



T.R.

NİĞDE ÖMER HALİSDEMİR UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

INVESTIGATION OF POTYVIRUS AND PHYTOPLASMA DISEASES ON
COMMON BEAN (*PHASEOLUS VULGARIS* L.) GROWN IN NİĞDE PROVINCE

İREM KOÇ

JANUARY 2023

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

INVESTIGATION OF POTYVIRUS AND PHYTOPLASMA DISEASES ON
COMMON BEAN (*PHASEOLUS VULGARIS* L.) GROWN IN NİĞDE PROVINCE

İREM KOÇ

Master Thesis

Supervisor

Prof. Dr. Çiğdem ULUBAŞ SERÇE

JANUARY 2023

The study titled “**Investigation of Potyvirus and Phytoplasma Diseases on Common Bean (*Phaseolus Vulgaris* L.) Grown in Niğde Province**” are presented by **İrem KOÇ** under supervision of **Prof. Dr. Çiğdem ULUBAŞ SERÇE** has been accepted as Master Thesis by juries, at the **Department of Plant Production and Technologies**, Niğde Ömer Halisdemir University Graduate School of Natural and Applied Sciences.

Head : Prof. Dr. Çiğdem ULUBAŞ SERÇE
Niğde Ömer Halisdemir University

Member : Prof. Dr. Miray SÖKMEN
Samsun Ondokuz Mayıs University

Member : Assoc. Prof. Dr. Eminur ELÇİ
Niğde Ömer Halisdemir University

CONFIRMATION

This thesis has been found appropriate at the date of /.... /2023 by the jury mentioned above who have been designated by the board of Directors of Graduate School of Natural and Applied Sciences and has been confirmed with the resolution of Board of Directors dated /..... /2023 and numbered

...../...../2023

Prof. Dr. Mustafa KARATEPE
DIRECTOR

THESIS CERTIFICATION

It is certified to “Investigation of Potyvirus and Phytoplasma Diseases on Common Bean (*Phaseolus vulgaris* L.) Grown in Niğde Province” thesis by İrem KOÇ was written based on principals of Niğde Ömer Halisdemir University - Graduate School of Natural and Applied Sciences, Department of Plant Production and Technologies. It is also certified that, all information and sources used in this thesis were cited and referenced definitively, by the author.



İrem KOÇ

SUMMARY

INVESTIGATION OF POTYVIRUS AND PHYTOPLASMA DISEASES ON COMMON BEAN (*PHASEOLUS VULGARIS* L.) GROWN IN NİĞDE PROVINCE

KOÇ, İrem

Niğde Ömer Halisdemir University

Graduate School of Natural and Applied Sciences

Department of Plant Production and Technologies

Supervisor : Prof. Dr. Çiğdem ULUBAŞ SERÇE

January 2023, 115 pages

Sixty potyvirus-positive samples were selected to be tested by RT-PCR. Fifty four samples were found to be infected with Bean common mosaic virus (BCMV), one sample infected with Bean yellow mosaic virus (BYMV). The sequenced isolate 186 showed 89% identity to a BYMV isolate from pea in Germany deposited in NCBI. In this study, ten common bean cultivars were inoculated with a BCMV isolate selected based on its high level of virulence trait, and their symptom appearance was evaluated after inoculation. Based on the phenotypic evaluations and PTA-ELISA screening, two cultivars were found to be susceptible to BCMV, while the eight cultivars were discovered to be resistant. Also, SCAR (SW-13, SBD-5, and ROC11) and CAPS (eIFE4) markers were used to detect resistance genes in 22 common bean cultivars. Results showed that 11 involved the *I* gene-specific product with SW-13 marker, while 13 contained the recessive *bc-1*² gene-specific product with SBD-5 marker. On the other hand, the recessive *bc-3* gene was found with ROC11 marker but not with eIFE4 in common bean cultivars tested. Moreover, 56 symptomatic common bean samples were tested for phytoplasma detection, no phytoplasma was detected in the samples tested. To our knowledge, this is the first study in the attempt at molecular identification of phytoplasma diseases in common beans in Türkiye.

Keywords: Potyvirus, Common bean, virus resistance, molecular marker, phytoplasma detection

ÖZET

NIĞDE İLİNDE YETİŞTİRİLEN FASULYE BITKİLERİNDE POTYVIRUS VE FITOPLAZMA HASTALIKLARININ ARAŞTIRILMASI

KOÇ, İrem

Niğde Ömer Halisdemir Üniversitesi

Fen Bilimleri Enstitüsü

Bitkisel Üretim ve Teknolojileri Anabilim Dalı

Danışman: : Prof. Dr. Çiğdem ULUBAŞ SERÇE

Ocak 2023, 115 sayfa

Altmış potyvirus pozitif fasulye bitkisi, RT-PCR için seçilmiştir. 54 örneğin Bean common mosaic virus (BCMV) ile enfekteli olduğu tespit edilirken, bir örneğin Bean yellow mosaic virus (BYMV) ile enfekteli olduğu belirlenmiştir. Sekans dizisi elde edilen izolat 186, NCBI'de depolanan ve Almanya'da bezelyeden elde edilen bir BYMV izolatı ile %89 benzerlik göstermiştir. Bu çalışmada, on fasulye çeşidi şiddetli virulanslık özelliği gösteren BCMV izolatı ile inokule edilmiş ve inokulasyondan sonra çeşitlerin oluşturduğu simptom belirtileri gözlemlenmiştir. Fenotipik değerlendirmeler ve ELISA sonuçlarına göre, iki çeşit BCMV'ye hassas bulunurken, sekiz çeşit dayanıklı bulunmuştur. Ayrıca 22 fasulye çeşidinde dayanıklılık genlerini araştırmak amacıyla SCAR (SW-13, SBD-5 ve ROC11) ve CAPS (eIFE4) markörleri kullanılmıştır. Sonuçlar, 11 çeşidin SW-13 markörü ile dominant *I* genine özgü ürün verdiğini, 13 çeşidin ise SBD-5 markörü ile resesif *bc-1²* genine özgü ürün oluşturduğunu göstermiştir. Diğer yandan, bu çalışmada kullanılan fasulye çeşitlerinde resesif *bc-3* geni ROC11 ile elde edilirken eIFE4 markörü ile bulunamamıştır. Ayrıca, simptomatik 56 fasulye bitkisi, fitoplazma teşhisi için test edilmiş fakat herhangi bir fitoplazma bulgusuna rastlanamamıştır. Bildiğimiz kadarıyla bu çalışma, Türkiye'de fitoplazma hastalıklarının moleküler tanımlanmasına yönelik yapılan ilk çalışmadır.

Anahtar Kelimeler: Potyvirus, fasulye, viruse dayanıklılık, moleküler markör, fitoplazma teşhisi

ACKNOWLEDGEMENTS

First of all, I would like to express my sincerest thanks to my supervisor Prof. Dr. Çiğdem ULUBAŞ SERÇE, who guided and supported me in all the time of my research works for Master's degree. Her views, endless patience, vision, dynamism, and motivation have always deeply inspired me.

I would like to thank my fellow labmates, Kadriye YURTASLAN, Bahar Makbule TEMELTÜRK, Eray ATALAY, and Muhammet EKEMEN for their support and input in laboratory work.

I would also like to thank Moin QURESHI who was always by my side whenever I needed him and has always believed in, supported, and helped me a lot in making this study.

I would also like to express my sincere thanks to my family for all the ways they have loved, prayed for, cared for, and spiritually supported me throughout my life. They kept me going on and this work would not have been possible without their input. Their prayers for me have always allowed me to get this far.

This study was produced from the BAP project with the number TGT 2021/20-LUTEP, named "Investigation of Potyvirus and Phytoplasma Diseases on Common Bean (*Phaseolus vulgaris* L.) Grown in Niğde Province". I would like to thank Niğde Ömer Halisdemir University, Scientific Research Projects Unit for their support for this project.

TABLE OF CONTENTS

SUMMARY	iv
ÖZET	v
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xii
SYMBOLS AND ABBREVIATIONS	xvi
CHAPTER I	1
INTRODUCTION	1
1.1 Objective of Thesis	5
CHAPTER II	6
GENERAL INFORMATION	6
2.1 Major Diseases of Common Bean	6
2.1.1 Viral diseases infecting common bean	7
2.1.1.1 <i>Potyvirus</i>	9
2.1.1.2 Potyvirus diseases of common bean in Türkiye	12
2.1.2 Resistance to potyvirus diseases in common bean	14
2.2 General Introduction to Phytoplasmas	19
2.2.1 Phytoplasma diseases infecting common bean	21
CHAPTER III	25
MATERIALS AND METHODS	25
3.1 Collection of Symptomatic Common Bean Plant Samples	25
3.2 Detection of Viruses in Common Bean Samples Using Potyvirus-Specific Antiserum	26
3.3 Total Nucleic Acid Isolation From Common Bean Samples	27
3.4 One-Step RT-PCR Method for the Detection of <i>Potyvirus</i> Species	28
3.5 Agarose Gel Electrophoresis	31
3.6 Nucleotide Sequence Analysis of BYMV Isolate	31
3.7 Phylogenetic Analysis of BYMV Isolate	31
3.8 Investigation of Reactions of Different Common Bean Cultivars to BCMV	32

