T.R.
VAN YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PROTECTION

INVESTIGATION AND MOLECULAR CHARACTERIZATION OF BARLEY YELLOW DWARF VIRUSES IN WHEAT FIELDS (*Triticum aestivum L.*) OF DIYARBAKIR PROVINCE BY MULTIPLEX RT- PCR

MASTER THESIS

PREPARED BY: Nawzad Omer HASSAN
FIRST SUPERVISOR: Assist. Prof. Dr. Mustafa USTA
SECOND SUPERVISOR: Prof. Dr. Emad M. AL- MAAROOF

VAN-2018

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ACCEPTANCE and APPROVAL PAGE

This thesis entitled "**Investigation and molecular characterization of Barley yellow dwarf viruses in wheat fields (*Triticum aestivum* L.) of Diyarbakir province by multiplex RT-PCR**" presented by **Nawzad OMER HASSAN**. under supervision of Assist. Prof. Dr. Mustafa USTA in the department of Plant Protection and Second Supervisor Prof. Dr. Emad M. Al-Maaroof in the department of Crop Science Sluaimani University has been accepted as a M. Sc. / Ph.D. thesis according to Legislations of Graduate Higher Education on 11th Jan 2018 with unanimity / majority of votes members of jury.

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This thesis has been approved by the committee of The Institute of Natural and Applied Science on ...13.../..01.../.2018.. with decision number 2018/19-1

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Director of Institute

THESIS STATEMENT

All the information's presented in the thesis were obtained according to the ethical behaviors and academic rules frame. And also, all kinds of statement and source of information that does not belong to me in this work prepared in accordance with the rules of thesies were cited to the source of information absolutely.

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Nawzad Omer HASSAN

ABSTRACT

INVESTIGATION AND MOLECULAR CHARACTERIZATION of BARLEY YELLOW DWARF VIRUSES IN WHEAT FIELDS (*Triticum Aestivum L.*) OF DIYARBAKIR PROVINCE BY MULTIPLEX RT- PCR

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In order to ascertain the presence of *Barley/Cereal yellow dwarf viruses* (BYDV-PAV, MAV, SGV, RMV, and CYDV-RPV), in wheat fields, a survey was conducted in the year of 2016 in Diyarbakir province. Presence of BYDV viruses (PAV, MAV, SGV, RMV, and CYDV-RPV) was investigated by using multiplex RT-PCR and RT-PCR methods. A total of 365 wheat leaf samples were randomly collected from wheat fields of Diyarbakir province. As a result of tests the wheat samples were found to be infected by BYDV-PAV, BYDV-SGV and CYDV-RPV with the percentage of (3.5 %), (2.4 %) and (1.3 %), respectively. Detected mix infections of BYDV-PAV+BYDV-SGV, BYDV-PAV+ CYDV-RPV, BYDV-SGV+ CYDV–RPV, and BYDV-PAV+ BYDV-SGV+ CYDV-RPV were 2.2 %, 0.8 %, 0.8 %, and 0.8 % respectively.

A virus isolate was selected randomly and its coat protein gene was characterized. Complete coat protein gene of BYDV-PAV virus (Genbank accession no. KX774424) was cloned and sequenced with the universal primers. BYDV-PAV Diyarbakir isolate was compared with 21 isolates from other regions in the world. The nucleotide sequence of BYDV-PAV Diyarbakir isolate was showed 81.43 % - 98.3 % similarities with the world isolates.

The present study is the first report for documentation of BYDV-PAV, BYDV-SGV and CYDV-RPV viruses in wheat fields in Diyarbakir province in Turkey.

Keywords: Diyarbakır survey wheat, Characterization, Cloning, Multiplex RT-PCR, Turkey, Viral diseases.

ÖZET

DİYARBAKIR İLİ BUĞDAY (*TRİTİCUM AESTİVUM* L.) TARLALARINDA ARPA SARI CÜCELİK VİRÜSLERİ'NİN MULTİPLEKS RT-PCR İLE ARAŞTIRILMASI VE MOLEKÜLER KARAKTERİZASYONU

HASSAN, Nawzad Omer
Yüksek Lisans Tezi, Bitki Koruma Ana Bilim Dalı
Tez Danışmanı: Dr. Öğr. Üyesi. Mustafa USTA
İkinci Danışman:Prof. Emad M. Al-MAAROOF
Ocak, 2018, 71 sayfa

Diyarbakır ili buğday alanlarında Arpa sarı cücelik virüslerini (*Barley/Cereal yellow dwarf viruses:* BYDV-PAV, MAV, SGV, RMV ve CYDV-RPV) belirlemek amacı ile 2016 yılında survey çalışmaları yürütülmüştür. BYDV (PAV, MAV, SGV, RMV) ve CYDV-RPV'nin varlığını araştırmak için multipleks RT-PCR ve RT-PCR yöntemleri kullanılmıştır. Diyarbakır ilinde varlığı tespit edilen virüslerde BYDV-PAV'ın kılıf protein geninin moleküler karakterizasyonuda da gerçekleştirilmiştir. Bölgedeki buğday tarlalarından rastgele 365 adet buğday yaprak örneği toplanmıştır. Testlenen 365 buğday örneğinden 13' ünün BYDV-PAV (% 3.5), 9'unun BYDV-SGV (% 2.4), 5'inin CYDV-RPV (% 1.3) ile bulaşık oldukları tespit edilmiştir.

Tespit edilen BYDV-PAV virüs türünden bir izolat rasgele seçilerek kılıf protein geni karakterize edilmiştir. BYDV-PAV (Gen bankası Ulaşım No. KX774424) virüsünün kılıf protein geninin tamamı klonlanarak üniversal primerler ile DNA dizilemesi gerçekleştirilmiştir.

BYDV-PAV Diyarbakır izolatının dünyadaki 21 farklı izolatın nükleik asitleri ile % 81.43-98.2 % arasında değişen oranlarda benzerlik gösterdiği belirlenmiştir.

Bu çalışma ile BYDV-PAV, BYDV-SGV, CYDV-RPV virüsleri Diyarbakır ilindeki buğday alanlarında ilk defa rapor edilmiştir.

Anahtar kelimeler: Diyarbakır buğday Survey, Karakterizasyon, klonlama, Multipleks RT-PCR, Türkiye,

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Firstly, I am thankful to the almighty Allah who showed me rays of light to complete my scientific project successfully.

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2018

Nawzad Omer HASSAN

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SYMBOLS AND ABBREVIATIONS

Some symbols and abbreviations used in this study are presented below, along with descriptions.

Symbols	Description
Mg	Microgram
µL	Microliter
µM	Micromole
Da	Dalton
M	Minute
g	Gram
kDa	Kilo Dalton
Kg	Kilogram
M	Molar
Kb	kilobase pair
mg/ml	Milligram/milliliter
mL	Milliliter
mM	Millimolar
MW	Molecular weight
Nm	Nanometer
Mol	Moles
U	Unit
V	Volt
v/v	Volume/volume
w/v	Weight/volume
˚C	Centigrade

Abbreviation	Description
A	Adenine
Aa	Amino acid
BMV	*Brome mosaic brome virus*
The USA	The United States of America
B/CYDV	*Barley/Cereal yellow dwarf virus*
BaMMV	*Barley mild mosaic virus*
BaYMV	*Barley yellow mosaic virus*
BSMV	*Barley stripe mosaic hordeivirus*
BYD	*Barley yellow dwarf*
BYDV	*Barley yellow dwarf luteoviruses*
BYDV-GAV	*Barley yellow dwarf virus-GAV*
BYDV-GPV	*Barley yellow dwarf virus-GPV*
BYDV-MAV	*Barley yellow dwarf virus-MAV*
BYDV-PAV	*Barley yellow dwarf virus-PAV*
BYDV-RMV	*Barley yellow dwarf virus-RMV*
BYDV-SGV	*Barley yellow dwarf virus-SGV*
BYSMV	*Barley yellow streak mosaic virus*
BMV	*Brom mosaic bromovirus*
C	Cytosine
cDNA	Complementary nucleic acids deoxyribo
CYDV-RPS	*Cereal yellow dwarf virus-RPS*
CP	Coat protein
CYDV-RPV	*Cereal yellow dwarf polerovirus-RPV*
DAS-ELISA	Double Antibody Sandwich –ELISA
dATP	Demoski adenosine tri phosphate
DNA	Deoxyribonucleic acid
DNase	Deoxyribonucleic
dNTP	Deoxy Nucleoside Triphosphate
EDTA	Ethylene diamine tetraacetic acid

ELISA	Enzyme-Linked Immunosorbent Assay
EMBL	Europe Molecular Biology Laboratory
EtOH	Ethanol
FAO	Food and Agriculture Organization Of the United Nations
HCI	Hydrochloric acid
IC-RT-PCR	Immune Capture RT-PCR
ITS1	Internal Transcript Spacer
KH2PO4	Potassium dihydrogen phosphate
MDMV	Maize dwarf mosaic virus
MP	Movement protein
MSpV	Maize stripe virus
NaI	Sodium iodide
NaOAc	Sodium acetate
Nt	Nucleotides
OGSV	Oat golden stripe virus
OMV	Oat mosaic virus
ONMV	Oat necrotic mottle virus
ORF	Open reading frame
PCR	Polymerase chain reaction
PZR	Polymerase chain reaction
rDNA	Ribosomal DNA
RFLP	Restriction fragment length polymorphism
RNA	RiboNucleic Acid
RNAse	Ribo Nuclease Acid enzyme
Rpm	revolutions per minute
RT	Reverse Transcriptase
RTP	Read Through Protein
RT–PCR	Reverse Transcriptase -Polymerase Chain Reaction

SBCMV	*Soil-borne cereal mosaic virus*
SBWMV	*Soil-borne wheat mosaic fur virus*
SDS	Sodium dodecyl sulfate
sgRNA	Subgenomic RNA
ssRNA	Single-stranded ribonucleic acid
T	Tim's
TAE	Tris-Acetate EDTA
Taq DNA polymerase	Thermos table DNA polymerase enzyme
TAS-ELISA	Triple antibody sandwich-ELISA
TBE	Tris-Borate EDTA
TNA	Total Nucleic Acid
TUĐK	Turkey Statistical Institute
U	Uracil
WDV	*Wheat dwarf virus*
WSMV	*Wheat streak mosaic tiritimovirus*
WSSMV	*Wheat spindle streak mosaic bymovirus*
WYMV	*Wheat yellow mosaic virus*
K	Potassium
Mg	Magnesium

1. INTRODUCTION

Wheat is the dominant crop in temperate countries that are being used for human food and livestock feed. Its success depends partly on adaptability and high yield potential but also on the gluten protein fraction which confers the viscoelastic properties that allow dough to be processed into bread, pasta, noodles, and other food products. Wheat also contributes essential amino acids, minerals, and vitamins, and beneficial phytochemicals and dietary fiber components to the human diet, and these are particularly enriched in whole-grain products (Shewry, 2009). *Wheat* (*Triticum spp*.) is a grass that is cultivated worldwide, It is important for human nutrition in most countries in the world. Common wheat (*Triticum aestivum* L.), and durum wheat (*Triticum durum Desf*.) is of the largest economic importance of all other species (Piergiovanni, 2007).

World production of durum wheat grain amounts is 32-34 million tons and pretends an upward penchant (Matuz and Beke 1996; Rachon and Szumilo 2006). The common bread wheat (*T. aestivum* L.) and durum wheat (*T. durum Desf.*) grains contain two sorts of major proteins: non-storage and storage proteins. The non-storage proteins also denominated metabolic proteins, include water-soluble proteins albumins and salt soluble globulins, show caused for 15– 20% of all of the grain proteins (Piergiovanni, 2007). Wheat is a member of the grass family that produces a dry one-seeded fruit commonly called a kernel. This grass is widely cultivated for its seed, a cereal grain which is a worldwide staple food. There are many species of wheat which together make up the genus *Triticum*; the most widely grown is common wheat (*T. aestivum*), scientific classification :

Kingdom: Plantae

Division: Magnoliophyt

Class: Liliopsida

Order: Poales

Family: Poaceae

Genus: ***Triticum.***

Many diseases can attack wheat and causes different levels of quantitative and qualitative losses in grain yield or another kind of abnormalities in the host plants.

Viral diseases occur when the virus RNA gets into the host-plant and engaged in the DNA of host and replicate their self. The symptoms of the viruses revealed on the host's cause of the virus involves with the symptoms of the action of the host in the result it causes dying and mutation of genetic Agrios, 2005: Burnett *et al* 1995. BYDV diseases can hint wheat leaves in the plant virology caused by the widespread use of molecular techniques for nucleic acid clarifications. By the isolation of symptom variants from areas with atypical symptoms in systemically infected plants (Kunkel 1971a, 1947b: Agrios 1978). Although the genetics and pathogenicity of plant viruses have been well studied in crop hosts, their diversity and ecology have received less attention, particularly in the natural ecosystems where symptoms are often less noticeable (Wren *et al*., 2006). There has been a recent flurry of studies on the prevalence and impact of plant viruses in wild host plants, partly as a result of ecological risk assessment for transgenic virus resistance in major crops (Cooper and Jones 2006). While the strength of the interactions between viruses and hosts can vary, these studies confirm the potential of viruses to influence the fitness of host population. (Friess and Maillet, 1996; Funayama *et al*., 1997; Maskell *et al*., 1999; Power and Mitchell, 2004; Malmstrom *et al*., 2005b, 2006).

To investigate the role of pathogen spillover in the ecology of plant viruses, a series of field experiments with BYDV-PAV, which infects a broad range of grass hosts. In experiments with constructed communities of wild annual grasses, the presence of a reservoir species wild oats, barley, and wheat), greatly increased the prevalence of BYDV-PAV in several other species, *Avena fatua* (Power and Mitchell, 2004). RNA recombination is one of the major forces in increasing plant virus variability and adaptation to new hosts, often leading to the emergence of new variants and resistance-breaking virus strains. RNA recombination can also increase viral fitness by repairing defective viral genomes or efficiently removing deleterious mutations that result from error-prone replication. RNA recombination is affected by several factors, including the viral replication proteins and various features of the viral RNA templates

involved. Host genes also affect RNA recombination, suggesting a complex interaction between a given virus and its host during viral adaptation and evolution. Future research will likely unravel further details of this evolutionarily important process in the emergence of new viruses or viral variants with altered pathogenicity. Nucleotide sequence determination and the development of methods for the comparison of distantly related sequences, led to phylogenetic analyses of proteins with a similar function in viruses belonging to different genera. These analyses first were done with RNA-dependent RNA polymerases (Kamer and Argos, 1984: Roossinck 2008: Wang 2011). Wheat serves as a natural host for many viruses that generally cause symptoms that are distinctive from other infectious diseases. There is more than one method for Detection, identification, and description of virus species. Multiplex reverse transcription polymerase chain reaction (M- RT-PCR) method was developed for the simultaneous detection and discrimination of viruses (Deb and Anderson, 2008). The Polymerase Chain Reaction (PCR) is a powerful technique used for the amplification of a specific segment of a nucleic acid.

Turkey is characterized by extreme geo-climatic diversity, which permits the production of a wide range of livestock and crops. There are several publications concerning the climatic zones of Turkey. These also have diverse agro-ecologies, and represent the largest wheat production provinces, accounting for 1.9 million hectares of the national total of 8.6 million hectares of cultivated wheat in 2007, with Konya leading (623.000 ha) followed by Ankara (512.000 ha), Diyarbakir (302.000 ha), Adana (263.000 ha) and Edirne (190.000 ha) (Karagöz, 2009).

Diyarbakir is in the South East Zone (AZ 6) with large fertile plains in the southern part. The production system is mainly rain fed, although the GAP project has invested in one of the biggest irrigation schemes in the zone.

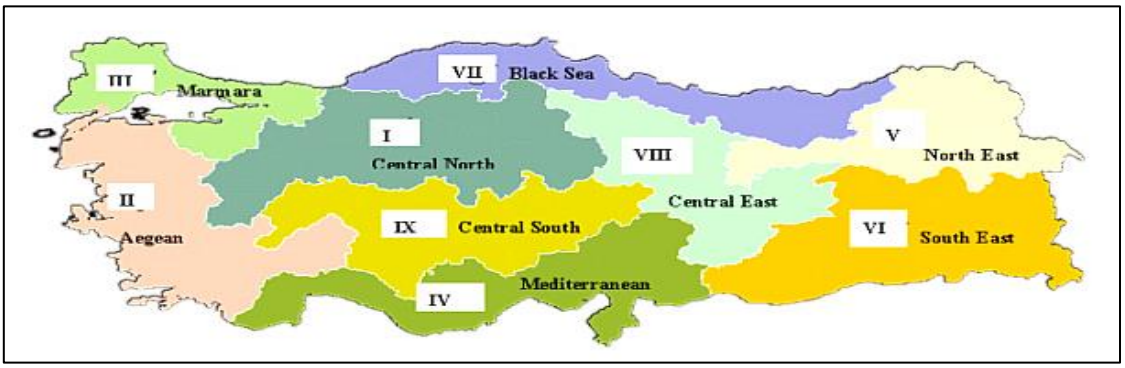


Figure 1.1. Agricultural Zones of Turkey

The world wheat production averages are almost it is around 550 million. By according to (FAO). Rare researches have been done in different areas of Diyarbakir, evenits suitable place for wheat production (Figure 1.1).

FAO data refer that the amount of manufacturing wheat in the world from 1996 to 2014 was (11985.2) million tons, and the highest amount of wheat production during these 19 years, the most produced one was in 2014 near 729 million tons and the lowest wheat production was in 2003 (Figure 1.2).

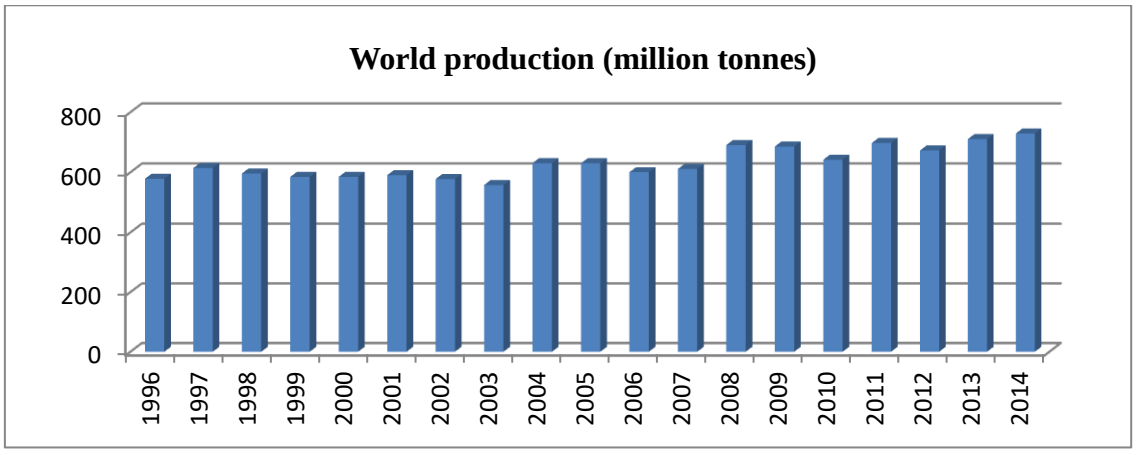


Figure 1.2. Wheat production in the world as of the years (FAO, 2015)

As reported by FAO the amount of producing wheat during 1996 to 2014 were (375) million tons, Wheat production in Turkey was 18.5 million tons in 1996. The highest amount of wheat production in Turkey near 22 million tons in 2013, While the lowest was 17.2 million tons in 2007 (Figure 1.3).

