefV, ArMV and GFLV nucleotide sequences

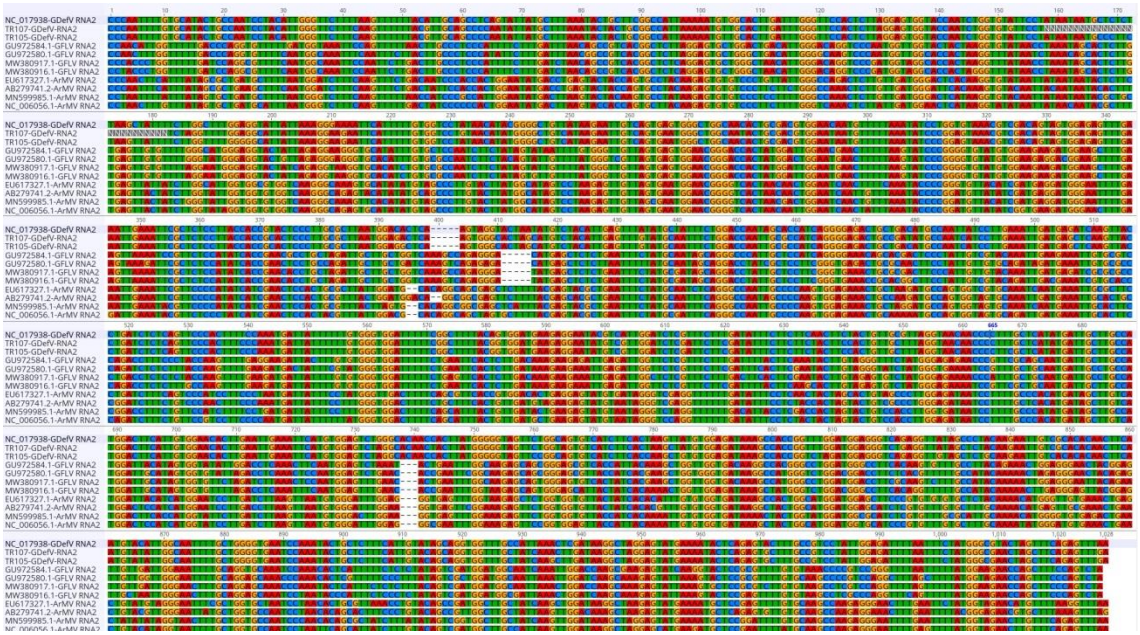


Figure 4.5. RNA-2 coat protein region nucleotide sequence alignment of GDefV, GFLV and ArMV isolates

Table 4.6. Identity table of GFLV, ArMV, anf GDefV isolates

	GdefV RNA2-NC_017938.1	105 RNA2 trimmed-not trimmed consensus Nucleotide alignment	107 RNA2 trimmed-not trimmed consensus Nucleotide alignment	GFLV RNA2-GU972584.1	GFLV RNA2-GU972580.1	GFLV RNA2-MW380916.1	GFLV RNA2-MW380917.1	ArMV RNA2-AB279741.2	ArMV RNA2-EU617327.1	ArMV RNA2-MN599985.1	ArMV RNA2-NC_006056.1
GdefV RNA2-NC_017938.1		**91%**	**90%**	69%	72%	71%	72%	68%	71%	69%	70%
105 RNA2 trimmed-not trimmed consensus	**91%**		**96%**	70%	73%	72%	73%	69%	71%	70%	70%
107 RNA2 trimmed-not trimmed consensus	**90%**	**96%**		70%	72%	71%	72%	69%	71%	70%	70%
GFLV RNA2-GU972584.1	69%	70%	70%		85%	85%	86%	69%	71%	73%	73%
GFLV RNA2-GU972580.1	72%	73%	72%	85%		89%	89%	66%	68%	69%	69%
GFLV RNA2-MW380916.1	71%	72%	71%	85%	89%		92%	66%	68%	69%	69%
GFLV RNA2-MW380917.1	72%	73%	72%	86%	89%	92%		66%	68%	69%	70%
ArMV RNA2-AB279741.2	68%	69%	69%	69%	66%	66%	66%		84%	82%	83%
ArMV RNA2-EU617327.1	71%	71%	71%	71%	68%	68%	68%	84%		83%	84%
ArMV RNA2-MN599985.1	69%	70%	70%	73%	69%	69%	69%	82%	83%		87%
ArMV RNA2-NC_006056.1	70%	70%	70%	73%	69%	69%	70%	83%	84%	87%	