3.8.1 Common bean seed sources used in the reaction studies to BCMV and in resistance gene analysis	32
3.8.2 Inoculum preparation and mechanical virus inoculation to common beans ...	34
3.9 Determination of Resistance Level of Common Bean Cultivars to BCMV	35
3.9.1 Determination of resistance level and resistance genes of common bean cultivars based on the symptoms	35
3.9.2 Determination of resistance level of common bean cultivars based on PTA-ELISA results.....	36
3.9.3 Screening resistance genes on common bean cultivars by PCR method.....	36
3.9.3.1 Digestion of PCR products obtained by eIFE4 marker	37
3.10 Detection of Phytoplasma in Common Bean Plants.....	37
3.10.1 Phytoplasma DNA-enriched isolation method	38
CHAPTER IV	42
RESULTS	42
4.1 Detection of Viruses in Common Bean Samples Using Potyvirus-Specific Antiserum.....	42
4.2 One-Step RT-PCR Method for the Detection of <i>Potyvirus</i> Species.....	49
4.2.1 PCR results for BCMV	49
4.2.2 PCR results for BCMV negative isolates tested against the other <i>Potyvirus</i> species.....	50
4.2.3 PCR results for BYMV	52
4.2.4 PCR results for BCMNV	53
4.2.5 PCR results for CABMV and PeMoV	54
4.2.6 PCR results for SMV	56
4.2.7 PCR results for CIYVV	57
4.3 Nucleotide Sequence Analysis of BYMV Isolate.....	57
4.4 Phylogenetic Analysis of BYMV Isolate.....	58
4.5 Investigation of Reactions of Different Common Bean Cultivars to BCMV	59
4.5.1 Determination of resistance level and resistance genes of common bean cultivars based on the symptoms	59
4.5.2 Determination of resistance level of common bean cultivars to BCMV based on PTA-ELISA results.....	65
4.5.3 Screening resistance genes on common bean cultivars by markers	67
4.6 Detection of Phytoplasma in Common Bean Plant Samples.....	72

CHAPTER V	76
DISCUSSION	76
CHAPTER VI.....	82
CONCLUSION AND FUTURE ASPECTS	82
REFERENCES	84
APPENDICES	108
CURRICULUM VITAE.....	115



LIST OF TABLES

Table 1.1. The main dry bean-producing provinces of Türkiye	1
Table 1.2. <i>Potyvirus</i> species infecting common bean.....	2
Table 2.1. Some of viruses infecting common bean worldwide modified from Meziadi et al. (2017).....	8
Table 2.2. The several studies in the world involving the detection of some potyviruses infecting common bean	11
Table 2.3. The studies in Türkiye related to the detection of some potyviruses infecting common bean	13
Table 2.4. Dominant <i>R</i> genes and resistant genotypes against some potyviruses infecting common beans modified from Meziadi et al. (2017)	15
Table 2.5. Recessive genes and resistant genotypes against some potyviruses infecting common bean modified from Meziadi et al. (2017).....	15
Table 2.6. The studies related to the detection of some phytoplasma diseases infecting common bean	24
Table 3.1. Collection sites, field numbers visited and the number of common bean plant samples studied in this research	26
Table 3.2. PTA-ELISA positive samples used in One-Step RT-PCR reactions.....	29
Table 3.3. Primer pairs used in One-Step RT-PCR amplification of <i>Potyvirus</i> species.	30
Table 3.4. The reaction mixture used in One-Step RT-PCR amplification of each potyvirus	30
Table 3.5. Amplification conditions for each <i>Potyvirus</i> species used in PCR tests	30
Table 3.6. BYMV isolates used in phylogenetic analysis	32
Table 3.7. The common bean cultivars with codes from one to ten are used in virus reaction studies as well as BCMV resistance analyses and also the common bean cultivars with codes from eleven to twenty-one are included in BCMV resistance analyses.....	33
Table 3.8. Markers used in PCR amplification of resistance genes	37
Table 3.9. Amplification conditions for each marker used in PCR.....	37
Table 3.10. Selected common bean samples for phytoplasma detection in both direct and nested-PCR analyses	39

Table 3.11. Nucleotide sequences of universal and specific primers used in direct and nested-PCR procedures for phytoplasma detection in common bean plants	41
Table 3.12. Quantities of the components for preparation of the reaction mixtures of direct and nested-PCR procedures.....	41
Table 3.13. Amplification conditions used in PCR tests for the detection of phytoplasma	41
Table 4.1. Potyvirus-specific PTA-ELISA results of common bean leaf samples collected from different places	44
Table 4.2. Potyvirus infection rates estimated by PTA-ELISA of the collected common bean samples in Niğde province.....	48
Table 4.3. BLASTn analysis of BYMV isolate obtained from this tudy.....	58
Table 4.4. The PTA-ELISA results for each bean cultivar having 3 different groups before BCMV inoculation to evaluate seed-transmitted potyviruses.....	60
Table 4.5. The results of systemic symptoms and PTA-ELISA results of inoculated common bean cultivars from the first inoculation trial	66
Table 4.6. The results of systemic symptoms and ELISA results of inoculated bean cultivars from second inoculation trial.....	66
Table 4.7. The results of dominant <i>I</i> , recessive <i>bc-1²</i> and <i>bc-3</i> genes by DNA markers in common bean cultivars.....	71

LIST OF FIGURES

Figure 2.1. Genome organization of potyviruses.....	9
Figure 3.1. Villages and towns where symptomatic common bean plant samples were collected within the scope of the research. Site 1 represents Alay, Site 2 Gölcük, Site 3 Konaklı, Site 4 Kiledere, and Site 5 Amas Bağları. In contrast, Site 6 represents Ağcaşar, Site 7 Çayırılı, Site 8 Elmalı, Site 9 Hasaköy, Site 10 Ovacık, Site 11 Yarhisar, and Site 12 Yeşilova.....	25
Figure 3.2. The virus inoculation trial on common bean cultivars	35
Figure 3.3. Schematic diagram of used primers sequentially in direct and nested-PCR procedures for the detection of phytoplasma in common bean plant samples. Primer pairs (P1/P7- R16F2n/R16R2) were tried first for four selected common bean samples with the isolate names 27, 28, 29, and 30, then the other primer pairs were used for the rest of the common bean samples in one reaction of direct and nested-PCR tests based on the gel result, respectively	40
Figure 4.1. Some of the principal symptoms belonging to common bean plants surveyed in the fields that were confirmed as potyvirus (BCMV) infected; chlorotic and necrotic lesions (a, b), mosaic and vein banding (b, c), mottling (d), leaf distortion and reduced size of internodes (e), dwarfing and yellowing (f)..	43
Figure 4.2. Some of the principal symptoms belonging to common bean plants surveyed in the fields that were confirmed as potyvirus (BYMV) infected; yellow mosaic caused by BYMV	43
Figure 4.3. The PCR result of common bean samples analyzed for BCMV in One-Step RT-PCR method. M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). W: Water control	49
Figure 4.4. Gel results of 24 infected common bean samples (a) and the last 30 common bean samples for BCMV (b). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). W: Water control	50
Figure 4.5. Agarose gel results of nine symptomatic common bean samples for BCMNV and BYMV (a) and the results for CABMV (b). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). W: Water control	51

Figure 4.6. Gel results of nine infected common bean samples for SMV and PeMoV (a) and the results for CIYVV (b). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). W: Water control	52
Figure 4.7. Gel results of 34 infected bean plants for BYMV (a) and the results for the remaining 17 samples with BYMV (b). M; GeneRuler 1kb DNA Ladder (Thermo Scientific). P: Positive control confirmed in this study (Isolate number 186) W: Water control	53
Figure 4.8. Gel results of 33 infected bean plants for BYMV (a) and the results for the remaining 27 samples for BYMV with new designed degenerate BYMV-pnsF1 primer (b). M; Hibrigen 100 bp DNA Ladder. P: Positive control confirmed in this study (Isolate number 186) W: Water control	53
Figure 4.9. Gel results of 35 infected bean plants for BCMNV (a) and the results for the remaining 19 samples with BCMNV (b). M; GeneRuler 1kb DNA Ladder (Thermo Scientific) in the first column of the gel and Hibrigen 100 bp DNA Ladder in the last column of the gel. W: Water control.....	54
Figure 4.10. Gel results of 37 infected bean plants for CABMV (a) and the results for the remaining 14 samples with CABMV (b). M: Hibrigen 100 bp DNA Ladder. W: Water control. *: The band in the last column in Figure 4.10b does not belong to CABMV, it belongs to another study	55
Figure 4.11. Gel results of 37 infected bean plants for PeMoV (a) and the results for the remaining 14 samples with PeMoV (b). M: Hibrigen 100 bp DNA Ladder. W: Water control.....	55
Figure 4.12. Gel results of four infected bean plants for PeMoV and CABMV at different annealing temperatures (50°C, 52°C, 54°C, and 58°C). M: Hibrigen 100 bp DNA Ladder	56
Figure 4.13. Gel results of 37 infected bean plants for SMV (a) and the results for the remaining 15 samples with SMV (b). M: Hibrigen 100 bp DNA Ladder. W: Water control.....	56
Figure 4.14. Gel results of 37 infected bean plants for CIYVV (a) and the results for the remaining 14 samples with CIYVV (b). M: Hibrigen 100 bp DNA Ladder. W: Water control.....	57
Figure 4.15. Phylogenetic tree of the Bean yellow mosaic virus isolate 186 constructed in MEGA-X. Bootstrap value was adjusted to (1000 replicates) which	