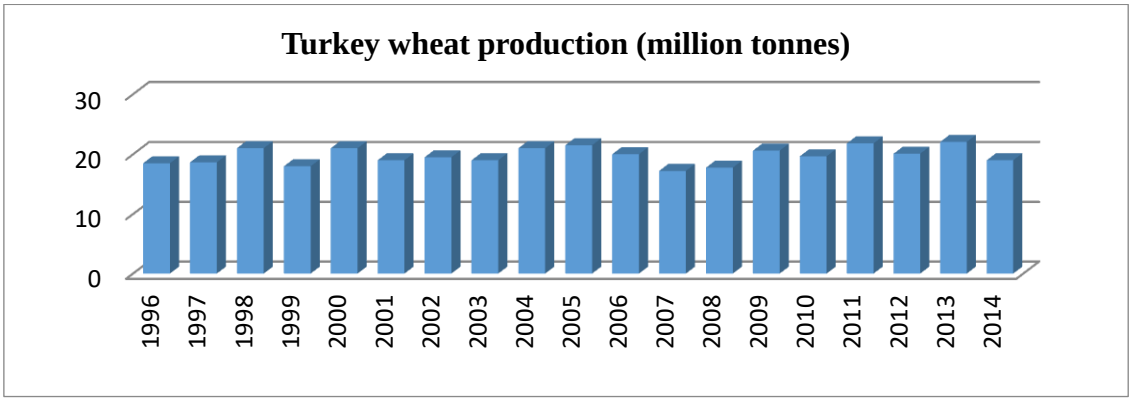


Figure 1.3. Wheat production in Turkey as of the years (FAO, 2015)

According to the statistics data, in agreement with the statistics of FAO find out that the production of wheat changes from one year to another. Generally, amount of wheat production has been to increase after 2010 in Turkey (Figure1.4).

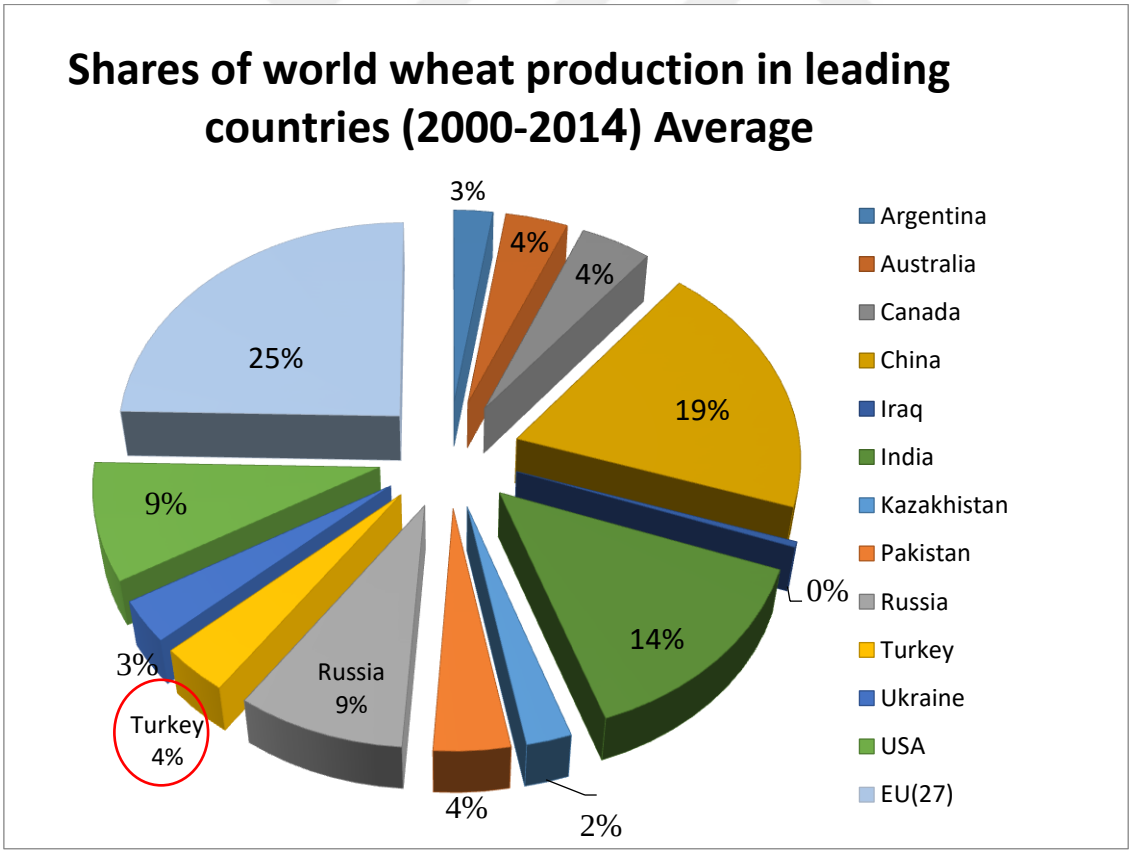


Figure 1.4. World wheat productions in leading countries (FAO, 2015)

FAO reports summarize that average yield average in the World from 1996 to 2014 was 5492.69 kg/ha comparing to 4348.98 kg/ha in Turkey. Also, wheat yield in Turkey in 2012 was 267.23 kg/ha 284.51in 2013 and 242.94 kg/h in 2014 (FAO 2015). The highest yield was detected in the years 2012, 2013, and 2014 (Figure 1.5).

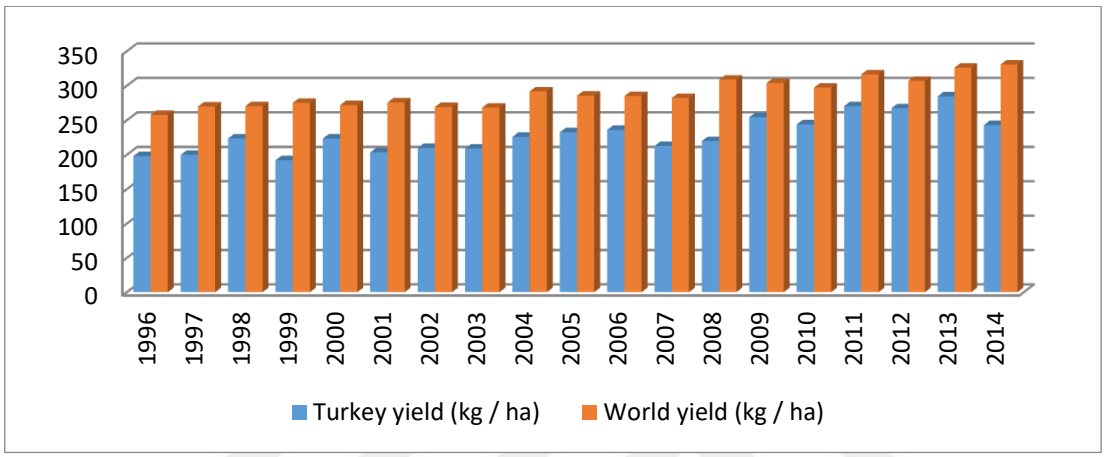


Figure 1.5. Wheat yield compared the world with Turkey (FAO, 2015)

Objectives of the Study;

- To investigate and determinate infection rates of *Barley yellow dwarf virus species* by multiplex RT-PCR method
- To elucidate the relationship of BYDV's with other BYDV species using partial genome sequence analysis
- To performed sequence and phylogenetic analysis of coat protein gene of some BYDV's species
- To better understanding the molecular evolution of BYDV-PAV and the taxonomic status of BYDV-PAV

2. LITERATURE REVIEW

Wheat serves as a standard host for many viruses that normally cause symptoms that are special from other infectious diseases. Although the incidence of viruses in wheat (or other cereals such as barley, oats, rye, triticale) in a given field perhaps relatively indistinct in some years, the viral infection might be quite obvious leading to dangerous economic losses. *Tritium aestivum* is susceptible to approximately 55 viruses naturally wheat defined as infected the virus is approximately 30 around (Wiese, 1987; Brunt *et al*., 1996).

Economic losses due to these viruses around the world are difficult to estimate because of insufficient molecular information and symptoms that resemble the effects of other biotic and abiotic factors. At the same time, the evolutionary potential of BYD-associated viruses is very high like other RNA viruses that cause diseases in humans, animals, and plants (Elena and Sanjuan, 2008). The kinds of *Barley yellow dwarf luteovirus* are (BYDV-PAV, MAV, RMV, SGV, and *Cereal yellow dwarf polerovirus* (CYDV-RPV). The Barley yellow dwarf disease of cereals is caused by several different viruses currently classified in two species, *Luteovirus* and *Polerovirus*, of the plant virus family *Luteoviridae*.

Lister and Ranieri (1995), studied for Yellow dwarf disease (YDD) is first of the most important viral diseases of cereal crops worldwide. These are only some of the economic importance and it has been caused damage to wheat in large areas. These *Barley yellow dwarf luteoviruses* are (BYDV-PAV, MAV, RMV, SGV, and *Cereal yellow dwarf polerovirus (*CYDV-RPV).

The availability of these methods allowing the differentiation of closely related genotypes, and the availability of biologically active complementary DNA (cDNA) clones of RNA genomes, definitively determined that virus populations are intrinsically heterogeneous owing to errors during replication. Following the trend with animal- and bacteria-infecting viruses, research focused on RNA viruses, and heterogeneity of cDNA-derived populations was initially shown for *Cucumber mosaic virus* (CMV) satellite RNA and for TMV (Aldahoud e*t al.,* 1989; Kurath and Palukaitis, 1989).

2.1. *Barley yellow dwarf viruses* (BYDVs)

2.1.1. General features

Mayo et al. (1999), experimented the *Barley yellow dwarf viruses* (BYDVs) is one of the ultimate significant viral diseases of cereal crops worldwide, and especially this (BYDVs) most economically important of wheat and it is effective in very major field crops. The Barley yellow dwarf disease of cereals is caused by several different viruses currently classified in two species, *Luteovirus* and *Polerovirus*, of the plant virus family *Luteoviridae*.

The evaluation of the *Barley yellow dwarf luteoviruses* is the only family *Poaceae* affects plants. *Poaceae* included more than 150 types of crops as it is grown. The virus mainly affects barley, wheat, corn, oats, rye and rice including many single and perennial crops culture on the outside of the plant in this family weeds. The virus also infects at least 100 other grass species, including maize and rice. It produces symptoms on wheat, barley, and other grain cereals characterized by small spikes, yellowing of leaves starting from the leaf tip and expanding to the base, and stunting. BYDV is transmitted by aphids in a persistent, circulative manner, but not mechanically and not through seeds BYDV European, Australia, Asia, New Zealand and Africa such as very wide geographical distribution show D Arcy and Burnett, (1995); Wiese (1987).

The study of genealogy naming and the initial grouping are done according to their industry. By feeding on a diseased plant for a minimum of 30 min and the virus life roust aphids are capable of transmitting the virus, after an incubation period of 12 has to 4 days. The virus is not transmitted by eggs and does not increase in the vector. BYDV strains have been differentiated according to their aphid vectors (Halberd *et al*., 1992; Osler *et al.,* 1992; Miller *et al.,* 2002; Gray *et al.,* 1998).

Fauquet *et al.* (2005), the recent studies are carried out research on the causes yellow dwarf changes in the genetic structure of the virus, which is classified again and it should be taking into consideration and each breed has been considered as some kind, *Barley yellow dwarf virus* (PAV, PAS, MAV, SGV, and RMV)'s *Luteovirus* and *Cereal yellow dwarf virus* (CYDV-RPV)'s It is classified again placed into the genus *polerovirus* (Van Regenmortel *et al*., 2000).

Chomič (2011), researched the BYDV serotypes were divided into two subgroups, which were thereafter reclassified as detached species. Actually, only BYDV-MAV (transmitted primarily by (*Sitobion avenae*) and BYDV-PAV (transmitted efficiently by (S. *avenae* and *Rhopalosiphum padi*) are *Barley yellow dwarf viruses*. Former BYDV serotype RPV (transmitted primarily by *R. padi*) was given a modern name, *Cereal yellow dwarf virus*-RPV (CYDV-RPV) and placed in gender *Polerovirus* along with four non-BYDV viruses in the *Luteoviridae.* A third genus, *Enamovirus,* consists only of RNA-1 of the bipartite *Pea enation mosaic virus* (PEMV). Its organism resembles polioviruses.

Domier *et al*. (2012), transmitted virus by the same species of aphids in a continual method, including *Rhopalosiphum padi*, which is the generally efficient. Other kind of aphids such as *Schizaphis graminum, Macrosiphum avenae, R.maidis* were also reported vectoring the virus the aphids can achieve the virus. *Luteoviridae* fall into three genera: *Luteovirus, Polerovirus,* and *Enamovirus.*

The most important virus in wheat cereal viruses with BYDV viruses single-stranded RNA has a structure; 25-28 nm in diameter has isometric particles. Isometric virus particles, the host plant in phloem cells it is localized. These viruses are electron microscope (Figure 2.1.) with the parenchyma cells, infected cells in the griddle; nucleus and cytoplasm can be seen. Outside of the *Luteoviridae* block, the viral genomes are completely different between *Polerovirus* and *Luteovirus* genera (Mille *et al*., 2002; Domier 2012; Wiese 1987; McCoppin, 2002).

According to McCoppin and Domier (2002), these viruses are single-stranded ribonucleic acid (ss RNA) genomes include of two proteins (major coat protein and minor read-through protein). Virus in RNA genome mRNA the assignment is made 5-6 gene or open reading frame (ORF) it has shown in (Figure 2.2.) Some proteins, genomic RNA (OFS) directly produced by the region, the other protein sub genomic RNA (sgRNA) named as shorter RNA it is expressed. BYD- connected viruses have a genome depending of positive sense single-stranded RNA (+ssRNA) and the total genome lenght is around 5.6 to 6.0 kb. The visions are hexagonal particles with a diameter of 24-25 nm. They are not enveloped and genomic RNA is encapsulated by CP with a molecular weight of 21 to 23kDa.

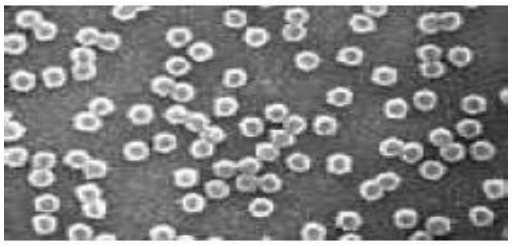


Figure 2.1 Electron microscope image of BYDV virions (D'Arcy and Domier, 2005).

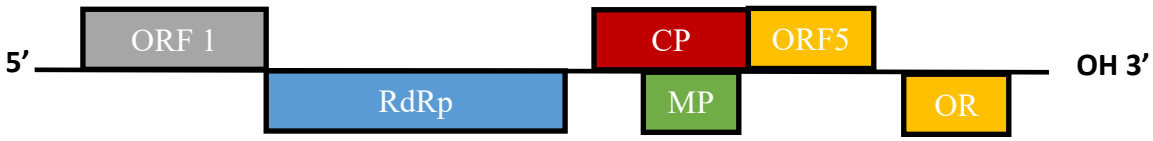


Figure 2.2 *Barley yellow dwarf viruses* (BYDVs*)* genome organization (D'Arcy and Burnett, 1995; Wiese, 1987).

2.1.2. Symptoms of BYDV's

The BYDV's symptoms which are caused by the environmental conditions, race infection in time of host plant's physiological state, age, varieties and the host species it varies depending such as temperature, soil moisture and soil fertility. Although the virus causes these symptoms, still it is very difficult to detect the virus and estimate yield losses using only symptomatology, which may be masked by other factors. Severe infection causes rolling, reddening, or yellowing of leaves, stunted plants, inhibited root formation, delayed heading and reduced yield. The most conspicuous symptom on infected hosts, loss of green color in leaves, is often more prominent on older leaves. However, disease symptoms may vary depending on the host plants and host plant genotypes. For instance, oats show reddening of the leaf blades along the vascular bundles, blasting of the florets, stunted growth, and late heading. Symptoms caused by BYDVs differ with the host species and cultivar, the period and the physiological status of the host plant at the time of infection, the strain and the environmental conditions and can be easily confused with nutritional and abiotic disorders. Symptoms include leaf discoloration from tip to base and from margin to center. The discoloration takes on different colors depending on the plant. In barley, the leaf turns bright yellow; in oat, an orange, red, or purple discoloration is seen and in wheat, rye, and triticale, the infected leaves are generally yellow and sometimes red. In maize, a conspicuous reddening

occurs on the lower leaves, while in rice; infected leaves turn yellow to orange, experimented the symptoms of BYD vary due to host species, host age, virus isolate, and climatic conditions. Usually the root system of the diseased plant does not develop. Heading is late or no heads are produced, yellow leaves are observed in 6-row barley and wheat, and reddening of leaves in 2-row barley has been seen frequently. In the field, wheat shows better resistance to BYDV than barley. Often 20 to 25 days after the first symptoms are observed, epidemic conditions will be seen, and, finally, in severe cases, the whole field will be infected (Nega, 2014;Mahlooji, 1990: Fauquet, et al., 1999).

2.1.3. Transmitted of BYDV's

Miller et al., (2002), evaluated the BYDV is in genus *Luteovirus*, family *Luteoviridae*. Which is BYDV seeds that are recognized as mechanics Virus on the stubble and more cannot sustain life on the soil, they can only survive on living plant tissue. The virus only infects the diffusion path aphids. (i) BYDV is transmitted only by aphids in a persistent manner and not mechanically ;(ii) circulate but does not replicate in the aphid; (iii) are confined to the phloem in the plant.