4.6 GDefV RNA2 Gene Motifs Search

Proteins generally have one or more functional region, which are commonly termed 'domains'. In pfam search we provided Nepovirus subgroup A polyprotein (NeA_P2 (PF12312)), Nepovirus coat protein, N-terminal domain (Nepo_coat_N (PF03689)), Nepovirus coat protein, central domain (Nepo_coat (PF03391) and Nepovirus coat protein, C-terminal domain (Nepo_coat_C (PF03688)) protein domains in sample 105 and 107 RNA2 consensus sequences validating the GDefV consensus sequences (Figure 4.6). The 105 and 107 GDefV isolate amino acid sequences were identical 97% to each other while sharing the identity of 93% and 91% with the reference isolate GDefV (NC_017938), respectively (Figure 4.7). However, the helicase and RNA dependent RNA polymerase (RdRp) domains on RNA1 sequences of GDefV were not found on the same frame, and not the whole RdRp domain indicating the necessity of the validation of RNA-1 consensus sequences.

Figure 4.6. Pfam search results of GDefV RNA-2 genoms of 105 (above) and 107 (below) isolates. Nepovirus subgroup A polyprotein (NeA_P2 (PF12312)), Nepovirus coat protein, N-terminal domain (Nepo_coat_N (PF03689)), Nepovirus coat protein, central domain (Nepo_coat (PF03391) and Nepovirus coat protein, C-terminal domain (Nepo_coat_C (PF03688)) protein domains

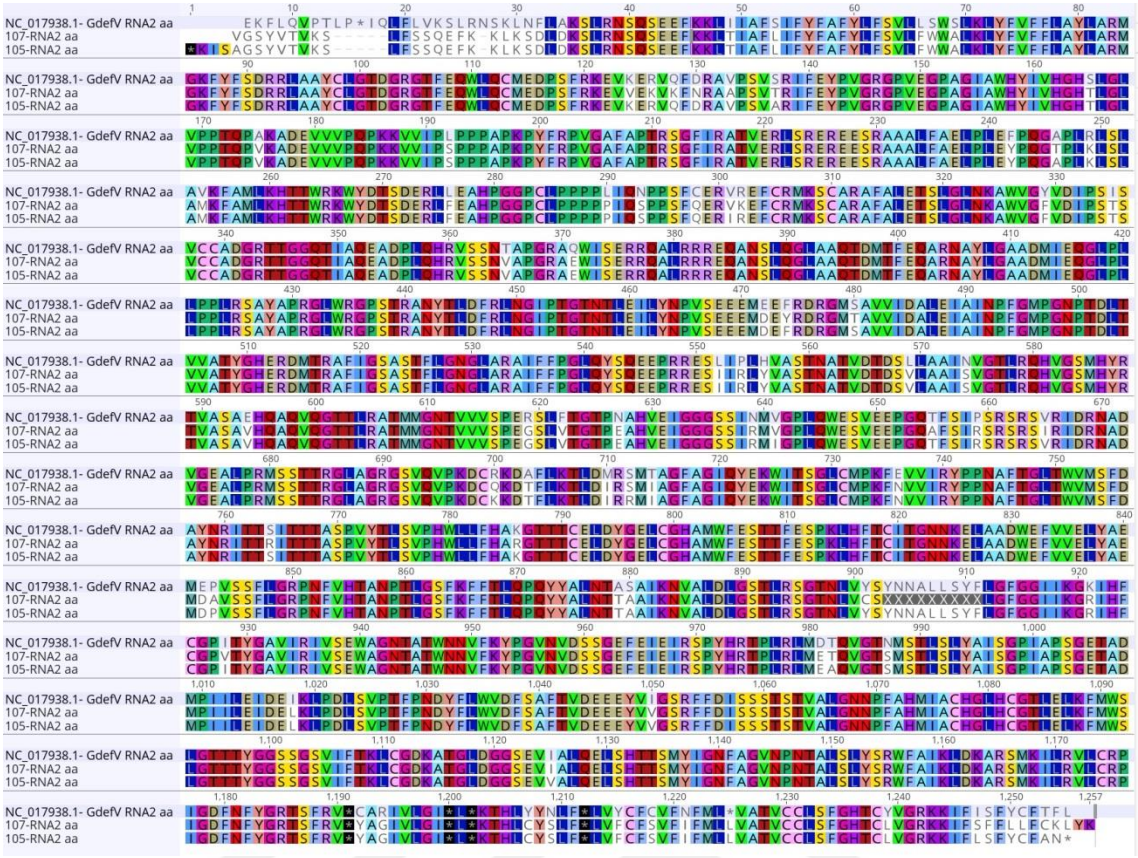


Figure 4.7 GDefV RNA-2 comlete genome amino acid multiple alignment results

CHAPTER V

DISCUSSION

We have collected samples in the summer in June 2020 according to symptoms such as malformation, asymmetrical, puckered, discoloration and vein banding of leave. As the virus could be masked in summer time, June is not suitable time to collect the sample. According to (Meng and Martelli, 2017) they indicated that the different symptoms could be occur in different time of season. For example;