shows the percentage of replicate trees in which the associated taxa clustered together	59
Figure 4.16. Symptoms belonging to the first inoculation trial. Vein clearing and leaf curling symptoms on inoculated leaves of cv. Güngör after 7 days of inoculation (a), mosaic symptoms with leaf curling three weeks after post-inoculation on the cv. Güngör (b), leaf curling symptoms on non-inoculated leaves of the cv. Akbulut (c)	61
Figure 4.17. Systemic mosaic symptoms on non-inoculated leaves of the cvs. Akman-98 and Sururbey three weeks after post-inoculation with BCMV (a, b), DL: Diseased Leaf; HL: Healthy leaf.....	61
Figure 4.18. Mild mosaic and leaf curling symptoms on non-inoculated leaves of the cvs. Cihan and Ergen three weeks after post-inoculation with BCMV (a, b), DL: Diseased Leaf; HL: Healthy leaf	62
Figure 4.19. Chlorotic symptoms on non-inoculated leaves of the cvs. Canipek and Borsa three weeks after post-inoculation with BCMV (a, b), DL: Diseased Leaf; HL: Healthy leaf	63
Figure 4.20. Vein clearing symptoms on non-inoculated leaves of the cvs. Adabeyazı and Zirve at three weeks after post-inoculation with BCMV (a, b), DL: Diseased Leaf; HL: Healthy leaf.....	63
Figure 4.21. Symptoms belonging to the second inoculation trial. Mild mosaic and leaf curling symptoms on non-inoculated leaves of Güngör and vein clearing and leaf curling symptoms on inoculated leaves of cv. Güngör three weeks after inoculation (a), systemic mosaic with leaf curling symptoms four weeks after post-inoculation on the cv. Güngör (b), leaf curling symptom on non-inoculated leaves of cv. Akbulut at three weeks after post-inoculation (c)	65
Figure 4.22. Gel results of common bean cultivars by using the SCAR marker SW-13 which is specific for the dominant <i>I</i> gene. Each cultivar has two rows except cv. Ergen. M: Hibrigen 100 bp DNA Ladder, HG1: Host Group 1, WC: Water control	67
Figure 4.23. Gel results of common bean cultivars by using the SCAR marker SBD-5 which is specific for <i>bc-1²</i> gene. Each cultivar has two rows except cv. Ergen. M: Hibrigen 100 bp DNA Ladder, HG1: Host Group 1, WC: Water control	68

- Figure 4.24. Agarose gel results of common bean cultivars by using the SCAR marker ROC11 which is specific for *bc-3* gene. Absence of signal indicates the presence of *bc-3* gene. Each cultivar has two rows except cv. Ergan. M: Hibrigen 100 bp DNA Ladder, HG1: Host Group 1, WC: Water control . 69
- Figure 4.25. Gel results of common bean cultivars by using the CAPS marker eIFE4 which is specific for *bc-3* gene. Each cultivar has two rows except cv. Ergan. M: Hibrigen 100 bp DNA Ladder, HG1: Host Group 1, WC: Water control 69
- Figure 4.26. Gel results of digestion by using *RsaI* enzyme for common bean cultivars. Each cultivar has two rows except cv. Ergan. Presence of two signals (381 and 160 bp) indicates the presence of *bc-3* allele. Presence of 541-bp fragment indicates the absence of *bc-3* allele. M: Hibrigen 100 bp DNA Ladder, HG1: Host Group 1, WC: Water control 70
- Figure 4.27. Gel images of nested-PCR products with R16F2n/R16R2 at the ratios of 1:50 (the first four samples on the left side) and 1:100 (the last four samples on the right side). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific) 72
- Figure 4.28. Gel images of nested-PCR products with M1/M2 primer pairs at the ratios of 1:50. M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). AP4 and AP5: Positive controls 73
- Figure 4.29. Gel images of nested-PCR products with U5/U3 primer pairs at the ratios of 1:50. M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). AP5 and AP4: Positive controls 74
- Figure 4.30. Gel images of nested-PCR products with M1/M2 primer pairs at the ratios of 1:50 (a, b). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). P: Positive control (AP3). W: Water control 74
- Figure 4.31. Gel images of nested-PCR products with R16F2n/R16R2 primer pairs at the ratios of 1:50 (a, b). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). W: Water control 75

SYMBOLS AND ABBREVIATIONS

Symbols	Descriptions
%	Percentage
bp	Base pair
°C	Celsius
cm	Centimeter
cv	Cultivar
d ₂ H ₂ O	Double-distilled water
h	Hour
ha	Hectare
kb	Kilobase
Kg	Kilogram
M	Molar
mg	Milligram
min	Minute
ml	Mililiter
mM	Milimolar
mm	Millimeter
Na ₂ SO ₃	Sodium sulfite
NaCl	Sodium chloride
ng	Nanogram
ng/μl	Nanogram per microliter
nm	Nanometer
nt	Nucleotide
pv	Pathovar
s	Second
u/μl	Units per microliter
w/v	Weight in volume
μl	Microliter

Abbreviations	Descriptions
AP	Alkaline Phosphatase
AP	Apple Proliferation
BLAST	Basic Local Alignment Search Tool
ClustalW	Clustal Alignment Format Without Base/Residue Numbering
CTAB	Cetyl Trimethylammonium Bromide
DAPI	DNA Staining With 4'-6- Diamidino-2-Phenylindole
DAS-ELISA	Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay
DBPh	Dry Bean Phyllody
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
HG1	Host Group 1
IC-RT-PCR	Immunocapture-Reverse Transcription-Polymerase Chain Reaction
IgG	Immunoglobulin G
IRPCM	International Research Programme On Comparative Mycoplasmaology
K ₂ HPO ₄	Dipotassium Hydrogen Phosphate
KASP	Kompetitive Allele Specific Polymerase Chain Reaction
NCBI	National Center For Biotechnology Information
OD	Optical Density
ORF	Open Reading Frame
PBST	Phosphate-Buffered Saline/Tween
PCR	Polymerase Chain Reaction
pH	Potential Of Hydrogen
pNPP	Para-Nitrophenyl Phosphate
PTA-ELISA	Plate-Trapped Antigen Enzyme-Linked Immunosorbent Assay
PVP-40	Polyvinylpyrrolidone-40
rDNA	Recombinant DNA
RFLP	Restriction Fragment Length Polymorphism

RNA	Ribonucleic Acid
rpm	Revolutions Per Minute
rRNA	Ribosomal RNA
RT-PCR	Reverse Transcription Polymerase Chain Reaction
TAE	Tris-Acetate-Ethylenediamine Tetraacetic Acid
TAS-ELISA	Triple Antibody Sandwich Enzyme-Linked Immunosorbent Assay
TE	Ethylenediamine Tetraacetic Acid
TEM	The Transmission Electron Microscopy
TNA	Total Nucleic Acid
TUIK	Türkiye İstatistik Kurumu
UV	Ultraviolet

CHAPTER I

INTRODUCTION

The dry bean or common bean (*Phaseolus vulgaris* L.), mainly consumed for its mature, shell, and green pods of beans (Gepts, 2001), is the most important crop of the member of the Leguminosae family, making it an excellent legume species around the world (Morales, 2003). The popularity of the crop originates from the fact that it is relatively easy to produce, it is flavorful and the flexibility with which it can be prepared (Myers and Kmiecik, 2017), and it is a good source of proteins, vitamins, and certain minerals (Reyes- Moreno et al., 1993). The dry bean has over 36 million hectares of cultivation area and 27.7 million metric tons of world production, and India is at the top among all other producer countries of dry beans with 6.12 million tonnes of production. It is followed by Brazil with 2.89 million tonnes and Myanmar with 2.48 million tonnes per hectare, respectively (FAO, 2021).

In terms of cultivation area in Türkiye, legumes occupy the most important place after cereals. Dry bean production in Türkiye takes place in the third rank after chickpeas and lentils among legumes (Küzeci, et al., 2019). Dry beans constitute 29% of the total legume production with a production of 305.000 tonnes (TUIK, 2021), and 67.6% of these cultivated dry beans were supplied by the Central Anatolia region of Türkiye. Niğde was responsible for 23.2% of the total production, followed by Konya with 18.8%, Bitlis with 13.7%, and Nevşehir with 11.3. Table 1.1 shows how much of a difference these four provinces made to Türkiye's overall dry bean production (Kanat, 2022).

Table 1.1. The main dry bean-producing provinces of Türkiye (Kanat, 2022)

Dry Bean Producing Provinces	2021 Production (Tonnes)
Niğde	70.905
Konya	57.285
Bitlis	41.703
Nevşehir	34.589

Common bean production is constrained by a variety of factors that differ by region, season, year, and cultivar. Diseases, pests, and abiotic factors are the main limiting factors for yields and seed quality. Disease damage, particularly in the early stages of

seedling development and pod formation, also causes significant yield losses (often 80-100%). (Beebe et al., 2012).

A wide variety of pathogens, both living and nonliving, can harm bean plants (Nyvall, 1989). Diseases such as angular leaf spot, anthracnose, and rust are widespread, while rhizoctonia web blight and ascochyta blight can be particularly severe in certain warm- and cool-humidity areas (Beebe et al., 2012). More than 140 viruses have been identified as targeting leguminous plants, with *P. vulgaris* L. being the most commonly affected species (Loebenstein and Carr, 2007).

The most prominent viral diseases infecting common beans are reported as alfalfa mosaic virus (AMV), bean common mosaic virus (BCMV), bean common mosaic necrosis virus (BCMNV), bean golden mosaic virus (BGMV), bean yellow mosaic virus (BYMV), cucumber mosaic virus (CMV), southern bean mosaic virus (SBMV), tobacco streak virus (TSV), and tomato aspermy virus (TAV) (Kumar et al., 1994; Güzel and Arlı-Sökmen, 2003; Ghorbani et al., 2010).

Potyviruses have gained great attention agriculturally, economically, and biologically. These viruses can be found not only in wild plants but also in cultivated ones all over the world (Roossinck, 2012). Some species and strains of potyviruses can infect common beans naturally or beans grown under experimental conditions, drastically reducing yield and quality (Table 1.2) (Schwartz et al., 2005).