The BYDV, serotypes were divided into two subgroups, which were subsequently reclassified as separate species. (i) Includes *Barley yellow dwarf virus*-PAV (BYDV-PAV), BYDV-MAV, and BYDV-SGV; and (ii) Subgroup II includes BYDV-RMV, *Cereal yellow dwarf virus*-RPV (CYDV-RPV), species. Currently, only BYDV-PAV (transmitted efficiently by *S. avenae* and *Rhopalosiphum padi*) and BYDV-MAV (transmitted primarily by *Sitobion avenae*) and *barley yellow dwarf virus*-SGV (transmitted primarily by *Schizaphis graminum)* are barley yellow dwarf viruses. Former BYDV serotype RPV (transmitted primarily by *R. padi*) was given a new name, *Cereal yellow dwarf virus*-RPV (CYDV-RPV) and placed in genus *Polerovirus,* and are *Barley yellow dwarf virus*- RMV (transmitted primarily by *R. maidis*) along with four non-BYDV viruses in the *Luteoviridae*. BYDV by four aphid species according to the state to move into fifth race is separated (Smith et al., 1999; Miller and Rasochová, 1997: Robertson, *et al*.,1991).

The environment of BYDV is generally likely to happen after a warm fall and temperate winter, which enhance the growth and development of host weed and aphid populations. BYDV as well can be crueler in not ill wheat planted into heavy corn wreckage than in wheat planted into soil free of surface corn wreckage. This may be because the paly corn wreckage attracts and shelters the aphids that transfer BYDV, Environmental factors functions have an important role in the disease cycle of BYDV. High light intensity relative temperatures 15-18 ^{o}C (Wiese, 1987; Miller and Rasochova 1997).

2.1.4. Control of BYDV's

The aphids are annihilation because the aphids are transferring the Virus, the diseases and bacterial (Isleib, 2015), we need:

- Cultural management**.** Control grassy weeds and volunteer cereals. These plants can harbor BYDV, which can then be transferred to the main crop by aphids. Small grains planted in mid-summer, as companion or cover crops can also become reservoirs of BYDV.
- Chemical control**:** used the chemical for the external or kills aphids' but according to when it has been seen a flay aphids in the fields
- Genetic resistance**:** Some wheat cultivars documented resistance or tolerance to BYDV. Other wheat family members like , oat and barley varieties have good levels of resistance or tolerance.
- Timely seeding: Planting winter wheat late in the season as practical in fall to avoid potential aphid activity in early falls as summer crops and grasses mature.

2.1.5. Studies on BYDV viruses in Turkey

There are few studies on wheat virus diseases in our country and these studies have been intensified in Thrace, Aegean, Central Anatolia and Western Black Sea Region. Despite cereals being the most important cultivated plants, research on wheat pests, diseases, weeds and especially virus diseases is not sufficient. In addition, it is not

known which viruses are infecting in which region and also the infection rates of the viruses are unknown.

In 2003, 5 different wheat viruses (BYDV-PAV, BYDV-MAV, CYDV-RPV, BSMV, WDV, BMV) were studied in Tekirdağ province. 260 wheat samples collected were tested by ELISA. In wheat samples 25 % BYDV-MAV, 22.3 % BYDV-PAV, 16.5 % WDV, 8.5 % CYDV-RPV, 3.1 % BMV and 1.5 % BSMV virus were detected, respectively (Köklü, 2004).

Maize, weed and vector samples were collected from corn fields in the Çukurova region between 2001-2003 and the presence of Barley yellow dwarf virus (BYDV), Maize dwarf mosaic virus (MDMV), Maize mosaic virus viruses (MSpV) have been investigated. MDMV with 8.04%, BYDV with 4.8 %, MSPV with 1.8 % and MMV with 1.6 % were determined in collected samples. Thanks to this study, it was stated that BYDV seriously harmed on corn (Fidan and Yılmaz, 2004).

Triticum aestivum L. and Triticum durum L. wheat species were surveyed in Turkey, especially in the Aegean region. Through this work, it is clear that Barley yellow dwarf virus (BYDV) is composed of five distinct virus strains rather than only one virus (İlbağı and Çıtır, 2004b).

These strains are Barley yellow dwarf virus-PAV (BYDV-PAV), Barley yellow dwarf virus-RMV (BYDV-RMV), Barley yellow dwarf virus- MAV (BYDV- and Cereal yellow dwarf virus-RPV (CYDV-RPV). Among them, it has been reported that the most commonly identified disease is BYDV-PAV and also the BYDV-RMV virus strain should be considered epidemiologically.

A study on corn virus diseases was conducted in Turkey's Thrace Region in 2004 and 2005. In 2004, 142 corn samples were collected and it was determined that 72 samples of MDMV, 2 of BYDV-PAV, 19 of MDMV and BYDV-PAV, 2 of MDMV, BYDV-PAV and SCMV were found in corn samples. It was also found that only one sample contained 4 viruses. In the surveys they conducted in 2005, 100 corn leaf samples were collected. As a result of the serological tests done, 50% of the samples were found to be infected with MDMV and SCMV (İlbağı *et al*., 2006).

In another study, 101 plant specimens were collected and tested serologically against Cereal yellow dwarf virus-RPV (CYDV-RPV) and Barley yellow dwarf virus-PAV (BYDV-PAV) viruses in bird seed fields in Tekirdag province. According to the

results of the serological tests, 48% BYDV-PAV, 2% CYDV-RPV and 14% mixed infection were found (İlbağı ve ark., 2008).

In the Central Black Sea Region, 220 plant samples were collected, showing signs such as dwarfing on wheat-cultivated fields, yellow-brown coloring and deformation on the leaves. As a result of the tests on the plant samples, the presence of BYSMV at 19.5 % at the BYDV and 1.3 % of the samples was reported (Düşünceli et al. 2009).

A survey for detection of *Barley yellow dwarf luteoviruses* (BYDV-PAV and BYDV-MAV), *Cereal yellow dwarf polerovirus* (CYDV-RPV) was carried out during May 2003 covering seven cereal growing counties of Tekirdag, Turkey. Two hundred sixty (260) wheat samples were collected from 26 wheat fields. Serological tests showed that three tested viruses, involving BYDV-MAV (25%), BYDV-PAV (22.3%), and CYDV-RPV (8.5%) were present in Tekirdag (Köklü, 2004).

Çıtır et al. (2005), 106 plant samples of cereal leaves exhibiting yellowing, dwarfing, streak mosaic, necrosis etc were collected in 2002 from Edirne, Kırklareli and Tekirdağ provinces. Infection rates were found that 32.33 % in winter wheat (*Triticum aestivum* L.), 26.52 % in barley (Hordeum vulgare L.), 8.32 % in oat (*Avena sativa* L.), and 8 % in triticale in 2001. Rates of virus infections in 2002 however were 17.27 % in winter wheat, 15.22 % in barley and 16.5 % in oats.

Ilbagı, H., (2006), collected the sample randomly for infections this plants and were of symptomatic plants were estimated at 3.7 to 63.6%, depending on locations. Biological and serological test results revealed the presence of Barley yellow dwarf virus-PAV (BYDV-PAV), Maize dwarf mosaic virus (MDMV), Sugarcane mosaic virus (SCMV), and Johnson grass mosaic virus (JGMV). Prominent and contained the four viruses of the samples were infected with MDMV, were infected with BYDV-PAV, with MDMV and BYDV-PAV with MDMV, BYDV-PAV, and SCMV. Individual MDMV, SCMV, BYDV-PAV and JGMV infections were detected in samples, respectively. Presence of MDMV was confirmed by Western blot analysis and IC-RT-PCR. SCMV was also detected by IC-RT-PCR.

In canary seed (*Phalaris canariensis* L.) fields in Tekirdag, a common virus infection causing dwarf and leaf yellowing was observed. One hundred plant samples showing the symptoms were collected and tested with DAS-ELISA and TAS-ELISA

using polyclonal antiserum against Barley yellow dwarf virus-PAV (BYDV-PAV) and Cereal yellow dwarf virus-RPV (CYDV-RPV). Results of both immunoassays were infected with BYDV-PAV in 48%, CYDV-RPV in 2%, and mixed infection in 14% of the samples. (Ilbagı et al., 2008).

A study was carried out between 2005-2006 to determine the virus diseases in wheat production region areas in Samsun province. In order to investigate Barley yellow dwarf virus (BYDV) PAV and MAV strain, 154 plant samples were tested by ELISA. Test results showed that five samples were infected with BYDV-PAV (3.4%) and three samples with BYDV-MAV (2%) whereas ile one sample was infected with BYDV-PAV+MAV double mix infection (0.7%) (Erkan *et al*., 2009).

A total of 116 and 100 leaf samples of wheat grown intensively in Samsun and Amasya provinces in 2006 and 2007, respectively, were collected in order to determine the presence of BYDV-PAV and BYDV-MAV and these samples were tested by enzyme-linked immunosorbent assay (ELISA). ELISA tests showed that ten samples were infected with BYDV-PAV (8.6%), six samples with BYDV-MAV (5.1%) and two samples (1.7%) with mixed-infection of BYDV-PAV+MAV in Samsun while four samples were infected with BYDV-PAV (4%), one sample with BYDV-MAV (1%) and four samples (4%) with mixed-infection of BYDV-PAV+MAV in Amasya (Deligöz *et al*., 2011).

A total of 900 wheat samples were collected from Eastern Anatolia Region in 2012. The collected samples were tested by the multiplex RT-PCR method to identify Barley / Cereal yellow dwarf viruses (BYDV-PAV, MAV, SGV and RMV, CYDV-RPV) viruses. As a result of the 900 tested samples; BYDV-PAV, BYDV-SGV, and CYDV-RPV were infected with 50(% 5.5), 44(% 4.8) and 4 (% 0.4) respectively(Usta, 2013).

2.1.6. Studies on BYDV viruses in world

Bekele et al. (2001), a survey was carried out in Ethiopia during the main rainy season of 1995 and the short rainy season of 1996. Randomly collected samples were tested by the tissue blot immunoassay (TBIA) for BYDVs. All five known BYDV (PAV, MAV, RPV, and SGV) serotypes were identified from Ethiopia.

In Malmstrom and Shu, (2014), the presence of different Barley and Cereal yellow dwarf viruses (B/CYDV) was investigated by multiplex RT-PCR. The basic multiplex can produce two fragments at the same time; a fragment of ~ 830 bp indicating the presence of BYDV-PAV, BYDV-MAV or BYDV-SGV viruses and a fragment of ~ 372 bp showing the presence of CYDV-RPV, BYDV-RMV or BYDV-GPV viruses. The enhanced multiplex RT-PCR produces two additional fragments, which further separating between BYDV-PAV, BYDV-MAV and BYDV-SGV. The enhanced multiplex produces two attachments further separating BYDV-PAV, BYDV-MAV and BYDV-SGV. Multiplex RT-PCR can be tested multiple samples at the same time and with less cost. The Multiplex RT-PCR is useful not only in the basic diagnosis of B/CYDV but also in studying long-term epidemiological studies of these viruses in nature (Malmstrom and Shu, 2014).

In another study in Egypt, total RNA of wheat leaf samples and aphids were used to investigate BYDV viruses by RT-Real-Time PCR technique for detecting and quantifying BYDV. The five serotypes (PAV, RMV, RPV, MAV, and SGV) were reported and the BYDV- PAV is the most common virus in the region. In addition, the sensitivity of RT- Real Time PCR is 3 to 5 fold higher than conventional PCR for detecting virus infection (Nagy *et al*., 2007).

According to Deb and Anderson (2008), Wheat spindle streak mosaic (WSSMV), Soil-borne wheat mosaic virus (SBWMV) and Wheat streak mosaic virus (WSMV) Barley and Cereal yellow dwarf viruses (B/CYDVs), which are economically important viruses were investigated using the multiplex RT-PCR test, a rapid and specific wheat virus diagnostic tool. M-RT-PCR method was developed for the concurrent detection and separation of eight viruses: five strains B/CYDV, WSSMV, SBWMV and WSMV. The protocol uses specific primer sets for each virus-producing five distinct fragments 295, 175, 400, 237, and 365 bp, indicating the presence of two strains of BYDVs, -PAV, -MAV, CYDV-RPV and two unidentified Luteoviridae BYDV-SGV and RMV, respectively. This system also readily detected WSSMV, SBWMV and WSMV specific amplicons at 154, 219 and 193 bp, respectively. Similarly, BYDV-GAV, GPV, PAV, BSMV (*Barley stripe mosaic virus*), WYMV (Wheat yellow mosaic virus), WDV (Wheat dwarf virus) and WBD (Wheat

blue dwarf phytoplasma) were identified by M-RT-PCR method in China (Tao *et al*., 2012).

Molecular characterization of BYDV-PAV and CYDV-RPV viruses causing significant product loss was performed in Iran. A genome segment of each isolate was amplified by PCR. The BYDV- PAV-IR fragment (1264 nt) covered a region containing partial genes for coat protein (CP), read through protein (RTP) and movement protein (MP), while the fragment of RPV-IR (719 nt) contained partial genes for CP, RTP and MP (Rastgou *et al*., 2005).

Ali et al. (2013), studied the Barley yellow dwarf virus (BYDV) is a potential threat to agriculture production. The amplified complete coat protein sequences of the isolate M07 and M12 determined to be 597 bp and 603 bp, respectively. Nucleotide sequence identity of 87.6 % (84.3 % amino acid sequence identity) to a Chinese isolate of BYDV-PAV. Whereas, the isolate M12 showed a maximum nucleotide sequence identity of 94.5% (94.0 % amino acid sequence identity) to French isolate BYDV-PAV. Since more than 10 % differences, among the amino acid level of any gene product, it's the sole criterion to discriminate between species within the family Luteoviridae, the isolate M07 that showed a maximum of 84.3% (less than 90%) amino acid sequence to identity with the previous known Luteovirus species, thus, it was recommend to be a distinct PAV species within the genus Luteovirus.

Sample was collected from winter cereal crops (wheat, barley, corn, and ryegrass). In different two years screened by Polymerase chain reaction (PCR) with primers designed on ORF 3 (coat protein - CP) for the presence of *Barley yellow dwarf virus* (BYDV) and Cereal yellow dwarf virus (B/CYDV). PCR products of expected size (~357 bp) for subgroup II and (~831 bp) for subgroup I were obtained for three and 39 samples, respectively. These products were cloned and sequenced. The subgroup II 3' partial CP amino acid deduced sequences were identified as BYDV–RMV (92 – 93 % of identity with "Illinois" Z14123 isolate). The complete CP amino acid deduced sequences of subgroup I isolates were confirmed a BYDV-PAV (94 – 99 % identity) and established high homogeneous group (identity higher than 99 %). These results support the prevalence of BYDV-PAV (Bernard et al., 2013).

Rastgou et al., (2005), studied the one isolate of BYDV-PAV from wheat (PAV-IR) and one isolate of CYDV-RPV from barley (RPV-IR) selected for molecular

characterizations. Genome segment of each isolate amplified by PCR, the PAV-IR fragment (1264 nt) covered a region containing partial genes for coat protein (CP), it should read through protein (RTP) and movement protein (MP). PAV-IR showed a high sequence identity to PAV isolates from USA, France and Japan (96–97 %). In a phylogenetic analysis, it is placed into PAV group I together with PAV isolates from barley and oats. The fragment of RPV-IR (719 nt) contains partial genes for CP, RTP and MP. The sequence information is to confirm its identity as CYDV. However, RPV-IR shows 90–91% identity with both RPV and Cereal yellow dwarf virus-RPS (CYDV-RPS). Phylogenetic analyses suggested that it was more closely related to RPS. These data comprise the first attempt to characterize BYD-causing viruses in Iran and southwest Asia.

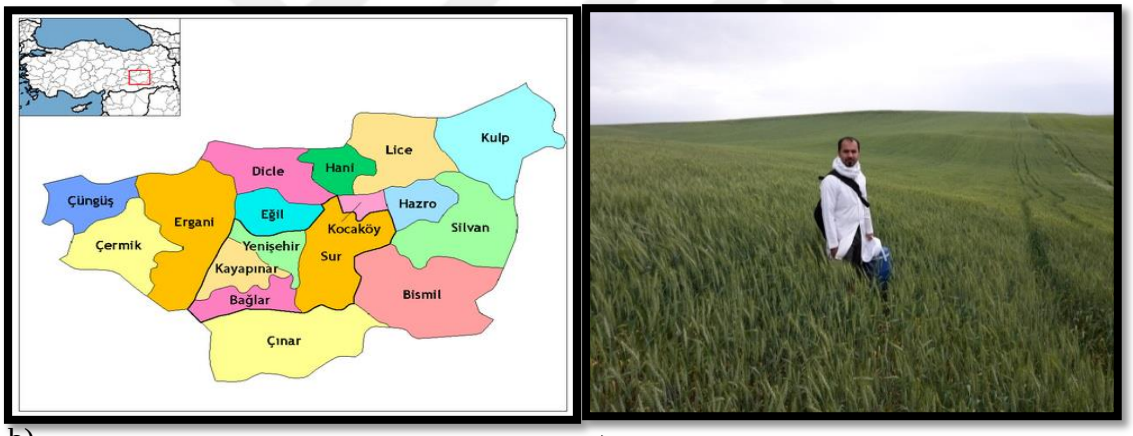
In a study conducted in Tunisia, 240 barley samples were randomly collected from 6 different barley fields to characterize B/CYDV isolates and analyzed by DAS-ELISA. The consequence of 40 barley specimens was infected with B/CYDV virus (BYDV-PAV (77.5 %), CYDV-RPV (25 %) and BYDV-MAV). Studies of the geographic distribution showed a high incidence of B/CYDV in the Tunisian Southern provinces (Bouallegue et al., 2014). To investigate the viruses of Barley yellow dwarf and Cereal yellow dwarf viruses (B / CYDVs) subgroup 1 (Barley yellow dwarf virus-PAV, BYDV-MAV and BYDV-SGV) and subgroup II (BYDV-RPV, CYDV- RPV, BYDV- GPV), multiplex RT-PCR was performed in Pakistan. In the study conducted, 13 of 37 samples of wheat were infected. Wheat samples positive for subgroup I were further tested by PCR, and results showed that 10 samples were positive for BYDV-PAV and three for BYDV-MAV. DNA sequences of CP region of nine isolates (BYDV-PAV) were compared with present sequences in GeneBank. Sequence analysis distinguished that three isolates had a maximum identity (92.8– 94.6 %) to BYDV-PAS, and six had a maximum identity (99.3– 99.7 %) to BYDV-PAV (Siddiqui *etal*.,2012).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Survey areas

The survey was conducted in wheat fields in four districts of Diyarbakır province at booting- heading stage during April-March, 2016. In each field, the wheat samples were randomly collected. Samples were collected from wheat plants that with or without BYDV's symptom. The samples were placed in icebox filled with ice then transferred to Virology lab of Plant Protection Department (Figure 3.1). A total of 365 samples were collected from district in Diyarbakir as it is shown in Table 3.1.



b) a)

Figure 3.1. Districts surveyed in Diyarbakir province (a), collecting wheat leaves sample randomly (b).