Infectious malformations could be appearance with a seriouse leave size deformation such as malformation, puckered, deep lobes and with open petiolar sinuses and usually with a chlorotic mottling in the old leaves. Moreover, not just leave but the shoots are also dramatically influensed and showed significant symptoms like malformation, double nodes appearing, branching of abnormality fasciations, short internodes, and zigzag growth. Berries ripen irregularly and small-sized and set poorly, bunches are smaller and fewer than normal. Although some masking may occur in summer, foliar symptoms overearly in the spring and continue all through the vegetative season.

Yellow mosaic when stains occur early in the spring and may influence every single vegetative part (tendrils, leaves, shoots,) and inflorescences. Chromatic adjustments of the leaves contrast from a couple of dispersed yellow spots, once in a while as lines or rings, to broad mottling of the interveinal regions, to entirely yellowing. The foliage and shoots show nearly nothing, contortion, yet packs are not many and little. With expanded the temperatures during summer, the canopy develops a normal green color and the yellowing fades away.

Vein banding is a third syndrome related inside all Nepoviruses presence. The manifestations seem like chrome yellow bits along the principle veins of mature leaves and afterward attack the interveinal regions. In spite of the initial two syndromes, this sort of staining shows up in mid to late summer in a set number of leaves with little or no malformation.

As a result of our GDefV survey, yellow mosaics and vein banding symptoms on GDefV infected vines were observed mainly, but not infectious malformations symptoms. Based on this knowledge, late in the spring is the best time to collection of the samples of grapevine for survey of the virus. It was concluded that June in which month we have collected our samples is being not a suitable period. As a suggestion, for a detection of GDefV the best period could be made in late of spring.

GDefV infection rate was estimated as 18% in Niğde and 12% in Nevşehir which are higher than the previous research reported as 1,43 % in Nevşehir and 1,2 % in Turkey among the samples collected during the years 2015-2016 (Ulubaş Serçe et al. 2020). On the other hand, in a survey performed in Adiyaman, Şanlıurfa GDefV was detected by ELISA in average infection 3.4% (Çiğşar et al., 2003). These infection rate differences could be explained as increase of virus incidance at the same locations and/or the sensitivity of the selected test chemicals in this reseach. In our reseach, FastStart SYBR Green master mix, which has haigher amplification power, used instead of Taq DNA ploymerase (Thermo Fisher Scientific). This PCR approch could be another reason to visulaize the amplified GDefV fragments.

BLAST analysis of the four amplicon sequences resulted 90.93%, 89.63%, 95.95% and 92.27% identity for the isolate-84, isolate 86, isolate-92 and isolate 104, respectively with only available GDefV isolate in NCBI. These results validated the PCR amplification for GDefV in our research.

The tranmission of GDefV to *N. tabacum* cv. *Xanthi* species with mechanical inoculation did not occur for multiplication of the virus. It is reported that *Chenopodium* species exhibited chlorotic and necrotic local lesions followed by systematic mottling and deformation symptoms after mechanical inoculation with GDefV (isolate-N66) (Çığşar 2003). The isolate-N66 was latent in tobacco plants such as *N. benthamiana, N. cavicola, N. clevelandii, N. glutinosa* and exhibited clorotic local symptoms and vein banding systemicaly in *N. occidentalis* species. Isolate-N66 induced symptoms in *C. amaranticolor* similar those elicited by GFLV and ArMV, but not with several other nepoviruses known to infect grapevine (Martelli 1993). In our case, it is proved that *N. tabacum* cv. *Xanthi* was not a usefull indicator for GDefV. There are many research where the *Nicotiana* species and cultivars were used in mechanical inoculation. For

example, tomato yellow leaf curl virus, cucumber mosaic virus, grapevine virus A and tobacco mosaic virus were inoculated to *N. benthamiana* (Edelbaum et al. 2009). Tobacco cv *Xanthi* was found to be a highly sensitive for the detection of ToMV showing local lesion (Hadas et al. 2004).