Table 1.2. *Potyvirus* species infecting common bean (Meziadi et al., 2017)

Family	Genus	Virus
<i>Potyviridae</i>	<i>Potyvirus</i>	<i>Bean common mosaic virus</i> (BCMV)
		<i>Bean common mosaic necrosis virus</i> (BCMNV)
		<i>Bean yellow mosaic virus</i> (BYMV)
		<i>Cowpea aphid-borne mosaic virus</i> (CABMV)
		<i>Clover yellow vein virus</i> (CIYVV)
		<i>Peanut mottle virus</i> (PeMoV)
		<i>Passionfruit woodiness virus-K</i> (PWV-K)
		<i>Soybean mosaic virus</i> (SMV)
		<i>Thailand passiflora mosaic virus</i> (ThPV)
		<i>Zucchini yellow mosaic virus</i> (ZYMV)
		<i>Watermelon mosaic virus 2</i> (WMV-2)

Bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) are two viruses that can drastically reduce bean yields by as much as 80%. Important from an epidemiological standpoint, BCMV is also seed transmitted by up to 83% in *P. vulgaris* (Morales, 2003). BCMV and BCMNV are among the most common and economically important viruses of beans in the world and our country (Yeken et al., 2018). BCMV and BCMNV are transmitted non-persistently by aphids, and can also be spread mechanically by plant sap, seeds, pollen, and agricultural equipments (Hall, 1994; Chew et al., 2010). The transmission of common bean disease via seed planting is significant because it serves as both a primary infection source and a means of regional and even transnational spread of the disease (Schwartz and Corrales, 1989). The occurrence of potyvirus infections limits bean production. Especially, BCMV is seen in almost every region where common beans are grown in Türkiye, and it is a serious issue in certified bean seed production (Deligöz et al., 2015).

There are different studies about the viruses causing diseases in common beans in Türkiye. Several potyviruses, including BCMV (Açıkgöz, 1984; Fidan and Yorgancı, 1990; Lisa et al., 1994; Güzel and Arlı-Sökmen, 2003), BCMNV (Güzel and Arlı-Sökmen, 2003), bean yellow mosaic virus “BYMV” (Açıkgöz, 1984; Lisa et al., 1994), cowpea aphid-borne mosaic virus “CABMV” (Yılmaz and Özaslan, 1987), cucumber mosaic virus “CMV” (Güzel and Arlı-Sökmen, 2003; Kılıç and Yardımcı, 2012), tomato black ring virus “TBRV” (Gümüş et al., 2001), and alfalfa mosaic virus “AMV” (Güzel and Arlı-Sökmen, 2003; Kılıç et al., 2013) were previously reported as infecting beans.

There are hundreds of plant species that are known to be infected by phytoplasmas. Legumes are one of the plant species affected by phytoplasmas severely. As a matter of fact, farmers consider pests and diseases to be among the major obstacles to increasing legume yield (Rubiales et al., 2015). When it comes to biotic stresses, viruses pose the greatest threat to all legume crops (Sen Gupta, 1974; Rubiales et al., 2015). Also, the spread of phytoplasma diseases has also become a major problem for legume crops (Bertaccini et al., 2014). Six different phytoplasma groups (16SrI-B, 16SrII-DC, 16SrV, 16SrVI-D, 16SrIX-A, and 16SrXII-B) have been reported as infecting various bean species such as broad bean, cowpea, chickpea, soybean, pea, mung bean, pigeon pea, lentil, and faba bean all over the world (Raj et al., 2006; Arocha et al., 2007; Akhtar et

al., 2008; Kumar et al., 2012; Pallavi et al., 2012; Zwolińska et al., 2012; Akhtar et al., 2016; Thorat et al., 2016; Omar, 2017).

Phytoplasma diseases have a substantial impact on many different types of crops, including ornamental plants, weeds, spices, medicinal plants, and others, and as a result, many instructive reviews have been written on various aspects of phytoplasma diseases (Bertaccini and Duduk, 2009; Rao et al., 2011, 2017c; Marcone, 2014; Liu et al., 2017; Rao and Kumar, 2017). However, there is no much information about the phytoplasma diseases of common beans. As yet, long stem, machismo, phyllody, and witches'-broom diseases have been detected in common beans. Nevertheless, the causal agents of both machismo and witches'-broom diseases were just demonstrated by electron microscopy. To date, no phytoplasma diseases have been specified on common beans in Türkiye.

The best way to combat viruses is through the development of genetic resistance. Bean breeding programs around the world aim to develop cultivars with increased resistance to biotic and abiotic stresses. Reduced pesticide use in high input systems, prevented yield loss from pests and diseases, and more consistent bean production in a wide range of challenging environments are all possible thanks to stress-resistant cultivars. Cultivar development programs have benefited from the identification of sources of resistance to numerous significant bean diseases (Beaver et al., 2003; Miklas et al., 2006). Disease-resistance genes can be deployed effectively by breeders, but only if they have access to information from plant pathologists about the virulence patterns of the pathogens (Coyne et al., 2003).

Globally, conventional breeding has made great strides toward developing disease-resistant cultivars. More effective use of breeding to create resistant cultivars is possible with the help of markers associated with established resistance loci. Resistance or resistance sources of some bean cultivars, genotypes, or breeding lines have been mostly studied against BCMV and BCMNV both in the world and in our country. Most recently, 350 common bean genotypes have been screened for their resistance to BCMV and only eleven genotypes viz., BD9116291, EC31084, EC400414, EC400445, EC540173, EC541703, ET4515B, ET8409, IC340947, IC356024, and IC360831 were found to be resistant as a result of this study (Manjunatha et al., 2021). In our country, one study has been conducted recently to evaluate the presence of resistance sources in

39 common bean cultivars, widely grown in Türkiye to BCMV and BCMNV with different molecular markers (SW-13, SBD-5, ROC11, eIFE4). According to results eleven common bean cultivars carried one single resistance gene ($bc-1^2$), whereas a gene combination of $bc-1^2$ and $bc-3$ was found as the main resistance factor in Fransız, Karabacak, and 40 Günlük cultivars (Palacioğlu et al., 2020). In another study, BCMV and BCMNV resistance levels were tested in 204 breeding lines, and 153 breeding lines were found to be resistant to NL-4 of BCMV and other 5 to NL-3 strains of BCMNV, while 46 breeding lines were susceptible to both strains, and it was revealed that most of the breeding lines (153) had the *I* gene, and other five and four breeding lines had $bc-2^2$ and $bc-1^2$, respectively (Deligoz et al., 2021).

The common bean is one of the most important grain legume crops cultivated in Niğde province, which is found in the first rank of bean production in the Central Anatolia Region of Türkiye. Because of the importance of the bean crop in Niğde's agriculture and the lack of information about the prevalence of viruses in this province and no information about the bean phytoplasma diseases in Türkiye, the current investigation has been conducted for the below-mentioned purposes.

1.1 Objective of Thesis

- Investigation of the existence of viral (potyviruses) and phytoplasma diseases in the main common bean production areas of Niğde province,
- Detection of viruses and phytoplasmas on collected common bean samples using appropriate serological and molecular methods,
- Determination of reactions of different common bean cultivars to available viral agents obtained from this study.

CHAPTER II

GENERAL INFORMATION

More than 7,000 years ago, in the highland areas of Latin America, the common bean (*Phaseolus vulgaris* L.) was domesticated (Kaplan, 1965; Gepts and Debouck, 1991). Three distinct gene pools (Mesoamerican, Andean, and Peruvian-Ecuadorian) characterize the *P. vulgaris* strains collected from the wild between northern Mexico and northwest Argentina (Debouck et al., 1993; Kami et al., 1995). Morphology, multi-locus genetic markers, seed proteins, allozymes, and nucleotide data studies on *P. vulgaris* point to two main gene pools, the Mesoamerican and the Andean, with regional structuring visible even in domesticated forms (Bellucci et al., 2014a). According to Bitocchi et al. (2012), wild forms of common beans were first domesticated in central Mexico (Mesoamerican gene pool) and then transported to South America, and common beans contain three gene pools (Andean, Mesoamerican, and northern Peru-Ecuador).

Beans are hardy plants that can thrive in a wide range of environments and can be grown anywhere from 50 to 3000 meters above sea level. The ideal conditions for this crop are a warm temperature range (14 to 26°C), a moderate amount of rain (400–1600 mm per year), and a slightly acidic soil pH (5-6). Beans have a relatively broad-spectrum of days to maturity (70-330 days) and potential grain yield (400-5000 kg ha⁻¹) (Wortmann et al., 1998). Domestication of common beans in the Americas led to their spread to other regions and countries around the world. Europe (Santalla et al., 2002; Angioi et al., 2010; Gioia et al., 2013), central-eastern and southern Africa, Brazil, and China have all been identified as secondary centers of diversity (Bellucci et al., 2014a).

2.1 Major Diseases of Common Bean

The aerial and underground components of the common beans are susceptible to a wider variety of disease-causing pathogens, including bacteria, fungi, and viruses (Schwartz and Corrales, 1989; Schwartz et al., 2005). In both tropical and temperate regions, growing common beans presents a challenge due to a bacterial disease known as common bacterial blight (CBB), which is caused by *Xanthomonas axonopodis* pv.

phaseoli. *Pseudomonas syringae* pv. *phaseolicola*, which causes halo blight, and *Pseudomonas syringae* pv. *syringae* van Hall, which causes bacterial brown spot, are two other significant bacterial diseases that can lead to significant yield losses, especially in slightly cooler and more humid regions. The most widespread fungal diseases in the world's bean-growing regions are angular leaf spot (caused by *Phaeoisariopsis griseola*), anthracnose (caused by *Colletotricum lindemuthianum*), and rust (caused by *Uromyces appendiculatus*) (Singh and Schwartz, 2010).

Apart from all these diseases, web blight (caused by *Thanatephorus cucumeris*) in the warm humid tropics (e.g., Costa Rica, Colombia), various root rots caused by fungi, including *Fusarium* in most common bean production regions, white mold (caused by *Sclerotinia sclerotiorum*) in the United States and Canada, and ascochyta blight (caused by *Ascochyta phaseolorum*) in cool wet regions including the humid highlands of Mexico, Central America, and the Andes may be the most predominant, highly destructive and endemic problems (Singh and Schwartz, 2010).