3.1.2. Plant material

Main material in this study was fresh leaves of wheat plants collected from the survey areas. Leaf samples were used as a material for detection of BYDV viruses. Ten wheat leaves samples were collected from each winter wheat fields in March 2016. Dwarfing and yellowing are the most commonly observed symptoms on the infected plants in the field, showed in the Table 3.1.

Table 3.1. Survey area and number of samples collected from different wheat fields of Diyarbakır districts.

No.	District Name	Total Area (Hectares)	Sowing Area Of Cereals And Other Crops (Acres	Collect Leaves No.Samples
1	Bismil	1246527	1233134	160
2	Çınar	610995	572244	80
3	Silvan	746290	708370	70
4	Sur	937529	904377	55
Total		6147045	5553326	365

3.1.3. Total nucleic acid (RNA) extraction

The total nucleic acid (RNA) means the process of biological method studies for extraction in the cells and tissues of the fresh wheat leaves. Total RNA Extraction is also the first step of molecular diagnosis. The resulting total RNA extraction was stored at -20 ° C in freezer. Total nucleic acid chemicals used during the study process.

3.1.4. Synthesis of Complementary DNA (cDNA)

The Complementary DNA (cDNA) synthesis was implemented using reverted First Strand cDNA kit of the company Fermentas (Thermo-Fermentas, Vilnius, Lithuania). cDNAs have been stored in a freezer at -20 ° C until PCR process.

3.1.5. Polymerase Chain Reaction (PCR)

The Obtained cDNA carried out by PCR tests for amplification target cDNA, using *Taq* DNA polymerase and dNTP mixtures (dATP, dGTP, dCTP, dTTP), supplied from the Thermo-Fermentas company (Vilnius, Lithuania). Eppendorf master cycler Personal Personal brand PC device was used for the amplification of nucleic acids.

3.1.6. Oligonucleotide primers

Sets of primer used to identify the BYDV infection are given in Table 3.2. Primers that used in this study are taken from primers used by various researchers. For perform to the characterization of virus isolates, primers amplifying the complete coat protein of the BYDV-PAV were designed with Vector NTI 10 Program. The designed primers synthesised through Sentegen firm (ANKARA). The primers used diluted with RNase- free water at a specified rate (100 pmol/ul concentration) before use and kept at -20 ° C.

Table 3.2. Primer and specific virus used for detection of wheat viruses.

Virus Primer	Name Sequence	Sequence	length	Source
Grup1 BYDV-PAV, MAV, SGV)	YAN-R SHU-F	5'-TGTTGAGGAGTCTACCTATTTG-3' 5'-TACGGTAAGTGCCCAACTCC-3'	832bp	(Malmstrom And Shu, 2004)
Group 2 (CYDV-RPV, BYDV-RMV)	S2A F S2B F	5'-TCACCTTCGGGCCGTCTCTATCAG-3' 5'-TCACCTTCGGGGCGTCTCTTTCTG-3'	372bp	(Malmstrom And Shu, 2004)
BYD-SGV	SGV-R	5'-ACATTTCTTCGTGTGTTGCG -3'	254bp	(Malmstrom And Shu, 2004)
BYDV-PAV	PAV-F	5'- ACCTAGACGCGCAAATCAAA-3'	590bp	(Malmstrom And Shu, 2004)
BYDV-MAV	MAV2-F	5'-AATAACCGCAGGAGAAATGG-3'	590 p	(Malmstrom and Shu, 2004)
BYDV-SGV	BYDV-SGV BYDV-SGV	5'-ACCAGATCTTAGCCGGGTTT-3' 5'-CTGGACGTCGACCATTTCTT-3'	237bp	(Deb and Anderson, 2008)
BYDV-RMV	RMV F RMV R	5'-ACGAGGACGACGACCAAGTGGA-3' 5'-GCCATACTCCACCTCCGATT-3'	365bp	(Deb and Anderson, 2008)
CYDV-RPV	RPV-F RPV-R	5'-ATGTTGTACCGCTTGATCCAC-3' 5'-GCGAACCATTGCCATTG-3	400bp	(Deband Anderson, 2008)
BYDV-PAV	PAV-F PAV-R	5'-CAGTGGATCCATGAATTCAGTAGGTCGTAG-3' 5'-CAGTAAGCTTGAGGAGTCTACCTATTTGGC-3'	614bp 614bp	(Usta. 2013)

3.1.7. Agarose gel electrophoresis

DNA products amplified by PCR were run by agarose gel electrophoresis (1%) with ethidium bromide. Medium-sized GT wide-sub cell apparatus belonging to the BIO-RAD firms was used in agarose gel electrophoresis. Amplified DNA products were imaged with Syngene analysis system (Synoptic Ltd. in Cambridge, GB). Imaged DNA product excised from the agarose gel using a razor.

3.1.8. Study of Characterization

The DNA DNA amplified by PCR is cleaned from the agarose gel using the Bioline purification kit (Catalog No.BIO-52029) and cloned into *E. coli* (JM 109) bacteria by electroporation (BioRad Micropulser) using pGEM T-Easy Vector system (Catalog No A3610) that was purchased from Promega Company.

Recombinant plasmids including insert gene after cloning were purified via Bioline isolate plasmid mini kit (Catalog No.BIO-52026).

3.1.9. Phylogenetic analysis by bioinformatics methods

The coat protein region of the sequenced BYDV-PAV virus was submitted to gene bank of NCBI website (http://www.ncbi.nlm.nih.gov). DNA nucleotide alignment and phylogenetic tree of BYDV- PAV isolate were created CLC Main Workbench 6.2.3. and Mega4.

3.2. Methods

3.2.1. Disease survey

This study was carried out in the wheat field in 4 different districts (Bismil, Silvan Çınar, and Sur) of Diyarbakır province in Turkey. A Total of 365 wheat leaf samples with or without symptoms were randomly collected from in each field different as mentioned in Table 3.1. The collected samples were placed in the icebox. Ten plant samples were collected from each field during April- May in 2016. A wheat leaf sample was stored in the deep freeze at -20^{o}C until total nucleic acid extraction.

3.2.2. Investigated the viral infection

3.2.2.1. Plant total nucleic acid (TNA) extraction

To investigate the BYDV's presence in wheat fields in Diyarbakir province, the total RNA extraction procedure was implemented to the collected plant samples. Total nucleic acid extraction of collected wheat samples carried out using silica-based extraction method according to Foissac *et al*. (2001). 100 mg of leaf or phloem tissue were ground in 1 ml of grinding buffer in plastic bags or in mortars. 500 µl of the extract were then transferred to an eppendorf tube and 100 µl of 10 % Sodium lauryl sarcosyl solution were added. Tubes were incubated at 70°C with intermittent shaking for 10 min and incubated on ice for 5 min. After centrifugation at 14000 rpm for 10 min, 300 µl of the supernatant were transferred to a new tube containing 150 µl of ethanol, 25 µl of resuspended silica and 300 µl of 6 M sodium iodine. The mixture was then incubated at room temperature for 10 min with shaking. After centrifugation at 6000 rpm for 1 min, the pellet was collected and washed twice with washing buffer. Then the pellet was eluted with 150 µl of RNase-free water and incubated for 4 min at 70°C followed by a centrifugation at 14000 rpm for 3min. Finally, the supernatant was transferred to new Eppendorf tubes to which 13 µl of sodium acetate 3M and 400 µl of cold ethanol were added and the mixture was stored at –20°C.

3.2.2.2. Synthesis of complementary DNA (cDNA)

The Synthesis of complementary DNA (cDNA), was realized as First Strand cDNA kit by using Thermo brand Revert aid (Thermo-Fermentas, Vilnius, Lithuania). *Barley yellow dwarf viruses (*BYDV-PAV, BYDV-MAV, BYDV-SGV, and CYDV-RPV, BYDV-RMV) were investigated using multiplex RT-PCR method. Malmstrom and Shu (2004) method were used to all *Barley yellow dwarf viruses* recognizing the universal Yan- Reverse primer **5'-TGTTGAGGAGTCTACCTATTTG-3'** using cDNA synthesis from total RNA. Thus, there is no need for separate cDNAs for each *Barley yellow dwarf viruses.*

For the synthesis of complementary DNA (cDNA); 5 µl of TNA, 1 µl 10 mM dNTP mix, 1 µl of 20 pmol /µl the Universal Yan Reverse primer, 5 µl of RNase free water put into Eppendorf tube. The mixture was incubated at 65 °C for 5 min and put in ice for 5 min. Denaturized total RNAs were reverse transcribed in cDNA with 4 µl of 5x RT strand buffer, 2 µl 0.1M of DTT, 1µl of RNAase inhibitors and 1µl reverse transcriptase enzyme reaction mixture in a completed by adding to 20 µl and incubated at 42 °C, for 50 min.

After incubation, to inactivate the reverse transcriptase enzyme and terminate the reaction, PCR machine was run at 70 °C, for 15 minutes.

3.2.2.3. Investigation of the viruses with RT-PCR or multiplex RT-PCR

Diagnosis of *Barley yellow dwarf viruses* (BYDV)s including BYDV-PAV, BYDV-MAV, BYDV-SGV, CYDV-RPV and BYDV-RMV was executed by multiplex RT-PCR based according to Malmstrom and Shu (2004), which basic and enhanced RT-PCR systems. The basic multiplex method gave two difference band, group 1 and group 2 viruses. According to this system, for detection or group 1 viruses (BYDV-PAV, BYDV-MAV, BYDV-SGV) were made with Shu-F and Yan–R primers (Table 3.2), while Yan-R with S2A-F and S2B-F primers were used for detection of group 2 viruses (CYDV-RPV, BYDV-RMV). More specific tests of BYDVs in group 1 and group 2 were targeted using species-specific primers.

Diagnosis of BYDVs using Multiplex RT-PCR, the followings were added to for the tubes.

- 35.6 µl RNase free water
- 5µl 10X PCR Buffer
- 3µl 25mM MgCl2
- 1µl 10mM dNTP
- 1µl 20µM Shu-F } group 1 (832 bp)
- 1µl 20µM Yan R
- 1µl 20µM S2a F } group 2 (372 bp)
- 1µl 20µM S2b F
- 0.4 µl *Taq* DNA polymerase enzyme (5U/µl)

2 µl of cDNA were added to the mixture as template DNA and the total volume of the PCR reaction was 50 µl. Then, the tubes were mixed without foaming, the liquids on the sides of the tubes were precipitated with a microcentrifuge. In the determination of group 1 and group 2 viruses, virus-specific primers and temperature cycles are given in Table 3.3.

Table 3.3. Some primers used in the diagnosis of viruses, amplification products, PCR types, Temperature cycles, and information on used sources (Malmstrom and Shu, 2004).

Viruses	Used Primers	Amplification product	PCR variant	Temperature Cycle and Source s
1. Group Viruses	Yan- R Shu-F	832 bp	the classic Multiplex RT- PCR	94°C …..... 5 min 94°C …..30 sec 60°C ……..30 se 72°C ……..30 sec 35 cycles
2. Group Viruses	S2a S2b	372 bp		72°C …….. 7 min (Malmstrom and Shu, 2004)
BYDV PAV SGV	Yan-R Shu-F SGV-R	832 bp 590 bp	Extended Multiplex RT- PCR	94°C …..... 5 min 94°C ……..60 sec 60°C ……..60 sec 72°C……..60 sec 35 cycles 72°C …….. 7 min (Malmstrom and Shu, 2004)
BYDV MAV BYDV SGV	Yan-R Shu-F MAV-F SGV-R	832 bp 590 bp 254 bp	Extended Multiplex RT PCR	94°C …..... 5 min 94°C ……..60 sec 55°C ….....60 sec 72°C ……..60 sec 35 cycles 72°C …….. 7 min (Malmstrom and Shu, 2004)

Table 3.3. The some primers used in the diagnosis of viruses, amplification products, PCR type temperature cycles and information on used sources (Deb and Anderson, 2008). (Table 3.3.' more continue).

Viruses	Used Primers	Amplification product	PCR variant	Temperature Cycle and Sources
BYDV RMV	RMV-F RMV-R	365 bp	RT- PCR	94°C …..... 2 min 94°C……30 sec 58°C……..60 sec 72°C ……..30 sec (36 cycles) 72°C ……..10 min (Deb and Anderson, 2008)
CYDV RPV	RPV-F RPV-R	400 bp	RT- PCR	94°C …..... 2 min 94°C ……..30 sec 53°C ……..60 sec 72°C ……..30 sec (40 cycles) 72°C ……..10 min (Deb and Anderson, 2008)
BYDV SGV	BYDV-SGV BYDV-SGV R	250 bp	RT- PCR	94°C …..... 2 min 94°C ……..30 sec 55°C…..…60 sec 72°C ……..30 sec (40 cycles) 72°C ……..10 min (Deb and Anderson, 2008)

3.2.2.4. Agarose gel electrophoresis of PCR products, DNA staining, and visualization

Agarose Gel electrophoresis was accomplished to separate PCR products. PCR products were loaded on 1.0% agarose gel. It was prepared by dissolving 1g agarose/100 ml of 1X TAE (Tris-acetate acid –EDTA) and the agarose was dissolved completely by heating in a microwave oven for 5 min. Then, the thawed solution was poured into gel tray with 2µl of ethidium bromide (EtBr) as the staining agent before to solidify the gel for 20-25 min. After the solidification of the agarose, the comb was removed and the gel was placed in the electrophoresis apparatus. 15µl of PCR products were mixed with 5µl of loading dye and loaded into the gel wells. Meanwhile, first or end well was loaded the DNA ladder with loading dye for checking PCR amplifications length. The gel was run at 80-120 Volt for 1 hr. Gels were photographed using a gel imaging and analysis system under UV light.

3.2.2.5. Molecular characterization of BYDV- PAV

An isolate was selected randomly among positive BYDV-PAV samples detected by Multiplex RT-PCR in wheat samples collected from Diyarbakır province and its complete coat protein genes were cloned and characterized. Primer design for the complete coat protein gene of BYDV-PAV was performed by using Vector NTI 10 program. For the primer design, DNA sequences were used that were previously entered into the gene bank. (Accession number) NC_004750.1. The correctness of these primers was checked by the BLAST program and then sent to the Sentegen company (ANKARA) for synthesis. Designed primers: BYDV-PAV – F(5'-CAGTGGATCCATGAATTCAGTAGGTCGTAG-3') and BYDV-PAV-R (5'-CAGTAAGCTTGAGGAGTCTACCTATTTGGC-3'), generating a 614 bp genome fragment for the primer design (Usta, 2013).

3.2.2.5.1. Cloning virus coat protein gene and identification

BYDV-PAV coat protein gene was amplified by RT-PCR using the designed primers were cloned into the pGEM-T Easy plasmid vector by T-A cloning method. The coat protein genes of BYDV-PAV were amplified by PCR method using Go Taq Hot Start polymerase enzyme which shows 5'-3' exonuclease activity which adds adenine base to the 3' end of PCR product.

For RT-PCR process, PCR mixture is comprised the 29 µl of RNase-free water, 10 µl of 5X Go Green Taq Flexi Buffer, 3µl 25mM MgCl2, 1µl 10mM PCR Nucleotide Mix, 1µl of forward primer (100 pmol), 1µl of reverse primer (100 pmol), 0.25 µl of Go Hot Start Taq enzyme (5 U / µl) and 5µl of cDNA. After the tubes were mixed, a short spin was made and placed in on the PCR cycler. To amplify the BYDV- PAV complete coat protein gene, the PCR cycler was programmed as described by Malmstrom and Shu (2004). The BYDV- PAV complete coat protein was generated by PCR with the following thermal cycling scheme: 5 min at 95 °C, 35 cycles of 1 min at 95 °C, 1 min at 42 °C, and 1 min at 72 °C followed by a final extension at 72 °C for 10 min. Obtained PCR products were electrophoresed on 1% agarose gel and by visualized on gel imaging system and photographed. The thickness and brightness of the amplified band

were checked by UV imaging and the DNA bands were cut out with a sterile scalpel (Figure 3.2).

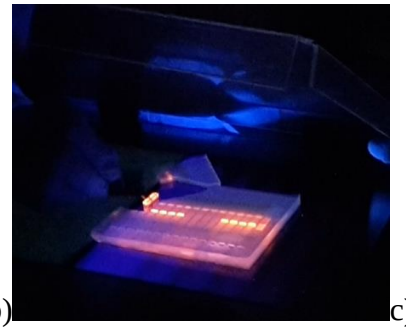
a)  b)  c) 

Figure 3.2. The amplified DNA is visualized with ultraviolet light and cut with scalpel a) cutting off the amplified BYDV-PAV coat protein bands b) transferring of the cut pieces into the Eppendorf tube c) control of DNA fragments.

For the purpose of purifying the agarose gel of BYDV-PAV coat protein gene is used the GeneJet Gel Extraction Kit (Fermantas, K0691) that based on the procedure suggested by the kit.

According to procedure,

- Excise gel slice containing the DNA fragment using a clean scalpel or razor blade. Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a pre-weighed 1.5 mL tube and weigh. Record the weight of the gel slice.
- Add 1:1 volume of Binding Buffer to the gel slice (volume: weight) (e.g., add 100 µL of Binding Buffer for every 100 mg of agarose gel).
- Incubate the gel mixture at 50-60 °C for 10 min or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process. Ensure that the gel is completely dissolved. Vortex the gel mixture briefly before loading on the column. Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution, and mix. The color of the mix will become yellow.
- Transfer up to 800 µL of the solubilized gel solution (from step 3 or 4) to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.

- Add 700 µL of Wash Buffer (diluted with ethanol as described on p. 3) to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
- Centrifuge the empty GeneJET purification column for an additional 1 min to completely remove residual wash buffer.
- Transfer the GeneJET purification column into a clean 1.5 mL microcentrifuge tube (not included). Add 50 µL of Elution Buffer to the center of the purification column membrane. Centrifuge for 1 min.
- Discard the GeneJET purification columnn and store the purified DNA at -20 °C.

3.2.2.5.2. Ligation

BYDV-PAV complete coat protein gene of RT- PCR amplified and pGEM -T Easy vector (Promega, A1360) is combined with a T4 ligase enzyme.
İnto a sterile tube;

	Quantity
Rapid 2x ligation buffer	5 µl
pGEM-T Easy vector	1 µl
BYDV-PAV DNA	5 µl
T4 DNA Ligase	1 µl
Total	12 µl

All of the mixtures was placed in the eppendorf tube and then allowed to incubate overnight at 4 °C for the formation of recombinant plasmids. The recombinant plasmids stored at- 20 °C until use.