There are many bioinformatics tools and facilitates big biological data anlaysis. In this research, CLC Geneomic workbench 11 and Geneious Prime licenced softwares were utilized due to user friendly PC interface who are not experinced on the operating systems. There main pipelines in bioinformatics analysis. Before sequencing data analysis, it is important to trim read ends to incorrect low quality calls at read ends. This application will potentially prevent improper assembly, increase the computation speet and time required to perform assembly. In CLC software, trimming was performed automatically, while BBDuk pluggin was used for this purpose in Geneiouse software. In trimming application it can be performed identification of adapters using presets for Illumina adapters, triming ends based on quality, elimination adapters based on paired read overhangs and discarding short reads (and associated pair mate). By the comparison of trimmed data from CLC and Geneious software and also not trimmed data between the softwares resulted approximately the same results. CLC software resulted more gap while Geneious software provided less gap and extended contigs belong to RNA1 and RNA2. The assembly of trimmed with not trimmed data resulted consensus with a high quality, and needed just a little manually cleaning. In both approach, it could be completed the one gaps which is not exist the other of one. The ORFs of GDefV RNA1 genome were not established completely, while the ORFs of RNA2 completed in the final consensus. As aresult, the RNA1 genome analysis should be performed in detail by application of different parameters in softwares. Beside this, the gap regions of RNA1 genome can be completed by amplification with designed specific primers targetting that region and sequencing again by sanger method.

Identity matrix and phylogenetic tree was proved that virus isolates in sample 105 and 107 were GDeFV which share a mosaic genome structure with ArMV and GFLV. This study demonstrated that the GDefV is belonging to *Nepovirus* is molecularly different from ArMV and GFLV. Moreover, it might be hypothesized that the result of bioinformatics analyses and phylogenetic analyses strongly support that we have archive our aim to complete whole genome of GDefV but just with a RNA2.

Since early, fast detection and management is exclusively important in plant disease decision systems, the knowledge on the genomic variation of viral agents helps to develop and improve detection and identification systems. The main goal of work were to research on complete genome variation of GDefV isolates in Turkey which is belong to Nepoviruses. The evaluated GDefV genomes in this research helped us to understand the variation among GDefV isolates. This variation has important effects for the detection of the virus and the reactions of the vine cultivars agains to virus.

CHAPTER VI

CONCLUSION AND FUTURE ASPECTS

As a result of this study, significant data about the prevalence of GDefV and its genomic variation were provided and evaluated. The best time for sampling of grapevine for detection of GDefV was late spring, the fact that the detection of the virus could be made late in the spring in this research. Tobacco species *N. tabacum* cv. *Xanthi* was not recommended for the propogation of GDefV in test plants and strongly advised using higher amplification power capacity DNA polymerase system.

As a final result of comparison of CLC Genomic Workbench 11 and Geneiouse Prime for the construction of comlete genome of GDefV, the used approaches provided in Geneious software less gap and extended contigs to generate almost complete genome. For future research, different parameters of the same software could be aplied and the resulted contigs assembled or also contigs produced by different parameters coming from different softwares could be assembled.

For a future studies, analysing more isolates' complete genomes would be ensure to elucidate genome variability of GDefV.

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SUPPLEMENTS

1. Sample 105 sequences:

>GDefV 105 RNA-1 genom nucleic acid sequences:
ATGAAAATTTTCTACAGGTTCTTACGTTACCGTGAAGTCAGCTTTTCTCCAGCCAAGAGTTTA
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>GDefV 105 RNA-2 genom complete nucleic acid sequences:
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>GDefV 105 RNA-2 complete genom amino acid sequences:
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2. Sample 107 sequences:

>GDefV 107 RNA-1 genom nucleic acid sequences:
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>GDefV 107 RNA-2 genom nucleic acid sequences:
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CURRICULUM VITAE

Sabina Mamedova was born in . She completed her preliminary and middle and . In she had the privilage to further her education in the department (English Option), at the Ömer Halisdemir University. In the first year, she completed the English Prep-class than she continued in the faculty. In this period, she had the opportunity to continue her educational training at under Programme put in place through Erasmus initiatives. After completion of the bachelor degree, she pursued a Msc. with thesis programme (English option) in the Plant Production and Technologies department, Graduate School of Natural and Applied Sciences at the Ömer Halisdemir University. In the course of her master period she focused on "Research towards elucidation of the complete genome of grapevine deformation virus (GDefV) isolates in Turkey" She successfully completed this programme with the supervisory role played by Prof. Dr. Çiğdem ULUBAŞ SERÇE.