2.1.1 Viral diseases infecting common bean

Under both natural and experimental conditions, the common bean is much more vulnerable to plant viruses. Therefore, viral diseases represent a significant obstacle to common bean productivity wherever this legume is planted (Schwartz et al., 2005). In developing countries, the viral pathogens and vectors of viruses that infect common beans have been effectively defined, and the majority have been appropriately examined in order to control them (Morales, 2003). More than thirty viral pathogens with single- or double-stranded RNA and DNA genomes have been naturally recorded in infected beans.

A huge variety of single- and double-stranded DNA and RNA plant viruses affect common bean over the world. *Alfamovirus*, *Bromovirus*, *Comovirus*, *Cucumovirus*, *Begomovirus*, *Endornavirus*, *Illarvirus*, *Luteovirus*, *Potyvirus*, *Sobemovirus*, *Tobamovirus*, *Necrovirus*, and *Tospovirus* are the genus of viruses that naturally infect common bean (Hall, 1991; Loebenstein and Thottappilly, 2004; Ghorbani et al., 2010; Okada et al., 2013).

Along with BCMV and BCMNV, some of the important viruses infecting common beans have been indicated in Table 2.1 with some modifications from Meziadi et al. (2017).

Table 2.1. Some of viruses infecting common bean worldwide modified from Meziadi et al. (2017)

Genome Type	Family	Genus	Virus	Transmission
+ssRNA	Potyviridae	Potyvirus	Bean common mosaic virus (BCMV)	Aphids, whiteflies, seedborne, mechanical
			Bean common mosaic necrosis virus (BCMNV)	Aphids, whiteflies, seedborne, mechanical
			Bean yellow mosaic virus (BYMV)	Aphids, whiteflies, seedborne, mechanical
			Cowpea aphid-borne mosaic virus (CABMV)	Aphids, whiteflies, seedborne, mechanical
			Clover yellow vein virus (CIYVV)	Aphids, whiteflies, seedborne, mechanical
			Peanut mottle virus (PeMoV)	Aphids, whiteflies, seedborne, mechanical
			Passionfruit woodiness virus-K (PWV-K)	Aphids, whiteflies, seedborne, mechanical
			Soybean mosaic virus (SMV)	Aphids, whiteflies, seedborne, mechanical
			Thailand passiflora mosaic virus (ThPV)	Aphids, whiteflies, seedborne, mechanical
			Zucchini yellow mosaic virus (ZYMV)	Aphids, whiteflies, seedborne, mechanical
			Watermelon mosaic virus 2 (WMV-2)	Aphids, whiteflies, seedborne, mechanical
	Bromoviridae	Alfavirus	Alfalfa mosaic virus (AMV)	Aphids
		Cucumovirus	Cucumber mosaic virus (CMV)	Aphids, seedborne
			Peanut stunt virus (PSV)	Aphids, seedborne, mechanical
			Tomato aspermy virus (TAV)	Unknown
		Bromovirus	Cowpea chlorotic mottle virus (CCMV)	Beetles
			Tobacco streak virus (TSV)	Thrips, seeds, pollen, dodder
	Secoviridae	Comovirus	Bean pod mottle virus (BPMV)	Beetles
			Bean rugose mosaic virus (BRMV)	Beetles
			Quail pea mosaic virus (QPMV)	Beetles
		Nepovirus	Tomato black ring virus (TBRV)	Nematodes
			Tobacco ringspot virus (TRSV)	Nematodes
	Unassigned	Sobemovirus	Southern bean mosaic virus (SBMV)	Beetles, seedborne
	Virgaviridae	Tobamovirus	Tobacco mosaic virus (TMV)	Mechanic
	Betaflexiviridae	Carlavirus	Cowpea mild mottle virus (CPMMV)	Whiteflies
-ssRNA	Bunyaviridae	Tospovirus	Tomato spotted wilt virus (TSWV)	Thrips
ssRNA (-/+ unknown)	Tombusviridae	Carmovirus	Bean mild mosaic virus (BMMV)	Beetles
dsRNA	Endornaviridae	Endornavirus	Phaseolus vulgaris endornavirus 1 (PvEV-1)	Seedborne
			Phaseolus vulgaris endornavirus 2 (PvEV-2)	Seedborne
ssDNA	Geminiviridae	Begomovirus	Bean dwarf mosaic virus (BDMV)	Whiteflies, mechanical
			Bean calico mosaic virus (BCaMV)	Whiteflies
			Bean golden mosaic virus (BGMV)	Whiteflies, mechanical
			Bean golden yellow mosaic virus (BGYMV)	Whiteflies, mechanical
			Calopogonium golden mosaic virus (CaGMV)	Weed
			Tobacco yellow dwarf virus (TYDV)	Leafhopper
			Tomato yellow leaf curl virus (TYLCV)	Whiteflies, mechanical
			Squash yellow mild mottle virus (SYMMoV)	Unknown
		Curtovirus	Beet curly top virus (BCTV)	Beet leafhopper
		Mastrevirus	Bean yellow dwarf virus (BeYDV)	Whiteflies, mechanical

Among these viral diseases, AMV, BCMV, BCMNV, BYMV, BGMV, CMV, SBMV, TSV, and *Tomato aspermy virus* (TAV) is considered the most significant viruses infecting common beans (Kumar et al., 1994; Güzel and Arlı-Sökmen, 2003; Ghorbani et al., 2010). In Türkiye, the viruses causing diseases on common beans have been revealed as BCMV (Açıkgöz, 1984; Fidan and Yorgancı, 1990; Lisa et al., 1994; Güzel and Arlı-Sökmen, 2003), BCMNV (Güzel and Arlı-Sökmen, 2003), BYMV (Açıkgöz, 1984; Lisa et al., 1994), CABMV (Yılmaz and Özasan, 1987), CMV (Güzel and Arlı-Sökmen, 2003; Kılıç and Yardımcı, 2012), TBRV (Gümüş et al., 2001), and AMV (Güzel and Arlı-Sökmen, 2003; Kılıç et al., 2013).

2.1.1.1 Potyvirus

Potyvirus is one of the largest genera in the family *Potyviridae*, containing over 150 different virus species (Sharma et al., 2021). Despite this, many newly discovered and named potyvirus species have emerged in recent years (Mederos et al., 2017; Parizad et al., 2017). Potyviruses are small, flexible viruses that are believed to have emerged between 15,000 and 30,000 years ago. Their flexuous filaments range in size from 680 to 900 nm in length and 11 to 20 nm in diameter (Gibbs et al., 2020). In the case of potyviruses, the P subunits of the coat protein enclose an 8-11 kilobase (kb) positive, single-stranded RNA genome (Sorel et al., 2014). At least 10 essential proteins are encoded by the single open reading frame (ORF) in *Potyvirus* genomes (Riechmann et al., 1992) (Figure 2.1).

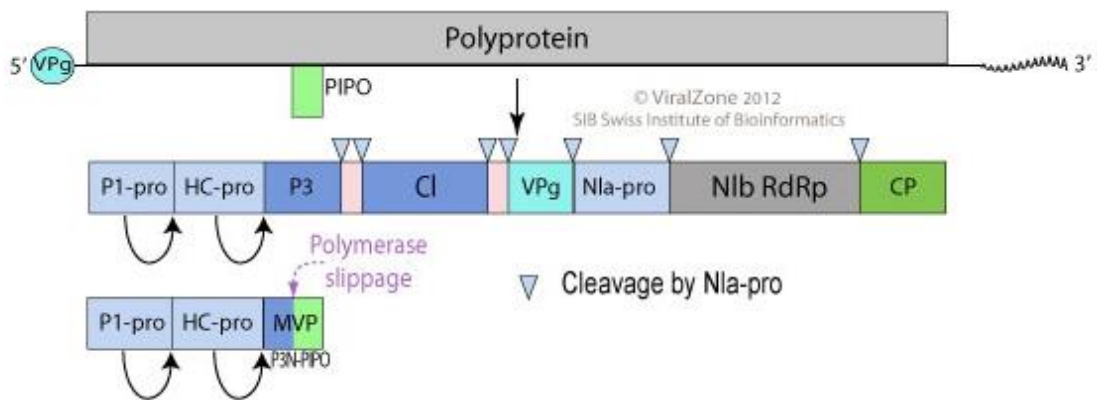


Figure 2.1. Genome organization of potyviruses (ViralZone https://viralzone.expasy.org/50?outline=all_by_species)

The majority of potyviruses are transmitted by aphids in a non-persistent manner with varying degrees of success. Over 4,000 aphid species have been recognized so far. There are over 4,000 known species of aphids. More than 200 of the nearly 300 species studied have been implicated in the spread of at least one virus (Eastop, 1983). Members of the *Aphidinae* subfamily, which includes the genera *Aphis*, *Myzus*, and *Macrosiphum*, make up the vast majority of known vector species. Potyvirus vector species that are polyphagous include *M. persicae*, *A. gossypii*, *A. craccivora*, and *A. faba* (Raccah et al., 2001). Some potyviruses are also spread by seed (Table 2.1). The virus can spread through its infected seeds, but only in certain host plant species and cultivars (Brunt et al., 1996).

Many potyviruses pose a significant hazard to major agricultural crops. The family *Potyviridae* covers commercially significant plant virus genera that represent a hazard to crops all over the world (Jones and Naidu, 2019). Potyvirus infections may also have a negative impact on common bean yield. Some of the potyviruses infecting beans include BCMV, BCMNV, BYMV, CIYVV, CABMV, PeMoV, and SMV.