3.2.2.5.3. Transformation

The obtained recombinant plasmids were transferred to *Escherichia coli* JM109 strain by electroporation. For this purpose, 5 µl recombinant plasmid and 100 µl bacterial suspension were mixed and incubated for 5 minutes on ice. The transformation was carried out using the Micropulser device Bio-Rad (Figure 3.3).

Figure 3.3 Process the transformation with micropulse device.

The specific cuvettes to be used in the transformation were washed with pure water to remove the ethanol completely before use. After being placed in the recombinant plasmid and bacteria mix baths, the EC2 program was selected and electric current was applied. After this step, which is described as shock phase, 900 µl SOC solution was rapidly added to the bacterial suspension and incubated on shaking platform for 2 h at 150 rpm at 37 ^{0}C. Solid LB Petri dishes containing the previously prepared ampicillin, IPTG and X-GAL were taken from the refrigerator and incubated for 1 hour at 37^{0}C. After the incubation, 200 µl of the bacterial suspension was cultured on the surface of the Petri dishes by drigaski bar as two plates for each sample.

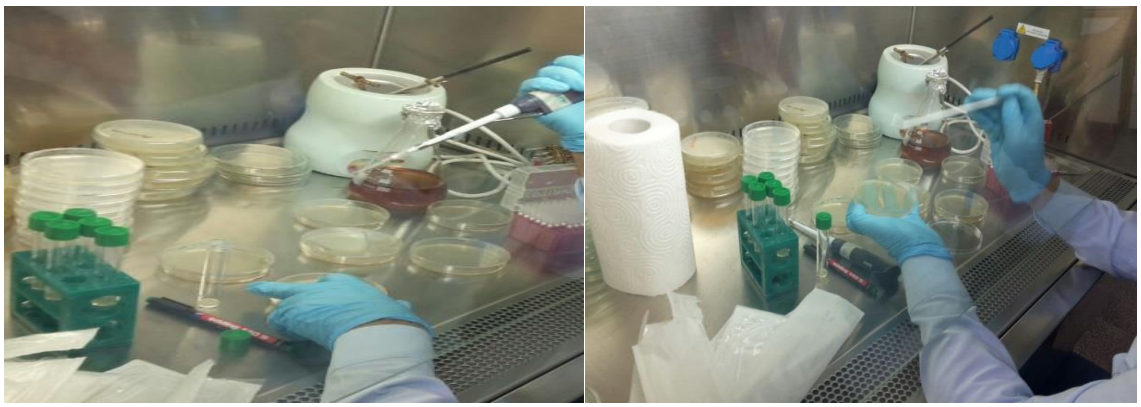


Figure 3.4. Planting of transformed bacteria with SOC to solid LB medium containing ampicillin after electroshock (a , spreading of bacterial suspension to solid medium with sterile drigaski bar (b .

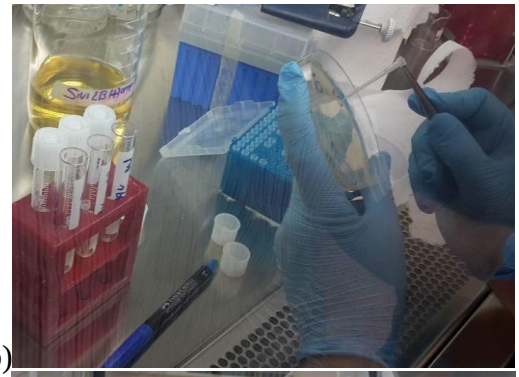
Petri dishes were inverted and incubated to the development of blue-white colonies for overnight at 37^{0}C.

3.2.2.5.4. Growing of single colonies from transformed bacteria

Ampicillin resistance is not found in transformed bacteria. For this reason, after transformation, because of the ampicillin resistance gene is only present in the pGEM-T Easy vector plasmid, bacteria containing only recombinant plasmids will develop in the solid LB medium containing ampicillin. In this case, two types of bacteria will develop in the solid LB medium containing ampicillin; namely plasmid + PCR product DNA-containing bacteria or circulating plasmid-containing bacteria. The first one is white and the other is a blue colony. If the PCR product is transferred to the pGEM-T Easy vector and inserted into Lac Z region, white proteins that causing not be able to the X-Gal and IPTG chemicals will be formed showing white colored bacteria. However, a bacterium including only the plasmatic bacterium is able to use X-Gal and IPTG chemicals and blue colored proteins because the Lac Z region on the plasmid contained therein is not damaged. On the other hand, since Lac Z region is not damaged, blue-colored proteins are formed by bacteria that inserting the only plasmid. Therefore, the color of the colonies is also blue. For this reason, whites are selected from developing colonies and cultured.

3.2.2.5.5. Blue-white selection and growing of the transformed colonies

Selecting the white single colonies that developed from the transformed bacteria that thought to carry insert DNA was added to 4 ml of liquid LB medium containing 100 µl / ml ampicillin and 0.8 ml glycerol into 15 ml capped sterile tubes. (Figure 3.8). In order to reduce contamination in these studies, it was performed in a sterile cabinet. After then, for the reproduce of transformed bacteria and plasmid replication that carry insert DNA was cultured 37 ^{0}C at 150 rpm for 18 hours. The ampicillin in the liquid LB medium was used to prevent the development of plasmid-free bacteria.

a)  b) 

c)  d) 

Figure 3.5 The blue and white colonies grow after transformation (a) select of white colony that from the blue and white colonies developed in solid LB (b) Planting of selected bacteria into liquid LB medium by sterile toothpick (c) incubation of the samples on the shaking platform.

3.2.2.5.6. Purification of recombinant plasmids

The recombinant plasmid which carries insert DNA from the amplified bacteria was carried out by GeneJET Plasmid Miniprep Kit (Fermantas, K0503) according to the company's instructions.

- Resuspend the pelleted cells in 250 µL of the Resuspension Solution. Transfer the cell suspension to a microcentrifuge tube. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
- Add 250 µL of the Lysis Solution and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear.
- Add 350 µL of the Neutralization Solution and mix immediately and thoroughly by inverting the tube 4-6 times.
- Centrifuge for 5 min to pellet cell debris and chromosomal DNA.

- Transfer the supernatant to the supplied GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate.
- Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
- Add 500 µL of the Wash Solution (diluted with ethanol prior to first use as described on p.3) to the GeneJET spin column. Centrifuge for 30-60 seconds and discard the flow-through. Place the column back into the same collection tube.
- Repeat the wash procedure (step 8) using 500 µL of the Wash Solution.
- Discard the flow-through and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.
- Transfer the GeneJET spin column into a fresh 1.5 mL microcentrifuge tube (not included). Add 50 µL of the Elution Buffer to the center of GeneJET spin column membrane to elute the plasmid DNA. Take care not to contact the membrane with the pipette tip. Incubate for 2 min at room temperature and centrifuge for 2 min.
- Discard the column and store the purified plasmid DNA at -20°C.
- Isolated genomic DNS is maintained at -20 °C for use in the other studies.

3.2.2.5.7. Control of colonies carrying insert DNA by colony PCR

The presence of recombinant plasmids in white colored colonies developed in solid LB was also determined by colony PCR, a more sensitive method with virus-specific primers. For each BYDV-PAV isolates, at least 5-10 white colonies were selected and tested by colony PCR method (Figure 3.8).

Accordingly, into sterile PCR tubes is added; 2.5 µl 10X PCR Buffer (200 mM Tris-HCl (pH 8.4), 500 mM KCl), 1.5µl 25mM MgCl2, 0.5µl dNTP mixture, 0.5µl each specific primer pair (100uM), 0.2 mµ of Taq DNA polymerase enzyme (5 U / mL), 18.3 µl of RNase-free water. In the sterile cabin, white colonies that grow in solid LB medium were selected and each colony was enumerated. The sterile toothpick or pipette

tip was touched in the middle of the white column and as using the template DNA was mixed into the PCR tube (Figure 3.9).

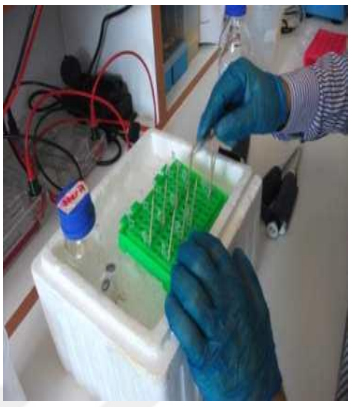
a)  b)  c) 

Figure 3.6. After the transformation, blue and white colonies (a) getting single colony from white colonies which developed in solid LB medium (b) putting of white bacteria into PCR tubes with a toothpick (c).

PCR tubes were placed into the PCR device after a short period of vortexing. The PCR device was programmed and was run in accordance with the following program;

- 94°C…. 5 min
- 94°C…. 60 sec
- 55°C ….60 sec
- 72°C ….60 sec
- 72°C…. 5 min

(94°C…. 60 sec, 55°C ….60 sec, 72°C ….60 sec) ┐ 40 cycles

If the cloned gene was not found in the first selected colonies, the same procedure was repeated as before. The PCR products obtained after the colony PCR were run on a 1% agarose gel with DNA ladder, separated by electrophoresis method, stained with ethidium bromide, and then displayed and photographed in gel imaging and analysis system under ultraviolet light.

3.2.2.5.8. DNA sequence analysis of the cloned PCR products

After molecular cloning, 25 µl of the recombinant plasmids were sent to IONTEK Inc. (Merter-Istanbul) for bi-directional DNA sequencing with a universal primer that specific to the promoter region of the isolate and the remainder was stored at -20 ° C for to be used later. The genome information obtained after sequence analysis

was analyzed with different bioinformatics programs as CLC Main Workbench, Mega 4 and Vector NTI programs.

Percent similarity rates of the nucleotide and amino acid sequences of the coat protein gene of the virus isolate and the phylogenetic tree was determined using the CLC Main Workbench 6 program. Thus, the similarities and differences between the BYDV-PAV Diyarbakır isolate and other virus isolates which submitted in NCBI GeneBank in the world were determined by conducting multiple sequence comparisons. Finally, the phylogenetic tree was created to reveal the genetic similarities and differences between our isolates and other world isolates.

3.2.2.5.9. Submit of DNA sequences into the GenBank

The sequence of amplified coat protein gene of BYDV-PAV Diyarbakır isolate was submitted to the National Center for Biotechnology Information (NCBI) Gene Bank.

3.2.2.5.10. Phylogenetic analysis of the coat protein gene

A random isolate was selected from the BYDV-PAV isolates detected in Diyarbakır province, and the complete coat protein genes were subjected to multiple comparisons (multiple nucleotide and amino acid sequence comparisons) by Geneious 6.1.5 program selecting virus isolates detected in different countries from the Genbank. The genetic distance model for the phylogenetic tree was constructed using the Tamura-Nei algorithm and a phylogenetic tree was created by neighbor-joining. In order to increase the reliability of the generated family trees, 100 recurrent bootstrap analyzes were applied. At the same time, when a phylogenetic tree is created, an out of group virus isolation was used in order to create an outgroup. BYDV-PAV Diyarbakır isolates were compared with other isolates in the world and genetic relationships were revealed.

3. RESULTS AND DISCUSSIONS

4.1. Disease Survey and the incidences of detected viruses

365 wheat samples were tested to determine the presence and rates of infections of BYDV-PAV, BYDV-MAV, BYDV-SGV, BYDV-RMV and CYDV-RPV viruses in Diyarbakir province (Bismil, Çınar, Sur, Silvan) in 2016.

Sampling studies were carried out in April-May 2015-2016 according to the wheat production amounts of all the district of Diyarbakır province. During surveys, viral infections, such as dwarfing, mosaic, reddening of flag leaves, and chlorotic streak patterns on leaves were observed rarely in the wheat fields (Figure 4.1).

Figure 3.1. General view of – disease symptoms on a wheat in the fields (a,b,c) Red colors on wheat leaves in Bismil (e) Wheat leaves exhibiting chlorotic stains in Bismil (d,.f) Chlorosis on wheat fields in Çınar.

4.2. Detection of *Barley yellow dwarf viruses* (BYDV- PAV, BYDV- SGV, BYDV-MAV, BYDV- RMV and CYDV- RPV) by Multiplex RT-PCR and RT- PCR methods

As a result of molecular tests, the presence of BYDV-PAV, BYDV-SGV and CYDV-RPV viruses were detected in 18 of 365 samples. BYDV-MAV and BYDV-RMV viruses were not detected in any of the tested samples. It has been found out that BYDV-PAV+BYDV-SGV mix infection has detected in 9 samples (2.4 %), BYDV-PAV+ CYDV-RPV mix infections were found in 3 samples (0.8 %), BYDV-SGV+CYDV–RPV mix infections were found in 3 samples (0.8 %) and BYDV-PAV+BYDV-SGV+CYDV-RPV mix infection in 3 samples (0.8 %). Districts infection rates, number of samples tested, detection rates of viruses and viruses detected during 2016 in Diyarbakır are given Table 4.1.

The highest disease incidence (5 %) was recorded in Bismil district. Among 160 collected samples 5 % (8 samples) were infected with BYDV-PAV, 3.1 % (5 samples) infected with BYDV-SGV and 3.1 % (5 samples) were infected with CYDV- RPV. 80 wheat samples were collected in Çınar district and 1.2 % were infected with (1 sample) with BYDV-PAV and 1.2 % (1 sample) infected with BYDV- SGV. From the Silvan district 70 samples were tested. BYDV-PAV was detected in 4.2 % (3 Samples), BYDV- SGV were detected in 4.2 % (3 samples). A sample was found infected by BYDV-PAV and 1.8 % 1 sample BYDV- SGV in 55 samples collected from Sur district (Table 4.1.)

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Table 4.1. Infection rates and detection of different group and types of *Barley yellow dwarf viruses* in wheat samples collected from different district of Diyarbakir during 2016.

Diyarbakir Location	NO. Test	BYDV-PAV		BYDV-SGV		CYDV-RPV		BYDV - PAV + BYDV-SGV		BYDV- PAV + CYDV-RPV		BYDV - SGV + CYDV - RPV		BYDV PAV+SGV + CYDV-RPV		General infection rate of Diyarbakir	
		No.(IP)	(PI) %	No.(IP)	(PI) %	No.(IP)	(PI) %	No.(IP)	(PI)%	No.(IP)	(PI) %	No.(IP)	(PI) %	No.(IP)	(PI) %	No.(IP)	(PI) %
Bismil	160	8	5 %	5	3.1 %	5	3.1 %	4	2.5 %	3	1.8 %	3	1.8 %	3	1.8 %	13	8.1%
Çınar	80	1	1.2 %	1	1.2 %	-	-	1	1.2 %	-	-	-	-	-	-	1	1.2 %
Silvan	70	3	4.2 %	3	4.2 %	-	-	3	4.2 %	-	-	-	-	-	-	3	4.2 %
Sur	55	1	1.8 %	1	1.8 %	-	-	1	1.8 %	-	-	-	-	-	-	1	1.8 %
Total	365	13	3.5 %	9	2.4 %	5	1.3 %	9	2.4 %	3	0.8 %	3	0.8 %	3	0.8 %	18	4.9 %

No.test: number of tests, No. IP: Number of the infected plants, PI: Infection rate.

Comprehensive surveys were carried out in the province of Diyarbakır in April and May 2016. Multiplex RT- PCR tests were performed to identify barley yellow dwarf viruses (BYDV- PAV, BYDV- MAV, BYDV- SGV, BYDV- RMV and CYDV- RPV). The gel images from obtained multiplex RT- PCR which positive reactions are given below (Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5, Figure 4.6, Figure 4.7, Figure 4.8,).

Figure 3.2. Multiplex RT-PCR analysis for wheat leaf samples from Bismil district (M:100-1000), 1:15, 2:20, 3:46, 4:54, 5:55, 6:63, 7:64, 8:65 (positive control), 9:64 negative control.

It was determined that sample No.8:65 at the position, which gave a DNA fragmentation of 832 bp in length, was infected with at least one of the BYDV viruses. No virus infection was detected in other samples.

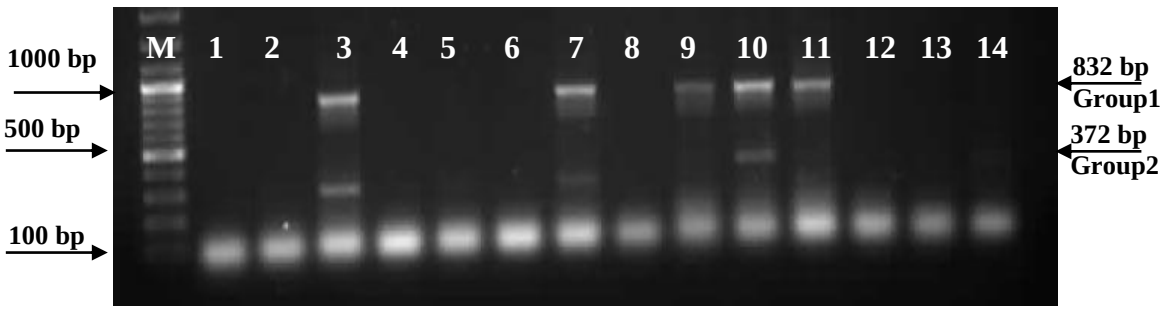

Figure 3.3. Multiplex-PCR analysis on agarose gel for wheat leaf samples from Bismil (M:100-1000), 1:1, 2:2, 3:3, 4:4, 5:5, 6:6, 7:7, 8:8, 9:166, 10:117, 11:105, 12:178, 13;65 and 14:65.

Samples of 3, 7, 9, 10 and 11, which give a DNA fragmentation of 832 bp in length, was infected with at least one of the BYDV viruses of group1 (BYDV- PAV, MAV, SGV). Only sample number 10 was found to be infected with the group 2 of viruses (BYDV-RMV, CYDV-RPV), giving a band of 372 bp in length. No virus infection was detected in other samples.

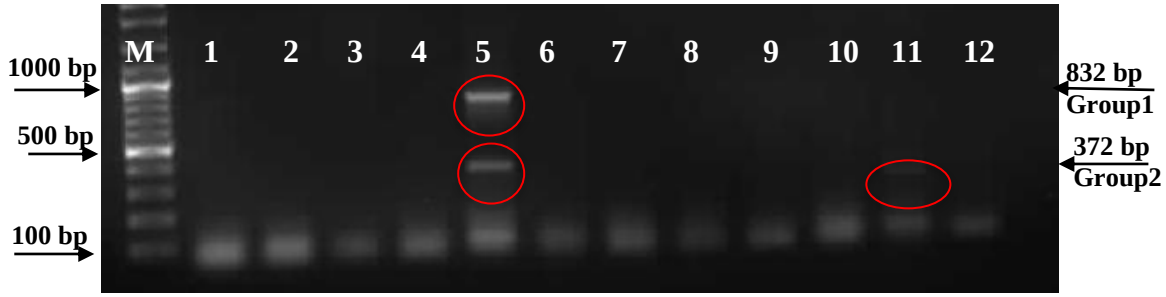

Figure 3.4. Multiplex RT-PCR analysis on agarose gel for wheat leaf samples from Bismil (M:100-1000), 1:121, 2:179, 3:159, 4:171, 5:163, 6:167, 7:102, 8:186, 9:177, 10:109, 11:221 and, 12:65.