The activities that are made to increase yield and quality contain the arrangement of environmental conditions, the application of agricultural practices in an appropriate way, and the protection of crop plants from disease-causing pathogens and pests. However, it is not easy to control and manage the viruses that cause significant diseases in crop plants. Cultural practices, the use of resistant and virus-free planting materials, as well as management of vectors that provide the transmission of viruses contribute remarkably to the management strategies of the viruses. Since the viruses cannot be brought under control even if all these strategies were applied and there are also no chemicals to control plant viruses, the importance of the management of plant viruses is getting more vital throughout the world. Therefore, the first issue to protect crop plants from viral diseases is to detect causal agents. Once the viral agents have been detected, the control measures may be taken earlier before the spread of viruses through infected plants or materials, as well as through their natural vectors (Kılıç and Yardımcı, 2014).

Many methods have been developed for the detection and identification of plant viruses. While a single test or assay may be sufficient for determining the identification of a virus, definitive identification usually necessitates the use of a mixture of methods

(Naidu and Hughes, 2003). For viral diagnosis, a variety of laboratory methods have evolved and been modified, but serology stands out as one of the most specific and simple techniques for obtaining a quick and exact identification (Purcifull et al., 2001). Apart from serological tests, along with the advancement of molecular techniques, Polymerase Chain Reaction (PCR) method has been developed, enabling the sensitive and accurate diagnosis of bean viruses from plant tissues (Choi et al., 1999; Petrovic et al., 2010; Mwaipopo et al., 2017).

Many studies have explored the occurrence of viruses in common bean plants using especially ELISA and Reverse transcription polymerase chain reaction (RT-PCR) methods from the past to present. There are many studies on the detection of some potyviruses infecting common beans around the world. However, the studies included in Table 2.2 have been summarized based on the use of molecular methods for detecting some potyviruses infecting common beans due to the similarity of molecular methods used in this study.

Table 2.2. The several studies in the world involving the detection of some potyviruses infecting common bean

Virus Species	Country	Symptom	Prevalence	Damage	Detection Method	Reference
SMV	Colombia	—	Data not found	Data not found	Mechanical transmission to the bean variety Dubbele Witte and to Monroe.	Castano and Morales, 1983
CIYVV	California	Yellow mosaic, leaf epinasty, and systemic necrosis	100% infection rate	Severe reduction in yield	ELISA, RT-PCR	Cmov and Gilbertson, 2001
PeMoV	USA	Systemic vein necrosis	Infection rates, ranging from 5 to 25 % in some snap bean fields	Significant yield loss	RT-PCR	Larsen and Miklas, 2010
BYMV	Iran	Mosaic and leaf distortion	The infection rate 20.7%	A major yield reduction factor in bean production	RT-PCR	Hosseini and Hosseini, 2014
BCMV and BCMNV	Mexico	Mosaic, dwarfing, leaf curling, chlorosis, systemic necrosis, top necrosis, and blackroot	63% incidence of BCMV, 45% incidence of BCMNV	Significant yield loss	RT-PCR for both BCMV and BCMNV	Flores-Estevez et al., 2003
BCMV and BCMNV	Kenya	Mosaic, leaf rolling, dwarfing, chlorosis, or a combination of these	35– 98% infection	100% yield losses	TAS-ELISA for BCMV DAS ELISA for BCMNV	Mangen, 2014

Table 2.2. (Continued) The several studies in the world involving the detection of some potyviruses infecting common bean

Virus Species	Country	Symptom	Prevalence	Damage	Detection Method	Reference
BCMV	Tanzania	Vein clearing, leaf distortion, and rugosity, mosaic, upward and downward leaf curl	Three fields (Mvomero, Lushoto, and Tarime) had the highest BCMV incidence (36.7%)	Cause yield losses as high as 100%	RT-PCR	Mwaipopo et al., 2018
BCMNV			The field with the highest incidence (76.7%) of BCMNV was in Gairo District			
BCMV, BCMNV, BYMV, and WVMV	Iran	Mosaic, mottling, chlorosis, vein banding and leaf deformation	22.7% <i>Potyvirus</i> infection in the samples tested. Amplifications have been obtained for four viruses	Not indicated	PTA-ELISA, RT-PCR	Valouzi et al., 2019

2.1.1.2 Potyvirus diseases of common bean in Türkiye

Among potyvirus species infecting common bean plants, those which BCMV (Açıkgöz, 1984; Fidan and Yorgancı, 1990; Lisa et al., 1994; Güzel and Arlı-Sökmen, 2003), BCMNV (Güzel and Arlı-Sökmen, 2003), BYMV (Açıkgöz, 1984; Lisa et al., 1994) and CABMV (Yılmaz and Özaslan, 1987) have already been investigated in our country. Among them, BCMV and BCMNV are considered the most crucial potyviruses, causing serious damage to common bean plants. BCMV and BCMNV have been linked to disease incidences as high as 100%, with yield losses ranging from 35-98% (Hampton, 1975; Wortmann et al., 1998; Albrechtsen, 2006). In Türkiye, there is no detailed research concerning the yield losses caused by both BCMV and BCMNV. Anyhow, especially BCMV is seen in almost every region where common beans are grown in Türkiye and it is a crucial problem in certified common bean seed production (Deligöz et al., 2015). Table 2.3 outlines the detection of some viruses infecting common beans in Türkiye.

Table 2.3. The studies in Türkiye related to the detection of some potyviruses infecting common bean

Virus Species	Country	Symptom	Prevalence	Damage	Detection Method	Reference
CABMV	Adana	—	Data not found	Data not found	—	Yılmaz and Özasan, 1987
BCMV, AMV, and TBRV	The Aegean region	—	24.1% infection for BCMV, 41.4% for AMV, 13.8% for TBRV	—	DAS-ELISA	Gümüş et al., 2001
BCMV	Tokat	—	59% infection	—	DAS-ELISA	Kutluk-Yılmaz et al., 2002
BCMV, BCMNV, BYMV, and CMV	Samsun	Mosaic, leaf curling, and leaf distortion	In leaf samples, 36% of infection for BCMV, 10.8% for CMV, 2.8% for BCMNV, In seeds, 2% for BYMV, 8.9% infection for BCMV, 17% for BCMNV+CMV	—	DAS-ELISA	Güzel and Arlı-Sökmen, 2003
BCMV and BCMNV	Samsun	Mosaic, chlorosis, dwarfing, leaf curling,	In 367 seed and leaf samples tested, 18.2 % was found to be infected with BCMV and only five (1.4 %) were infected with BCMNV	Significant yield loss	DAS-ELISA and RT-PCR	Arlı-Sökmen et al., 2011
BCMV, AMV, and CMV	Muğla	Leaf deformation, leaf curling, mosaic, and dwarfing	17.85% of infection for BCMV, 3.57% for AMV and 1.78% for CMV In case of mix-infection, 0.89% for AMV+CMV and 0.89% for BCMV+CMV	25% yield losses	DAS ELISA	Kılıç et al., 2013
BCMV	Burdur	Leaf deformation, leaf curling, vein clearing, necrosis, mosaic, and dwarfing	23.52 % infection has been detected in 24 samples.	—	DAS ELISA, IC-RT-PCR	Kılıç and Yardımcı, 2014
BCMV, AMV, and CMV	The West Mediterranean Region of Türkiye	Mosaic, deformation, distorted leaves, vein necrosis, yellowing, brown spotting and wilting	35.91% for CMV, 9.06% for BCMV, 5.70% for AMV and In case of mix-infection, 16.44% for BCMV+CMV. The three viruses have been detected by IC-RT-PCR	—	DAS ELISA, IC-RT-PCR	Kılıç et al., 2015
BCMV, CMV, AMV, and CMV	İzmir	Mosaic, mottling, chlorosis, vein banding and leaf deformation	62.86% for BCMV, 7.14% for CMV, 8.57% for BCMV+CMV In seeds, 61.43% for BMCV, 17.14% BCMV+CMV	—	DAS ELISA RT-PCR	Saraçoğlu and Erkan, 2016
BCMV and BCMNV	In the Lakes Region of Türkiye	Mosaic, mottling, curling, yellowing, chlorotic and necrotic lesions, and deformation	26.18% infection for BCMV in leaf sample, 15.27% for BCMNV In seed samples, 2.32% for BCMV, 6.97% for BCMNV In IC-RT-PCR tests only BCMV has been amplified	—	DAS ELISA, IC-RT-PCR and Biological Assays	Kılıç et al., 2020
BYMV	Burdur	Mosaic, mottling, curling, yellowing, chlorotic and necrotic lesions, and deformation	DAS-ELISA revealed that BYMV was present in 97 out of 443 samples (21.89%).	—	DAS-ELISA	Ulum et al., 2020

2.1.2 Resistance to potyvirus diseases in common bean

Creating disease- and insect-resistant cultivars of common bean is a challenging issue for plant breeders. Since the use of phytosanitary treatments is constrained in the present ecological context of protecting biodiversity and the environment, genetic resistance is the best and cheapest solution for managing viral diseases. Utilizing virus-resistant cultivars is, thus, the most efficient strategy in the common bean breeding program. Numerous research has been undertaken over the past 80 years to uncover viral resistance genes in common beans, and some of these genes are already being used in plant breeding. Some were investigated just in case it turned out that new genes were needed to replace the ones that had been defeated and restore full resistance. Other methods of preventing the spread of viruses in common beans include dominant and recessive resistance genes (Bourguet and Guillemaud, 2016).