Figure 3.5. Multiplex-PCR analysis of wheat leaf collected the samples in collected from Bismil M:100-1000 ,1;17, 2:18, 3:170, 4:65, 5:19, 6:13, 7:185, 8:276, 9:16, 10:14 and, 11:283

Figure 3.6. Multiplex RT-PCR analysis of wheat leaves samples collected from Çınar M:Marker (100-1000 bp),1:125, 2:111, 3:123, 4:166, 5:106, 6:112, 7:12, 8:104, 9:9, 10:180, 11:10, 12:128 and, 13:225, from Çınar District samples number 4:123, were positives.

Figure 3.7. Multiplex RT-PCR analysis of wheat leaves samples collected from Silvan M:Marker (100-1000 bp), 1:150, 2:151, 3:152, 4:153, 5:154, 6:155, 7:157, 8:158, 9:156, 10:650, 11:161, 12:162, 13:123 and, 14: 164.

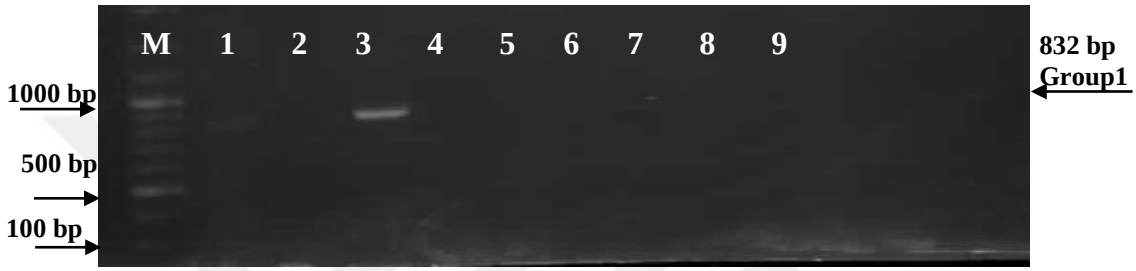

Figure 3.8 Multiplex RT-PCR analyze of field wheat leaves samples from M:Marker (100-1000 bp), 1:166, 2:168, 3:169, 4:172, 5:173, 6:174, 7:175, 8:246 and, 9:300, the number 1:P166, 3:P169 were positive.

As shown in all pictures above (Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5 Figure 4.6, Figure 4.7, and figure 4.8), eighteen samples which no 3, 7, 65, 105, 117, 123, 152, 161, 163, 152, 164, 166, 221, and 283 were reacted positively. The samples number 3, 7, 65, 105, 117, 123, 152, 161, 163, 152, 164, 166 and 169 were infected with group 1 viruses (BYDV- PAV, BYDV- MAV, BYDV- SGV) exhibited DNA bands of 832 bp in length. In total, 5 samples (65, 117, 163, 221, 283) were infected with group 2 viruses (BYDV-RMV, CYDV- RPV), exhibiting DNA bands of 372 bp in length.

The sample number 65, 117, and163, were exhibited two bands, 832 bp and 372 bp DNA fragment that suspected with infected at least two viruses, samples number 221 and 283 were exhibited only 372bp. All the positively reacted samples were represented in Table 4.1

4.1.1. Findings for the identification of BYDV species (BYDV-PAV, BYDV-MAV and BYDV-SGV) specifically by multiplex RT-PCR

All samples that gave 832 bp and 372 bp DNA fragment in multiplex RT-PCR were subjected to multiplex RT-PCR using species-specific primers. BYDV-PAV infections were detected in a total of 13 samples by RT-PCR testing using primers sets belonging to Usta (2013), exhibiting DNA fragment of 614 bp in length (Figure 4.9).

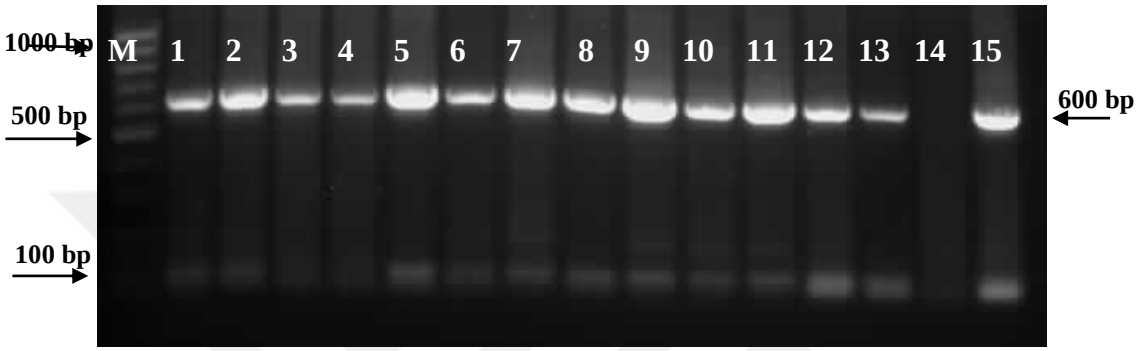

Figure 3.9. RT-PCR analysis for detection of BYDV-PAV in wheat leaf samples M:Marker (100-1000 bp) 1;3, 2;7, 3;105, 4;117, 5;123, 6;152, 7;161, 8;163, 9:164, 10:166, 11:169, 12:170, 13:65.14:Negative control and 15:Positive control.

Likewise, BYDV-PAV virus has been detected in samples 1:3, 2;7, 3;105, 4;117, 5;123, 6;152, 7;161, 8;163, 9:164, 10:166, 11:169, 12:170, 13:65.14:100 and 15:65 (Figure 4.10).

Multiplex RT-PCR tests using Yan R, Shu F, MAV-F and SGV-R primers did not found BYDV-MAV virus giving DNA fragment at 590 bp in any of the samples. BYDV-MAV infection was not found in any of the samples (Figure 4.10).

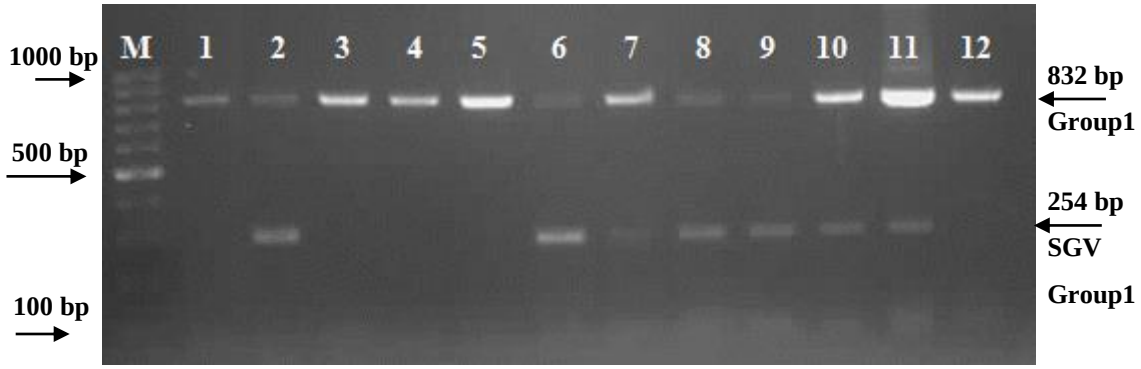

Figure 3.10. Multiplex RT-PCR for of (BYDV-MAV and BYDV-SGV) in wheat leave samples 1;164, 2;165, 3;166, 4;169, 5;170, 6;183, 7;221, 8;256, 9:260, 10:261, 11:263 and 12:163 .

As shown Figure 4.11, the primers used for detecting BYDV-SGV virus did not give the desired quality results in the multiplex RT-PCR tests, A different set of primers for better results, was used for the detection of these viruses (Deb and Anderson, 2008) (Figure 4.11).

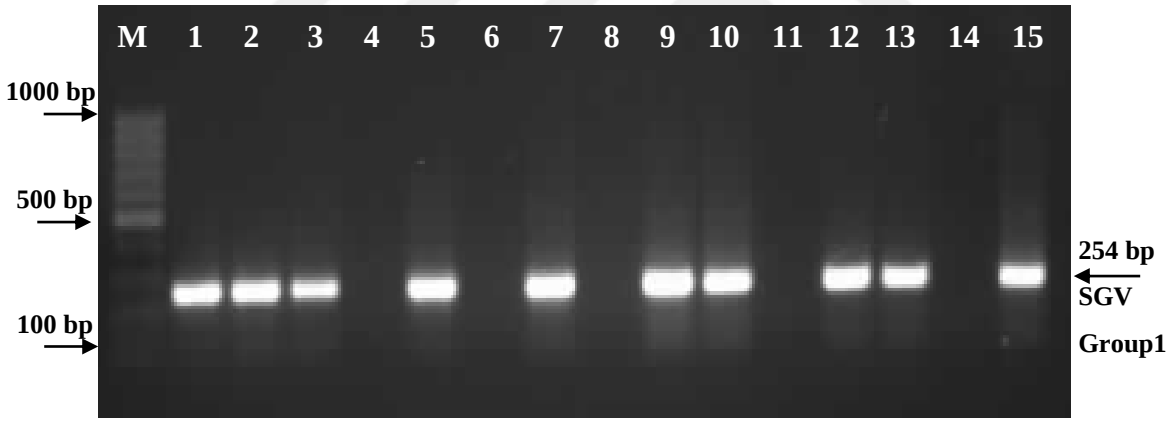

Figure 3.11. Multiplex RT-PCR analysis for detection of BYDV-SGV in wheat leave samples M:Marker (100-1000 bp) 1;3, 2;7, 3;117, 4;105, 5;123, 6;152, 7;161, 8;163, 9:164, 10:166, 11:169, 12:170, 13:65.14:Negative control and 15:Positive control.

As a result, the BYDV-SGV was found in 9 samples and BYDV-PAV was found in 18 samples in wheat samples collected from Diyarbakır province.

4.2.2. Findings for investigation of *Cereal yellow dwarf virus*-RPV (CYDV-RPV) by RT-PCR

Samples are giving 372 bp suspect DNA fragments by multiplex RT-PCR were researched by RT-PCR to identify the group 2 (BYDV-RMV and CYDV-RPV) viruses. PCR tests confirmed that 5 wheat samples (sample no 65, 117, 163, 221, 283) were infected with CYDV-RPV virus appearing a fragment of 400 bp in length (Figure 4.13). BYDV-RMV virus was not detected in any of the wheat samples.

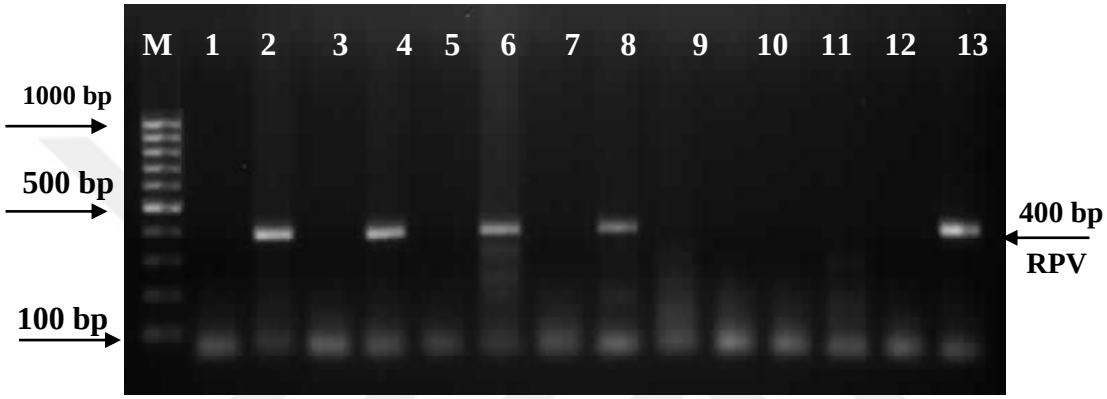

Figure 4.12. Determination of CYDV-RPV virus by RT-PCR 1:3, 2:163, 3:7, 4:221, 5:105, 6:283, 7:110, 8:117, 9:320, 10:230, 11:123, 12:256, and 13:65 (positive control)

Survey studies conducted in our country and in different parts of the world have shown that wheat viruses reveal infection rates at different levels in the world. It is stated that the most common virus of Barley yellow dwarf virus is BYDV-PAV (Conti et al., 1990, El-Yamani and Hill, 1990). In a study conducted by Dot-blot hybridization method in China, BYDV-PAV was observed to be 7.92 % in 2004, 27.50 % in 2005 and 31.82 % in 2006 (Liu et al., 2007). In this study, BYDV-PAV virus was detected as the most common virus in agriculture wheat in Diyarbakır.

In Hungary, BYDV-PAV the infection rate in wheat specimens was investigated by PCR and BYDV-PAV virus infection was detected as 58 % (Ay et al., 2008). In Pakistan, 45 of Wheat, Barley, Trikala and oat samples were collected to investigate BYDV (PAV, MAV, RPV, RMV, and SGV). According to the results of the ELISA test, samples were infected with 64.4 % BYDV-PAV, 40 % BYDV-MAV and 4.4 % BDV-SGV (Bashir et al., 1997). One of the most important viruses encountered in

cereal production in Turkey is BYDV-PAV (İlbağı *et al*., 2003, 2005, Pocsai *et al*., 2003).

A multiplex RT-PCR method was used to investigate BYDV viruses in 900 wheat samples collected in Eastern Anatolia in Turkey. As a result of the tests, wheat samples were found to be infected with 5.5 % BYDV-PAV, 4.8 % BYDV-SGV and 0.4 % CYDV-RPV. No BYDV-MAV or BYDV-RMV viruses were detected in any of the tested samples (Usta, 2013). The results of this work carried out in the province of Diyarbakır are similar to those in the Eastern Anatolia Region. Although the infection rates of viruses differ, the same viruses have been detected in Eastern Anatolia Region and Diyarbakır province. However, the detection rates of viruses were found to be lower than the other regions.

260 plant samples were collected from wheat-cultivated fields in the central and surrounding districts of Tekirdag in Trakya region in 2003. These samples were tested by ELISA and the presence of wheat samples was determined as 25 % BYDV-MAV, 22.3 % BYDV-PAV and 8.5 % CYDV-RPV (Köklü, 2004). Another study conducted in Trakya region reported that 63 of 90 leaf samples collected in Edirne, Kırklareli, and Tekirdağ were infected with BYDV-PAV by ELISA in 2001 (İlbağı, *et al*., 2008). The rates of virus infections detected in the samples tested in this study are much lower than the rates of infection detected in our country and in the world. Also, in other agricultural products in the Eastern Anatolia Region, virus infections have been reported to be at a very low level (Sipahioğlu, 2011). Infection rates of virus diseases may vary depending on the sensitivity of the plant, the development of the plant, the favored condition of the vector and other environmental factors (Köklü, 2004). Product losses caused by BYDV infections can reach up to 9-79 % depending on the infection period and plant's variety (Sutic, 1999). Maize, weed and vector samples were collected from corn fields, and the presence *Barley yellow dwarf virus* (BYDV), *Maize dwarf mosaic virus* (MDMV), *Maize mosaic virus viruses* (MSpV) have been investigated. MDMV with 8.04 %, BYDV with 4.8 %, MSPV with 1.8 % and MMV with 1.6 % were determined in collected samples (Fidan and Yılmaz, 2004).

Nowadays, RT-PCR testing is routinely preferred in the identification of plant viruses. Many researchers have used multiplex PCR to detect two or three virus strains in different hosts in addition to detecting wheat viruses (Grieco and Gallitelli, 1999, Saade *et al*., 2000, Sharman *et al*., 2000). Thanks to a PCR reaction involving compatible primers, three or more viruses can be detected at the same time, quickly and cost-effectively (Bariana *et al*., 1994, Nie and Singh, 2000, Canning *et al*., 1996, Gilbert, 2002). Although multiplex PCR has advantages such as cost and time saving, it is less sensitive than classic PCR. In the multiplex RT-PCR test, the components required for an amplification reaction such as polymerase buffer, Mg2 +, dNTP and Taq polymerase enzyme are simultaneously consumed more. For this reason, the efficiency of each reaction is lower than that of classic PCR (Tao et al., 2012). Deb and Anderson (2008) have successfully diagnosed and distinguished BYDV (PAV, MAV, SGV and RMV), CYDV-RPV, WSSMV, WSMV and SBWMV simultaneously by multiplex RT-PCR. Malmstrom and Shu (2004) have developed two new multiplex RT-PCR methods that can differentiate barley and cereal yellow dwarf viruses (B/CYDV). In wheat samples collected from the Eastern Anatolia region, the diagnosis of barley yellow dwarf viruses was carried out according to the method developed by Malmstrom and Shu (2004). In this method, cDNA synthesis was performed on all samples using the universal Yan-R reverse primer, which recognizes all types of barley yellow dwarf viruses. In this way, the deterioration and contamination of total RNAs are prevented due to repeated thawing and freezing as well as labor and cost savings.

4.2.3. Molecular test for wheat samples

4.2.3.1. Characterization of the coat protein gene of BYDV-PAV Diyarbakir isolate

Sample no 65 of Diyarbakır BYDV-PAV isolate were selected randomly for complete coat protein gene characterization. The coat protein gene of Diyarbakır BYDV-PAV isolate was amplified by RT-PCR using sense and antisense primers and was cloned into the pGEM-T Easy vector system (Figure 4.13).

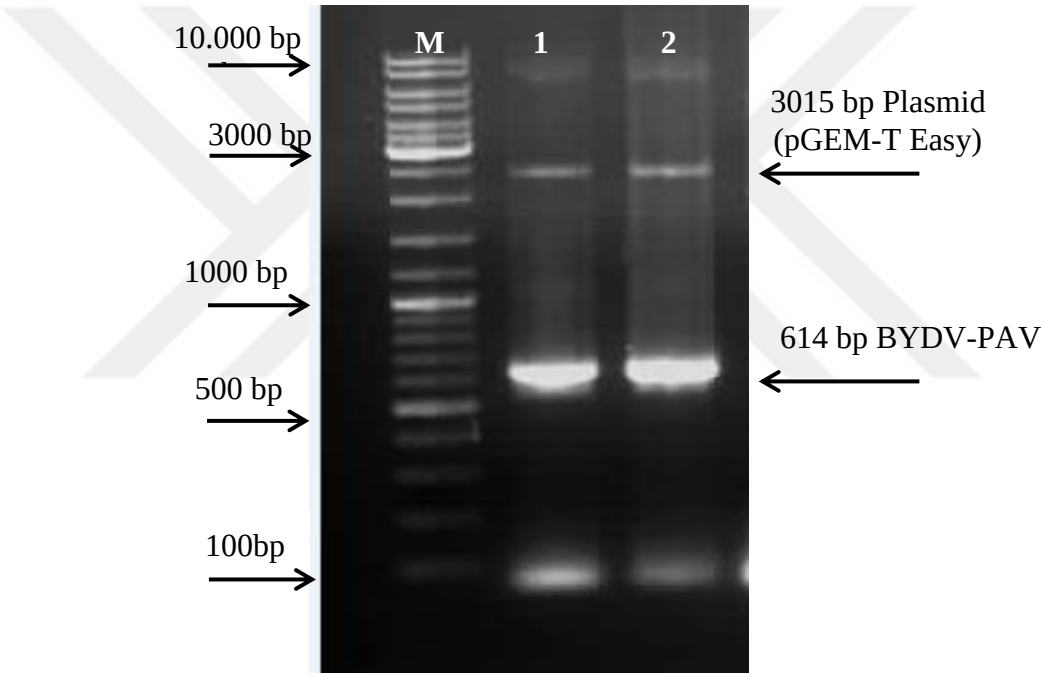

Figure 3.13. An agarose gel image showing the amplification complete coat protein gene of the BYDV-PAV isolate (sample no 65).

The coat protein gene of BYDV-PAV Diyarbakır isolate (No. 65) was found to be 603 bp in length. The recombinant plasmid containing the coat protein gene was subjected to bidirectional DNA sequencing (Sentegen Biotech- ANKARA). The DNA sequence was of BYDV-PAV submitted to the NCBI gene bank (http://www.ncbi.nlm.nih.gov) with the accession number of KX774424 (Table 4.2).

Table 4.2. The complete coat protein gene sequence of BYDV-PAV Diyarbakır isolate (No. 65).

Base sequence bp	Nucleic acid sequence of BYDV-PAV Diyarbakır isolate
1	atgaattcag taggtcgtag aggacctaga agagcaaacc aaaatggccc aagaaggcgg
61	caccgtagag caattcggcc agtggttgtg gtccaaccca atcgagcagg acccagacga
121	cgaaatggtc gacgcacacg aagaagaggg ccaaattcta tacttagacc aacaggcggg
181	tctgaggtat tcgtattctc aatcgacaac attaaagcca actcttccgg ggcaatcaaa
241	ttcggcccca gtctatcgca atgcccagcg ctttcagacg gaatacttaa gtcctaccac
301	cgttacaaga tcacaagtat ccgtgttgag tttaagtcac acgcgtcctc cactacggca
361	ggcgctatct ttattgaact cgacaccgcg tgcaagcaat cagccctggg tagctacatt
421	aattccttca ccatcagcag gaccgcctca aagaccttca gagccgaggc aattaacggg
481	aaggaattcc aagaatcgac gatagaccaa ttctggatgc tctacaaagc caatggaacc
541	acaactgaca ccgcaggaca atttatcatc accaagaaag tcaatttgat gactgccaaa
603	tag

It has been shown that the coat protein gene of BYDV-PAV Diyarbakır isolate has the same length as the coat protein gene lengths of other isolates in the world. As shown in Table 4.3. 21 different isolates were selected in the gene bank database, previously identified in different regions of the world to compare with our isolate (Table 4.3). The similarity between BYDV-PAV Diyarbakır isolate and 21 other isolates were demonstrated by phylogenetic analysis (Figure 4.14).

Table 3.3. The access number, host, country, gene and length of the BYDV-PAV complete coat protein gene in the Gen Bank.

NO.	Access number	Host	Country	Gene	Length
1	AJ007918	-	Francie	Coat protein	603 bp
2	AJ007926	-	Francie	Coat protein	603 bp
3	AJ223587	*Lolium multiflorum*	Francie	Coat protein	603 bp
4	AJ223588	*Hordeum vulgare*	Francie	Coat protein	603 bp
5	AJ295639	*Hordeum vulgare*	Greece	Coat protein	603 bp
6	AY167109	-	Francie	Coat protein	603 bp
7	DQ285674	-	America	Coat protein	603 bp
8	FJ875303	*Triticum aestivum*	China	Coat protein	603 bp
9	JQ811487	*Triticum aestivum*	Pakistan	Coat protein	603 bp
10	JX067842	*Hordeum vulgare*	Brazil	Coat protein	603 bp
11	JX067845	*Lolium spp.*	Brazil	Coat protein	603 bp
12	JX067846	*Triticum aestivum*	Brazil	Coat protein	603 bp
13	JX067847	*Triticum aestivum*	Brazil	Coat protein	603 bp
14	JX067849	*Triticum aestivum*	Brazil	Coat protein	603 bp
15	JX067850	*Zea mays*	Brazil	Coat protein	603 bp
16	JX067851	*Avena sativa*	Brazil	Coat protein	603 bp
17	JX067852	*Triticum aestivum*	Brazil	Coat protein	603 bp
18	JX473287	*Sorhgum halapense*	Pakistan	Coat protein	603 bp
19	JX473288	*Lolium multiflorum*	Pakistan	Coat protein	603 bp
20	KX900900	Triticum aestivum	Turkey	Coat protein	603 bp
21	KX774424	*Triticum aestivum*	Turkey	Coat protein	603 bp
22.	KP096226	*Bougainvillea spectabilis*	Turkey	Antiviral protein	893 bp

As a result of multiple nucleic acid comparisons, the BYDV-PAV Diyarbakır isolate was found to be similar to other isolates in the world at 81.41-98.34%. BYDV-PAV Diyarbakir isolate is showed similarity with 98.34% maximum Turkey isolate (KC900900) and with 81.41% minimum with China isolate FJ875303 (Figure 4.1).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
JX067842	1		100.00	100.00	99.83	99.83	99.67	99.67	99.67	98.34	98.34	95.36	94.69	96.35	95.36	94.69	92.87	92.37	90.22	90.38	89.55	81.43
JX067846	2	100.00		100.00	99.83	99.83	99.67	99.67	99.67	98.34	98.34	95.36	94.69	96.35	95.36	94.69	92.87	92.37	90.22	90.38	89.55	81.43
JX067852	3	100.00	100.00		99.83	99.83	99.67	99.67	99.67	98.34	98.34	95.36	94.69	96.35	95.36	94.69	92.87	92.37	90.22	90.38	89.55	81.43
JX067847	4	99.83	99.83	99.83		99.67	99.83	99.83	99.83	98.34	98.34	95.36	94.69	96.35	95.36	94.86	93.03	92.54	90.05	90.22	89.39	81.26
JX067849	5	99.83	99.83	99.83	99.67		99.50	99.50	99.50	98.51	98.51	95.52	94.86	96.52	95.52	94.86	93.03	92.54	90.22	90.22	89.55	81.43
JX067845	6	99.67	99.67	99.67	99.83	99.50		99.67	99.67	98.18	98.18	95.52	94.69	96.19	95.19	94.69	92.87	92.37	89.88	90.05	89.22	81.09
JX067850	7	99.67	99.67	99.67	99.83	99.50	99.67		100.00	98.18	98.18	95.52	94.86	96.52	95.52	95.02	93.20	92.70	90.22	90.38	89.55	81.09
JX067851	8	99.67	99.67	99.67	99.83	99.50	99.67	100.00		98.18	98.18	95.52	94.86	96.52	95.52	95.02	93.20	92.70	90.22	90.38	89.55	81.09
AJ007918	9	98.34	98.34	98.34	98.34	98.51	98.18	98.18	98.18		98.34	94.69	94.03	96.02	94.69	94.20	92.04	91.54	88.72	88.72	89.39	79.93
AJ223587	10	98.34	98.34	98.34	98.34	98.51	98.18	98.18	98.18	98.34		95.36	94.69	96.35	95.69	94.53	92.70	92.21	89.22	89.39	89.05	80.60
JX473288	11	95.36	95.36	95.36	95.36	95.52	95.52	95.52	95.52	94.69	95.36		98.01	98.01	97.35	94.53	92.87	91.87	89.55	89.55	88.89	80.60
JX473287	12	94.69	94.69	94.69	94.69	94.86	94.69	94.86	94.86	94.03	94.69	98.01		97.35	96.68	93.86	91.87	91.04	88.72	88.72	88.06	80.27
DQ285674	13	96.35	96.35	96.35	96.35	96.52	96.19	96.52	96.52	96.02	96.35	98.01	97.35		98.34	95.19	93.86	93.03	90.38	90.38	90.05	80.27
AJ295639	14	95.36	95.36	95.36	95.36	95.52	95.19	95.52	95.52	94.69	95.69	97.35	96.68	98.34		95.19	92.70	91.87	89.72	89.88	89.22	80.43
JQ811487	15	94.69	94.69	94.69	94.86	94.86	94.69	95.02	95.02	94.20	94.53	94.53	93.86	95.19	95.19		94.20	93.03	90.71	90.88	90.55	81.43
KX900900	16	92.87	92.87	92.87	93.03	93.03	92.87	93.20	93.20	92.04	92.70	92.87	91.87	93.86	92.70	94.20		98.34	91.21	91.71	91.38	81.59
KX774424	17	92.37	92.37	92.37	92.54	92.54	92.37	92.70	92.70	91.54	92.21	91.87	91.04	93.03	91.87	93.03	98.34		90.71	91.21	90.88	81.43
AJ223588	18	90.22	90.22	90.22	90.05	90.22	89.88	90.22	90.22	88.72	89.22	89.55	88.72	90.38	89.72	90.71	91.21	90.71		99.34	98.84	81.92
AY167109	19	90.38	90.38	90.38	90.22	90.22	90.05	90.38	90.38	88.72	89.39	89.55	88.72	90.38	89.88	90.88	91.71	91.21	99.34		99.17	82.26
AJ007926	20	89.55	89.55	89.55	89.39	89.55	89.22	89.55	89.55	89.39	89.05	88.89	88.06	90.05	89.22	90.55	91.38	90.88	98.84	99.17		81.76
FJ875303	21	81.43	81.43	81.43	81.26	81.43	81.09	81.09	81.09	79.93	80.60	80.60	80.27	80.27	80.43	81.43	81.59	81.43	81.92	82.26	81.76	

Figure 4.14. Similarity rates the nucleic acid sequences of the coat protein genes of BYDV-PAV Diyarbakır isolate (KX774424) with other isolates in the world by the CLC Main Workbench program.

Furthermore, the nucleic acid sequence of the BYDV-PAV Diyarbakır isolate (KX774424) was carried out the multiple alignments with the nucleic acid sequences of other isolates in.

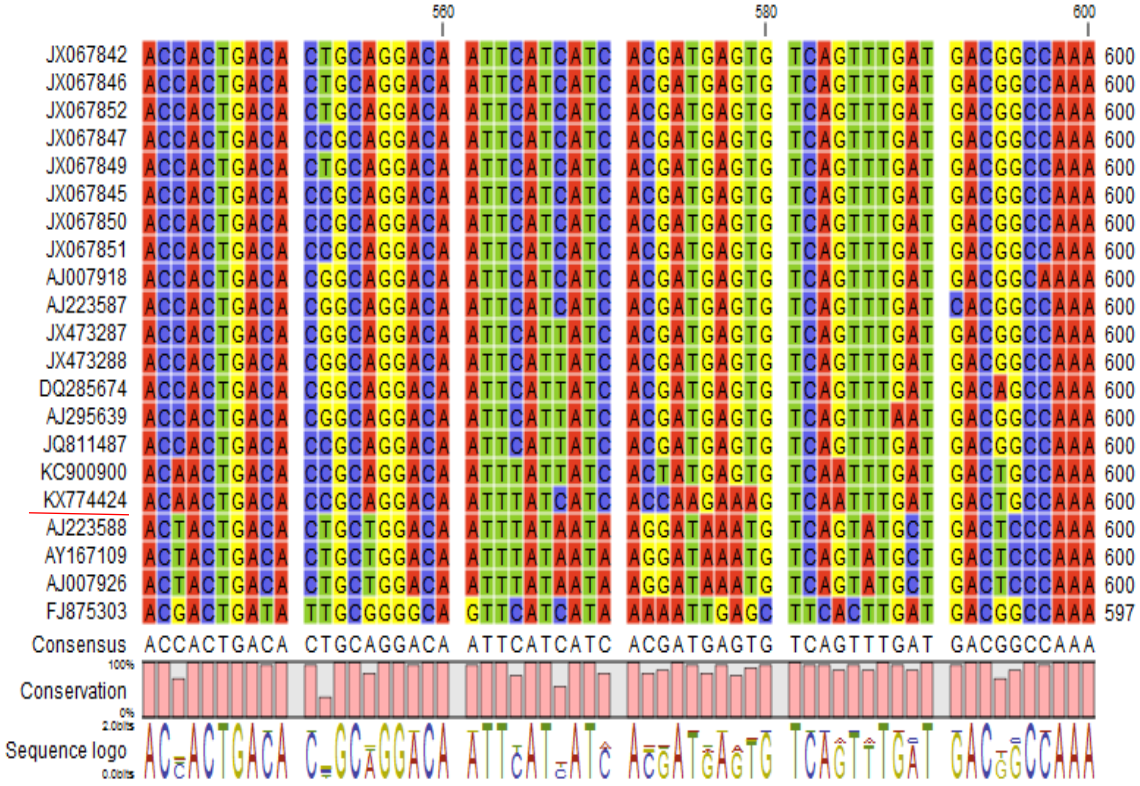

Figure 3.15 Comparison of multiple nucleotide sequences of the coat protein genes of BYDV-PAV Diyarbakır isolate (KX774424) with other BYDV-PAV isolates in the world.

4.3. Phylogenetic Analysis

A phylogenetic analysis was performed result of BYDV-PAV Diyarbakir isolate with the world isolates of BYDV-PAV Van isolates revealed that this isolate is similar to the Turkish isolate with 100% (Figure 4.14). BYDV-PAV Diyarbakir isolate was different from all other world isolate groups, that's why it is not positioned in any world groups, the Recent study of the diversity of BYDV-PAV showed that the isolates of this species could be divided into thirty groups based on the CP nucleotide sequence (Bisnieks et al., 2004).

Phylogenetic BYDV of Diyarbakir isolates with Van isolates is in the same group with 100 %. In general, the difference between isolation coat protein of BYDV Diyarbakir with the whole world coat protein isolations is ranged from 48 % to 100% the difference will make in terms of phylogenetic for instance, Diyarbakir isolation BYDV with Pakistan that each one of them belongs to the same family or section but they refer to same gender or genetic.

According to MEGA 4.1 program, genetic distance divided into six main groups. Each group divided into sub-min groups will be separated. The high similarity between species was KX774424 and KX900900. .

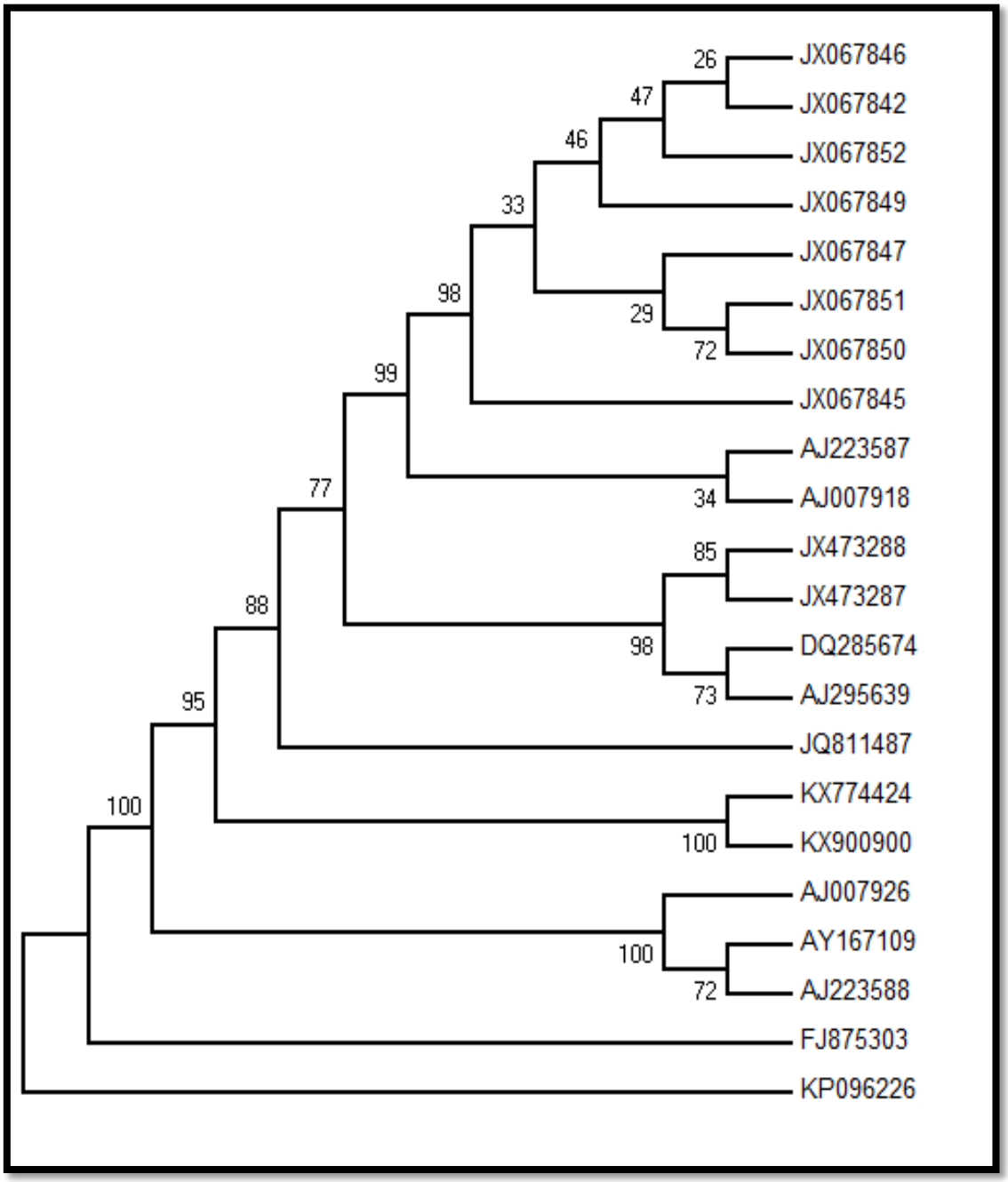

Figure 4.16. Phylogenetic analysis using the CP amino acid sequences of BYDV- PAV Diyarbakir isolate KX774424 with twenty world isolates of CP amino acid sequence.