Since the identification of the *I* locus at the end of chromosome Pv02 (Ali, 1950; Kyle and Dickson, 1988), dominant *R* genes against potyviruses have received the greatest attention in common beans. The resistance to at least 10 potyviruses (BCMV, BCMNV, BYMV, CABMV, CIYVV, SMV, passionfruit woodiness virus-K (PWV-K), thailand passiflora mosaic virus (ThPV), watermelon mosaic virus 2 (WMV-2), and zucchini yellow mosaic virus (ZYMV) is conferred by the dominant *I* gene (McKern et al., 1992; Fisher and Kyle, 1994; Berger et al., 1997; Hart and Griffiths, 2015). Virus-resistance genes that protect against BCMV or BCMNV are the most well-known in the common beans. Most research efforts have been focused on the *bc* genes. Drijfhout (1978) discovered that BCMV and BCMNV resistance in beans is controlled by a total of 6 recessive *bc* genes, all of which are located at one of 4 different loci. When plants have the *bc* genes integrated, either alone or in combination with the *I* locus, they are resistant to common mosaic disease and black root systemic necrosis. The four recessive genes are *bc-u*, *bc-1*, *bc-2* and *bc-3* with two alleles for *bc-1* (*bc-1* and *bc-1*²) and *bc-2* (*bc-2* and *bc-2*²). One of the recessive genes, *bc-u*, alone does not offer resistance to known BCMNV and BCMV strains. It seems that *bc-u* is a non-specific allele that is responsible for the expression of other *bc* genes (Strausbaugh et al., 1999). Among the recessive *bc* genes, *bc-1*, *bc-1*², *bc-2*, *bc-2*² and *bc-3* are specific to the virus strain. The *bc-u* gene, on the other hand, is a gene that is not specific to the virus strain and is responsible for the emergence of resistance created by other *bc* genes in the

absence of the *I* gene. Unlike *bc-2*, which doesn't have a known genomic location, *bc-u* and *bc-1* genes have been mapped to one end of chromosome Pv03 (Miklas et al., 2000; Meziadi et al., 2016), while *bc-3* has been mapped to chromosome Pv06 and identified as a member of the eIF4E gene family (Naderpour et al., 2010; Meziadi et al., 2016). The resistant genotypes with dominant *R* and recessive genes against some potyviruses infecting common beans were shown in Table 2.4 and Table 2.5 modified from Meziadi et al. (2017).

Table 2.4. Dominant *R* genes and resistant genotypes against some potyviruses infecting common beans modified from Meziadi et al. (2017)

Genus	Virus Name	Name of <i>R</i> gene	Resistant genotype	Reference
<i>Potyvirus</i>	BCMV	<i>I</i> *	'Black Turtle-1'	Drijfhout, 1978; Ali, 1950; Kyle et al., 1986
	BCMNV	<i>I</i> *	'Black Turtle-1'	Drijfhout, 1978; Kyle and Provvidenti, 1987; Provvidenti, 1974
	BYMV	<i>By-1</i>	'Amanda'	Park, 1991
	BYMV/CIYVV	<i>By-2</i> *	<i>P. coccineus</i> 'B28S2C', 'Amanda'	Hart and Griffiths, 2015; Dickson and Natti, 1968
	CabMV	<i>Cam</i> *	'GN 1140'	Provvidenti et al., 1983; Kyle et al., 1987
	PeMoV	<i>Pmv</i>	'Black Turtle-1'	Provvidenti and Chirco, 1987; Li et al., 1991
	PWV-K	<i>PvPwvk</i> * <i>m</i>	'Black Turtle-1'	Fisher and Kyle, 1994
	SMV	<i>Hss</i> *	'Black Turtle-1'	Provvidenti et al., 1982
		<i>Smv</i>	'GN1140'	
	ThPMV	<i>PvTpmv</i> * <i>m</i>	'Black Turtle-1'	Fisher and Kyle, 1994
	ZYMV	<i>PvZymv</i> * <i>m</i>	'Black Turtle-1'	Fisher and Kyle, 1994
	WMV-2	<i>Wmv</i> *	'Great Northern 1140'	Kyle et al., 1987

*: *R* genes located at the *I* locus; m: name proposed in Meziadi et al. (2017).

Table 2.5. Recessive genes and resistant genotypes against some potyviruses infecting common bean modified from Meziadi et al. (2017)

Genus	Virus Name	Name of Recessive gene	Resistant Genotype	Reference
<i>Potyvirus</i>	BCMV	<i>bc-1</i>	'Redlands Greenleaf C'	Drijfhout, 1978; Strausbaugh et al., 1999; Miklas et al., 2000
	BCMV/BCMNV	<i>bc-u</i>	'Olathe', 'Stringless Green Refugee'	Drijfhout, 1978; Strausbaugh et al., 1999; Miklas et al., 2000
		<i>bc-1</i> ²	'Nodak', 'Redlands Greenleaf B'	Drijfhout, 1978; Miklas et al., 2000; Feng et al., 2018
		<i>bc-2</i>	'Michelite', 'Sanilac'	Drijfhout, 1978; Miklas et al., 2000; Feng et al., 2018
		<i>bc-2</i> ²	'N85120'	Drijfhout, 1978; Kelly et al., 1995
		<i>bc-3</i>	'B85009', 'IVT7214'	Drijfhout, 1978; Mukeshimana et al., 2005
	BYMV	<i>by-3</i>	'GN1140'	Provvidenti and Schroeder, 1973
	CIYVV	<i>cyv</i>	'Clipper'	Hart and Griffiths, 2013

There are various research studies in the world to examine the reactions of different common bean cultivars and their available resistance genes to BCMV, BCMNV, BYMV, CABMV, CIYVV, PeMoV, and SMV. In one research study conducted by Provvidenti et al. (1983), resistance to blackeye cowpea mosaic virus (BICMV) and cowpea aphidborne mosaic virus (CAbMV) was conferred separately by single dominant factors in populations derived from crosses of resistant and susceptible plants of the bean cv. 'Black Turtle Soup'. The gene symbol *Bcm* was given for BICMV resistance, whereas the gene symbol *Cam* was given to CABMV. Testcross plants were inoculated with both viruses on separate leaves in order to establish the linkage. The presence of BICMV and CAbMV in each susceptible plant was determined using an ELISA due to the fact that the two viruses cause similar symptoms but do not cross-protect against each other. When resistant plants were approach-grafted onto BICMV or CAbMV-infected plants, or when they were mechanically inoculated and maintained in a 35°C environment, it was discovered that they still displayed a lethal systemic hypersensitive response.

Resistance to seven strains of BYMV from a broad geographical region was investigated in green and dry bean accessions. According to the results of this study, the beans were divided into four groups based on their resistance level. While group 1 comprised the accessions with a high level of resistance to most BYMV isolates, group 2 included the accessions containing previously known sources of resistance in germplasm derived from the cv. 'Great Northern', which was primarily susceptible to the BOS 25 isolate, yet had high levels of resistance to most other isolates. Besides, groups 3 and 4 demonstrated increased levels of susceptibility with the accession Red Mexican. When the seven BYMV isolates were inoculated into a variety of plant species, they induced varied responses, showing that they had various pathogenicity genes (Walkey et al., 1983).

Kyle and Provvidenti (1993) carried out one research study to find out the dominant factors in *P. vulgaris* L. cv. 'Black Turtle Soup,' line BT-I. As a result of this study, a single dominant factor, *Hss*, that enables a quick fatal necrotic response to SMV has been detected. Regardless of ambient temperature, inoculated plants having *Hss* factor produced localized necrotic lesions on inoculated tissue, followed by systemic vascular necrosis and plant death within 7 days. Moreover, since BT-I also carries potyvirus

resistance due to closely related or identical components *I*, *Bcm*, *Cam*, and *Hsw*, its association with *Hss* was investigated. There were no recombinants found among 381 F_3 families segregating for potyvirus susceptibility, indicating that if *Hss* is a separate factor, it is firmly connected to *I*, *Bcm*, *Cam*, and *Hsw*. Furthermore, when BT-I was also crossed with the line 'Great Northern 1140' ('GN 1140'), which was the first to identify the dominant gene, *Smv*, for systemic resistance to SMV. *Smv* and *Hss* evolved separately and are now co-dominant. While the ('GN 1140' x BT-1) F_1 populations demonstrated a seasonal shift of the codominant phenotype, the ('GN 1140' x BT-1) F_2 population under circumstances where *Smv* is substantially dominant permitted the separation of new phenotypic groups.

In one study, preliminary screening resistance was used to determine how germplasm materials responded to PeMoV, and it was discovered that the G 122 landrace and BelNeb-RR-1 great northern both possessed resistance. In this study, a recombinant inbred line (RIL) population made up of 75 F_9 -derived lines from a cross between the PeMoV-resistant BelNeb-RR-1 and the PeMoV-susceptible A55 (Ariyaratne et al., 1999) and as well as a second RIL population made up of $F_{5;7}$ RILs made by Johnson (1997) from a cross between the PeMoV-resistant landrace cultivar G122 and the A55 (AG population) were used. The Texas PeMoV isolate was mechanically inoculated at the primary leaf stage into each RIL population along with their respective parents. All susceptible plants showed systemic necrosis (top necrosis) in secondary trifoliolate leaves and stem 10 to 14 days after inoculation, which ultimately led to plant death. Despite the absence of viral systemic movement, resistant plants in both groups displayed a hypersensitive response on inoculated leaves. However, one line (recombinant) in the second population mentioned above showed a variable reaction of systemic mosaic and no systemic necrosis (Larsen and Miklas, 2010).

Additionally, a study was performed to validate a previously designed KASP for future *bc-3* resistance allele mining and to expand the characterization of CIYVV resistance in common bean through 391 USDA *P. vulgaris* Core Collection accessions, a collection of 99 snap bean cultivars, and an extended host differential panel of 75 genotypes with known response to other viral pathogens. In a summary, the snap bean cultivars and the *P. vulgaris* core collection were inoculated when the primary leaves were fully developed in order to conduct a quick screening (7-10 days after planting). The

symptoms of CIYVV infection in the inoculated plants were observed daily. This led to the confirmation of CIYVV-NY resistance in 19 dry bean genotypes, an additional snap bean breeding line, and one Core Collection accession by the observation of physical symptoms and the use of ELISA. In addition, PveIF4E mutations matching *bc-3* or *bc-3²* were present in all asymptomatic genotypes over the course of the investigation. Resistance phenotypes to CIYVV have also been demonstrated to be conditioned by the *bc-u*, *bc-2²* allele combination, and the *By-2* allele. These results support the use of the KASP tests for widespread *bc-3* allele mining, and they add up to a new paradigm for CIYVV genetic resistance (Hart and Griffiths, 2014).