5. CONCLUSION

Up to date, there have been studies investigating wheat viruses in Diyarbakır province. The present study is the most comprehensive work carried out in the districts of Diyarbakır provinces to investigate wheat viruses.

A total of 365 wheat leaf samples from wheat fields were collected in 2016 in Diyarbakır province, and the presence of *Barley yellow dwarf viruses* (BYDV-PAV, SGV, MAV, RMV and CYDV-RPV) was investigated by multiplex RT-PCR and RT-PCR methods.

Three different virus types (BYDV-PAV, BYDV-SGV, CYDV-RPV) were detected in wheat samples tested in the districts of Diyarbakır. BYDV-MAV and BYDV-RMV viruses were not detected in any of the 365 wheat samples tested.

BYDV-PAV was detected in 13 samples (3.5 %), BYDV-SGV was detected in 9 samples (2.4 %) and CYDV-RPV was detected in 5 samples (1.3 %), BYDV-PAV has been identified as the most common virus in Diyarbakır province as are in different regions of the world and in Turkey as well. It has been found that mixed infections of BYDV-PAV + BYDV-SGV in 9 (2.4 %) and BYDV-PAV + BYDV-SGV + CYDV-RPV in 3 (0.8 %) of samples. The incidence of viruses detected in Diyarbakır districts, it varied between 0.6% and 13.3 %. According to the results obtained, Bismil district of Diyarbakır was determined as the highest infected districts.

During the field survey, on wheat samples, redness of flag leaves, chlorotic spots, spotting, rolling and dwarfing symptoms were frequently observed. Since *Barley yellow dwarf viruses* have been detected in the region, it is necessary to determine vector aphid species in order to demonstrate their role and importance in their spread.

The coat protein gene of the virus detected in this study was registered to the NCBI Genebank as BYDV-PAV Diyarbakır isolate KX774424. A random BYDV-PAV isolate was selected from the *Barley yellow dwarf virus* (BYDV) detected in Diyarbakır province and the entire coat protein gene was cloned by T-A cloning method. The coat protein gene of Diyarbakır isolate was 603 bp in length as other isolates around the

world. Nucleotide sequence comparisons of the BYDV-PAV Diyarbakır isolate showed a similarity between 81.43-98.2 % with the world isolates.

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APPENDIX

EXTENDED TURKISH SUMMARY
(GENİŞLETİLMİŞ TÜRKÇE ÖZET)

DİYARBAKIR İLİ BUĞDAY (*Triticum aestivum* L.) TARLALARINDA ARPA SARI CÜCELİK VİRÜSLERİ'NİN MULTİPLEKS RT-PCR İLE ARAŞTIRILMASI VE MOLEKÜLER KAREKTERİZASYONU

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Ekmeklik buğday (*Triticum aestivum* L.) ve kışlık makarnalık buğday (*Triticum durum* Desf.), tüm diğer türlerin en geniş ekonomik öneme sahip olan türledir. Durum buğdayın dünya üretimi 32-34 milyon ton ve yukarı doğru artış eğiliminde olduğu ifade edilmiştir. Ekmeklik buğday (*Triticum aestivum* L.), kışlık makarnalık buğday daneleri, iki önemli depolanan ve depolanmayan büyük proteini türünü içerir. Depolanmayan proteinler, metabolik proteinler olarak isimlendirilir. Bunlar, suyla çözünebilen proteinler albuminler ve tuzla çözünebilen globulinleri içeren tüm tahıl proteinlerinin % 15-20 sebep olur.

Buğday üretimini ekonomik olarak sınırlayan virüslerin başında, afitler ile etkili olarak taşınan Arpa sarı cücelik virüsleri (*Barley yelllow dwarf viruses*; BYDVs), buğday üretimini tehdit eden önemli virüsler arasında yer almaktadır.

Türkiye'de hemen hemen tüm bölgelerinde buğday üretimi yapılmaktadır. Buğday üretiminde ülke olarak önemli bir yere sahip olunmasına rağmen buğday virüs hastalıkları ile ilgili olarak yeterli sayıda çalışma bulunmamaktadır. Yapılan çalışmalar genelde Trakya, Ege ve İç Anadolu ve kısmen de Orta Karadeniz bölgesinde buğday üretim alanlarında yoğunlaşmaktadır. Özellikle Güneydoğu Anadolu Bölgesinde bugüne kadar yapılan çalışma olmadığından dolayı bu bölgelerde hangi virüslerin infeksiyona yol açtığı ve yayılışları hakkında bilgiler bulunmamaktadır.

Diyarbakır ilinde önemli ölçüde buğday üretimi yapılan alanlarda infeksiyon oluşturulan Arpa sarı cücelik virüs (*Barley yellow dwarf virus*, BYDV)'lerinin

multipleks RT-PCR yöntemi ile belirlenmesi ve buğdaydaki virüs hastalıklarının yayılış alanlarının ortaya konulması ve BYDV-PAV virüsünün genomunda yer alan kılıf protein genin tamamının klonlanarak DNA dizileme ile ortaya konması ve filogenetik analizlerinin gerçekleştirilmesi amaçlanmıştır.

MATERYAL VE YÖNETIM

Bu çalışma, Diyarbakır ilinin dört farklı ilçelerinden (Bismil, Silvan, Çınar ve Sur) buğday tarlalarında gerçekleştirilmiştir. Simptom gösteren ve simptom göstermeyen tarlalardan rasgele 365 buğday yaprak örneği toplanmıştır. Toplanan örnekler buz kutusunda laboratuvara getirilmiştir. 2016 yılının Nisan ve Mayıs aylarında her tarladan on buğday yaprak örnekleri toplanmıştır. Yaprak örnekleri, total nükleik asit ekstraksiyonu yapılıncaya kadar -20^{o}C saklanmıştır. Toplanan buğday örneklerinin total nükleik asit ektraksiyonu, silika temelli yöntem Foissac et al. (2001)'nın önerdiği protokole göre gerçekleştirilmiştir.

Tamamlayıcı DNA (cDNA) sentezi, Thermo marka First Strand cDNA kit (Thermo-Fermentas, Vilnius, Lithuania) ile gerçekleştirilmiştir. Arpa sarı cücelik virüsleri (BYDV-PAV, BYDV-MAV, BYDV-SGV ve CYDV-RPV, BYDV-RMV) multipleks RT-PCR metodu ile araştırılmıştır. Total RNA'dan cDNA elde etmek için Malmstrom and Shu (2004)'nın kullanmış oldukları Yan- Reverse primer 5'-TGTTGAGGAGTCTACCTATTTG-3' i kullanarak tüm BYDV'leri ortak cDNA'lar üretilmiştir. Böylece, herbir arpa sarı cücelik virüsleri için ayrı cDNA yapmaya gerek kalmamıştır.

Arpa sarı cücelik virüs (BYDV)'leri BYDV-PAV, BYDV-MAV, BYDV-SGV, CYDV-RPV ve BYDV-RMV multipleks RT-PCR ile teşhisleri Malmstrom ve Shu (2004)' e göre yapılmıştır. Malmstrom ve Shu (2004), temel ve genişletilmiş multipleks RT-PCR olmak üzere iki farklı sistem kullanmışlardır. Temel multipleks yöntemine göre, grup1 ve grup2 virüslerini belirleyen ve iki farklı DNA fragmenti çoğaltan bir sistem geliştirmişlerdir. Bu sistemde Shu-F ve Yan–R primerleri kullanılarak grup1 virüsleri (BYDV-PAV, BYDV-MAV, BYDV-SGV) tespit edilirken, Yan-R ile birlikte S2a-F ve S2b-F primerleri kullanılarak grup 2 virüslerinin (CYDV-RPV, BYDV-RMV) teşhisleri yapılabilmektedir. Türe özgü primerler kullanılarak gerçekleştirilen genişletilmiş multipleks RT-PCR yöntemi ile grup1 ve grup2 içerisindeki virüslerin

daha spesifik teşhislerinin yapılması hedeflenmiştir. Şüpheli durumlarda farklı araştırıcıların kullanmış oldukları tür spesifik primer ile de doğrulama yapışmıştır.

PCR sonrası çoğaltılan DNA ürünleri %1'lik agaroz jel'de elektroforez yapılarak analiz edilmiştir. Yüklenen örnekler %1'lik agaroz jel içerisinde elektroforez yöntemiyle koşulduktan sonra jel görüntüleme ve analiz sisteminde görüntülenip fotoğraflanmıştır.

Diyarbakır ilinde gerçekleştirilen ilçelerden toplanan buğday örneklerine uygulanan Multipleks RT- PCR sonucu tespit edilen Arpa sarı cücelik virüs (BYDV)' lerin olan BYDV-PAV'dan rastgele birer izolat seçilerek kılıf protein genlerinin tamamı klonlanarak karakterizasyonu gerçekleştirilmiştir.

Bu amaçla, virüslerinin genomuna yer alan kılıf protein geninin tümünü çoğaltacak primer dizaynı Vector NTI programı ile gerçekleştirilmiştir. Primer dizaynı için daha önce gen bankasına girilmiş tüm genomu ortaya çıkarılmış DNA dizilerinden faydalanılmıştır. Tespit edilen BYDV-PAV virüsünün kılıf protein geni için Gen Bankasında NC_004750.1 (Accesion number) ulaşım numaralı dizi, referans alınmıştır.

Dizayn edilen primer kullanılarak RT-PCR yöntemiyle çoğaltılan ve sonrasında ticari kitlerle saflaştırılan BYDV-PAV kılıf protein geni T-A klonlama yöntemi ile pGEM-T Easy plazmid vektöründe klonlanmıştır. RT-PCR yöntemi çoğaltılan kılıf protein genlerinin pGEM-T Easy plazmit vektörü ile birleştirilmesi ligaz enzimi ile gerçekleştirilmiştir. Bu işlemde, Promega firmasının pGEM-T Easy vector sistemi (Katalog No, A1360) kullanılmıştır.

DNA fragmentinin plazmide aktarılması ile elde edilen rekombinant plazmidler, kitle sağlanan *Escherichia coli* bakterisinin JM109 suşuna elektroşok uygulaması ile bakterinin içerisine girmesi sağlanmıştır.Bakerilerin ölmemesi içi 900 µl SOC solüsyonu ilave edilmiştir. Hazırlanan petrilere 2 saat süren inkübasyon sonrası elde edilen bakteri süspansiyonundan 200 µl alınarak her bir numune için iki plate olacak şekilde petrilerin yüzeylerine ile ekim yapılmış ve drigaski çubuğu ile petri yüzeyine yayılmıştır.

Petriler ters çevrilip bir gece 37^0C' de inkübasyona bırakılarak mavi beyaz kolonilerin gelişmesi sağlanmıştır. Gelişen kolonilerden beyaz olanlar seçilerek kültür oluşturulmuştur. Plazmitlerin saflaştırılmasında, Bioline firmasının saflaştırma kitinden faydanılmıştır. Katı LB petrilerinde gelişen beyaz renkli kolonilerde gerçekten

rekombinant plazmitleri içerip içermediği daha hassas bir yöntem olan koloni PCR yöntemiyle de belirlenmiştir. Moleküler klonlama sonrası rekombinant plazmitler çift yönlü DNA dizilemesi yapılması için Sentegen Biotech (ANKARA)'ye gönderilmiştir. Dizi analizi sonrası elde edilen genom bilgileri farklı bioinformatik programlar ile CLC Main Workbench 6.1, Mega 4 ve Vector NTI 10 programları ile analiz edilmiştir.

CLC Main Workbench 6.1 programı ile çoklu dizi karşılaştırma yapılarak dünyadaki diğer virüs izolatlarıyla benzerlikler ve farklılıklar belirlenmiştir.

Son olarak filogenetik ağaç oluşturularak Diyarbakır izolatın diğer dünya izolatlarıyla olan genetik benzerlikleri ve farklılıkları ortaya çıkarılmıştır. Farklı buğday virüslerinin BYDV-PAV virüsünün çoğaltılan kılıf protein genlerinin sekans analizi sonrası elde edilen dizisi National Center for Biotechnology Information (NCBI) Gen bankasına kaydı gerçekleştirilmiştir.

SONUÇ

2016 yılında Diyarbakır ilinde, BYDV-PAV, BYDV-MAV, BYDV-SGV, BYDV-RMV ve CYDV-RPV virüslerinin bulunma ve infeksiyon oranını belirlemek için 365 buğday örneği testlenmiştir.Diyarbakır ilinin ilçelerinde buğday üretim miktarına göre örnekleme çalışması yapılmıştır. Survey esnasında, yapraklar üzerinde klorotik çizgi leke, bayrak yaprakların kızarması, mozaik, cüceleşme gibi viral infeksiyonlar nadirde olsa gözlenmiştir. Moleküler test sonuçlarına göre, 365 örneğin 18'inde BYDV-PAV, BYDV-SGV ve CYDV-RPV virüsleri belirlenmiştir. Testlenen örneklerin hiçbirinde BYDV-MAV ve BYDV-RMV virüsleri tespit edilmemiştir. Diyarbakır ilinin toplam 18 örnekte genel infesiyon oranı % 4.9 olarak bulunmuştur.

Diyarbakır ilinden toplanan 365 örneğin 13'ünde BYDV-PAV (% 3.5), 9'unda BYDV-SGV (% 2.4), 5'inde CYDV-RPV (% 1.3) virüs enfeksiyonu tespit edilmiştir. Dünya'da ve Türkiye'nin farklı bölgelerinde olduğu gibi BYDV-PAV en yaygın virüs olduğu belirtilmiştir. Dokuz örnekte BYDV-PAV+BYDV-SGV virüslerinin karışık infeksiyon oranı % 2.4 ve üç örnekte BYDV-PAV+ CYDV-RPV, BYDV-SGV+CYDV-RPV, BYDV-PAV+BYDV-SGV+CYDV-RPV virüslerin birlikte karışık infeksiyon oranları ise % 0.8 olarak bulunmuştur. Diyarbakır ilinin ilçelerinde belirlenen virüslerin infeksiyon oranları % 0.6 ile % 13.3 arasında değişmektedir. Elde edilen test sonuçlarına göre, Diyarbakır'ın Bismil ilçesi en BYDV-PAV'ı en yoğun

görüldüğü ilçe olarak belirlenmiştir. Bölgede bu bu virüslerin tespit edildiği için muhtemel yayılımı sağlayan vektör türlerinin belirlenmesi mücade içinde önemlidir.

Bu çalışmada belirlenen virüsün kılıf protein genin nükleik asit dizisi NCBI Genbank'a BYDV-PAV Diyarbakır izolatı olarak KX774424 erişim numarası ile kaydı yapılmıştır. Diyarbakır ilinde tespit edilen pozitif BYDV-PAV izolatları arasından rastgele biri seçilerek kılıf protein genin tamamı T-A klonlama yöntemi ile klonlanmıştır. Diyabakır izolatının kılıf protein geni dünyadaki diğer izolatlar gibi 603 bp olduğu belirlenmiştir.

Buffer Solutions Used in Total RNA Extraction

Electrophoresis buffers are used at work

Harness 50X TAE buffer (stock solution)

- Tries-base (C4H11NO3) pH (8.0) 242 gr
- Glacial Ascetic acid (CH3COOH) 57.1 gr
- 0.5 M EDTA (C10H14N2O8Na2.2H2O) 100 ml

After the above amount of the chemicals dissolved in 1 liter of water was stored at room temperature in sterile autoclave.

Preparation of 1X TAE buffer harness (to prepare 1 liter)

50 X TAE stock solutions of 20 ml of the sterile purified water used to complete to 1000 ml.

Buffer Resolutions Used in Electrophoresis Studies

Cloning working in the media used and the preparation of chemicals

Preparation of liquid LB medium (1 liter)

- 10 g Bacto tryptone (Fisher Bio Reagents BP1421 500g)
- 5 g Bacto yeast (Applichem)
- 5 g NaCl (Sigma)

Given who weighed components in the amounts indicated in 1 liter flask issues and more.

Make up to 1 liter with distilled water. At 121 °C for 15-20 minutes it is autoclavable. More after cooling to 50-55 °C, over the magnetic mixers at room temperature It is provided.

At this stage, according to the study aims to ampicillin, IPTG or X- gal It may be added in the required amounts.

Liquid LB media and competent cells prepared for the salt, ampicillin, IPTG and Xgal It is not participating.

Solid preparation LB medium (1 liter)

- 10 g Bacto tryptone
- 5 g Bacto yeast extract
- 5 g NaCl

The study used the cloning of Environment and Chemical Preparation

- 15 gr Agar (For solidifying)

1'litre components bottles above issue and who weighed about 1 liter of pure water. It is completed. At 121 °C for 15-20 minutes it is auto cleavable. After autoclaving , cool until room temperature 50-55 °C is provided on the magnetic disturbing . It stage, according to the study aims to ampicillin, IPTG or X- Gal required amount can be added.

Competent cells were prepared for salt solid LB medium, ampicillin, IPTG and X Gal is participate.

X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside)

20 mg X -Gal chemical weighed within 1 ml Dimethyl form amide Dissolved. To protect from the light chemical Eppendorf tube wrapped with aluminum foil. X Gal prepared was stored at -20 °C.

IPTG (Isopropyl- β -D-thiogalactoside)

100 mg IPTG chemicals are dissolved in 1 ml of sterile water weighed. To protect from the light chemical Eppendorf tube wrapped with aluminum foil. The prepared IPTG was stored at -20 °C.

SOC media preparation (for 1 liter)

- 20 g Bacto Tryptone
- 5 g Bacto Yeast Extract
- 2 ml of 5M NaCl.
- 2.5 ml of 1M KCl.
- 10 ml of 1M MgCl2
- 10 ml of 1M MgSO4
- 20 ml of 1M glucose

Placing those components with sterile distilled water into a bottle of TINALab It is completed to 1 liter with water. At 121 °C for 15-20 minutes it is autoclavable. After autoclaving, Cooling to room temperature 50-55 degrees is provided. Then, Stored at 4 ^{o}C in refrigerator.

CURRICULUM VITAE

He was born in Hajyawa of Sulaymaniyah - Iraq, in 1991. He completed the primary education in Hajyawa Town and secondary education in Hajyawa., During the years of 2010-2014, He had studied in Sulaymaniyah University, the college of Agriculture and Department of field crops . In 2014 he had graduated from here. At the September of 2014 He started his master study in Van Yuzuncu Yil University.

UNIVERSITY OF VAN YUZUNCU YIL
THE ISTITUTE OF NATURAL AND APPLIED SCIENCES
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