BCMV and BCMNV are the most widely distributed and well-studied viruses infecting common beans all over the world. BCMV was first discovered in *P. vulgaris* in the United States in 1917. However, the geographic distribution of BCMNV is more limited and its incidence noticeably lower in most common bean-growing areas of the world as compared to BCMV. Much of the research studies have been undertaken in common beans to detect both viruses and screen the reactions and resistance genes of common bean accessions, genotypes, or cultivars against these viruses ever since.

In one recent study, researchers screened for resistance to BCMNV in sixteen widely planted bean cultivars in addition to cowpea, peanut, and soybean using a completely randomized block design with three replicates. The plants were infected with a BCMNV BG12 ELISA-positive isolate when they had three leaves. For three weeks, data was collected weekly on the kind of symptoms and the prevalence of the disease in plants. Ten bean cultivars were identified as vulnerable based on data analysis due to the presence of virus-typical symptoms like leaf mosaic, downward leaf curl, and yellowing. Since the BCMNV BG12 isolate from western Kenya did not cause any symptoms in four bean varieties (Imbeko, KK/RIL5/Red 13, Okwoto, RIL05/CAL 194), but positive with DAS ELISA, their tolerance was established. DAS ELISA results were deemed negative for BCMNV in two bean varieties (KK RIL05 and KK 072), hence they were recognized as resistant (Collins et al., 2019).

A total of 350 common bean genotypes were screened in two separate studies over the course of three consecutive growing seasons (2016–2017, 2017–2018, and 2018–2019) using both natural epiphytotic conditions and artificial inoculation. Twenty days after

being inoculated, test germplasms in observation were studied. Plants that showed signs of blistering, leaf rolling, green vein banding, mosaic, and mild mosaic were deemed susceptible, while those that showed no signs were deemed resistant based on visual assessments. Eleven genotypes (BD9116291, EC31084, EC400414, EC400445, EC540173, EC541703, ET4515B, ET8409, IC340947, IC356024, and IC360831) were consistently resistant. Amplification of the coat protein gene by reverse transcription-polymerase chain reaction confirmed the presence of BCMV infection. After sequencing the amplified region, it was found that BCMV-K had a nucleotide sequence similarity to BCMV strains from Uttar Pradesh (MT265069) and Himachal Pradesh (FJ387162, EU492546) that ranged from 94% to 99.70%. (Manjunatha et al., 2021).

In Türkiye, one study has been conducted recently to evaluate the presence of resistance sources in 39 common bean cultivars, to BCMV and BCMNV by different molecular markers (SW-13, SBD-5, ROC11, eIFE4). According to the results, eleven common bean cultivars carried a single resistance gene of *bc-1*². Moreover, when the cultivars have been evaluated for resistance gene combinations to these viruses, twenty-five cultivars acquired a gene combination of *I+bc-1*². On the other hand, the gene combination of *bc-1*² and *bc-3* was seen as the main resistance factor in cvs. ‘Fransız’, ‘Karabacak’, and ‘40 Günlük’ (Palacioğlu et al., 2020).

Another recent study analyzed 204 breeding lines for BCMV and BCMNV resistance. The BCMNV NL-3 and BCMV NL-4 strains were utilized for sap-inoculation of each breeding line, and the symptomatic viral infection responses of each plant were evaluated 21 days after inoculation. Based on the results of the biological tests, it was determined that 153 of the breeding lines were resistant to the NL-4 strain and 5 were resistant to the NL-3 strain, while 46 of the breeding lines were vulnerable to both strains. Resistance gene-associated molecular markers were also employed to corroborate the phenotypic evidence. The results showed that the majority of breeding lines (153) carried the *I* gene, while the remaining five breeding lines carried *bc-2*² and the remaining four breeding lines carried *bc-1*² (Deligöz et al., 2021).

2.2 General Introduction to Phytoplasmas

Phytoplasmas are plant pathogenic bacteria lacking cell walls that cause severe damage

in more than 700 plant species throughout the world (Maejima et al., 2014). Since they are the least characterized organisms among other plant pathogens, and since they cause large economic losses worldwide, they are considered agriculturally important plant pathogens. Infected plants with phytoplasmas show a wide variety of symptoms, including virescence/phyllody (the development of green leaf-like structures instead of flowers), the proliferation of axillary buds resulting in "witches broom" symptoms, the sterility of flowers, generalized stunting, and abnormal internode elongation (Bertaccini, 2007). Both extensive phloem necrosis and excess formation of phloem tissue is a symptom of severe phloem necrosis, which can be caused by phytoplasma infections (Lee et al., 2000). Some plant species are tolerant or resistant to phytoplasma infections and may be symptomless or exhibit only moderate symptoms, but it is obvious that the presence of phytoplasma has underlying harmful consequences on plants.

Some insects and plant phloem tissue host the obligatory parasites known as phytoplasmas. Phytoplasma infections are spread by the insect families *Cicadellidea* (leaf-hoppers) and *Fulgoridea* (plant-hoppers), which are sap-sucking (Brack, 1979; Grylls, 1979; Nielson, 1979). Insects that feed on phloem can acquire phytoplasmas from infected plants and transmit them to other plants. Infected vectors or perennial plants can serve as reservoirs for phytoplasmas over the winter, allowing the pathogens to be spread further in the spring. The parasitic plant dodder is another potential vector of phytoplasma infection (*Cuscuta* sp.). The first was this sort of transmission accomplished in the spread of coconut lethal yellowing (Cordova et al., 2003). Phytoplasmas may also be transferred vegetatively by cuttings, bulbs, rhizomes, or storage tubers (Lee and Davis, 1992): phytoplasmas in ornamental and fruit trees are often disseminated in this manner.

Diagnosis is often performed via symptom observation, insect or dodder/graft transmission to host plants, and electron microscopy of ultrathin slices of phloem tissue. In the 1980s, phytoplasmas were also identified by the use of serological techniques, most notably ELISA with polyclonal or monoclonal antibodies. However, serological approaches have not been widely used in the detection and identification of phytoplasmas because of insufficient specificity and sensitivity in the creation of unique high titre polyclonal antisera. Consequently, the detection of many phytoplasmas has shifted toward the nucleic acid technique based on PCR technology. Conventional

phytoplasma detection relies on universal (generic) PCR primers based on the highly conserved 16S ribosomal RNA (rRNA) gene sequences (Ahrens and Seemüller, 1992; Davis and Lee, 1993; Lorenz et al., 1995). In contrast, group-specific primers were developed based on rRNA genes encoding different ribosomal proteins (Jomantiene et al., 1998; Bertaccini and Martini, 1999).

Nested-PCR, a molecular methodology based on the conserved (16S rDNA) ribosomal phytoplasma area, is utilized for general classification to generate a comprehensive and reliable method of phytoplasma detection and identification (Lee et al., 1998; Seemüller et al., 1998). In nested-PCR, one universal primer pair is used for the initial amplification, and then another universal primer pair is used for the subsequent amplification. Using a universal primer pair followed by PCR with group-specific primer pairs, a technique called nested-PCR may be able to distinguish between different phytoplasmas in a mixed infection (Lee et al., 1994; 1995). To further differentiate suspected phytoplasmas on the 16S rRNA gene, Restriction Fragment Length Polymorphism (RFLP) analysis of PCR-amplified DNA sequences by various endonuclease restriction enzymes is now routinely utilized (Lee et al., 1998). Because the RFLP patterns of each phytoplasma are kept, it is possible to identify previously undiscovered phytoplasmas by comparing their patterns to those of known phytoplasmas.

The IRPCM Phytoplasma/Spiroplasma Working Team-Phytoplasma Taxonomy Group has proposed naming a single unique phytoplasma 16S rRNA gene sequence as a *Candidatus* Phytoplasma species (IRPCM, 2004). Additionally, if a phytoplasma strain's 16S rRNA gene sequence is more similar to the previously described one by more than 97.5%, it should be regarded as a novel '*Ca. Phytoplasma*' species. The use of RFLP or phylogenetic analysis of 16S rRNA gene sequences from a wide variety of phytoplasma strains has revealed 19 RFLP groups and more than 20 distinct ribosomal groups (Montano et al., 2001; Arocha et al., 2005; Al Saady et al., 2008).

2.2.1 Phytoplasma diseases infecting common bean

As a result of phytoplasma infections, the productivity and quality of important legume crops around the world have suffered significantly (Bertaccini et al., 2014). Broad bean,

chickpea, cowpea, faba bean, lentil, mung bean, pea, pigeon pea, and soybean are just some of the major legume crops that are infected by one of six distinct phytoplasma groups (16SrI-B, 16SrII-D-C, 16SrV, 16SrVI-D, 16SrIX-A, and 16SrXII-B) (Raj et al., 2006; Arocha et al., 2007; Akhtar et al., 2008; Kumar et al., 2012; Pallavi et al., 2012; Zwolińska et al., 2012; Akhtar et al., 2016; Thorat et al., 2016; Omar, 2017). There are a variety of helpful papers out there on the numerous aspects of phytoplasma infections and their major impacts on a wide range of crops, including medicinal plants, spices, ornamental plants, weeds, and others (Bertaccini and Duduk, 2009; Rao et al., 2011, 2017c; Marcone, 2014; Liu et al., 2017; Rao and Kumar, 2017). However, there is a lack of data regarding the common bean phytoplasma diseases. A recent electron microscope research has shown the machismo and witches' broom diseases' respective causative components. To our knowledge, there has been no research and reported cases of common bean plant diseases caused by phytoplasmas in Türkiye.

In the Columbia Basin of Washington, it has been revealed that a new disease called dry bean phyllody (DBPh) was discovered among dry bean (*Phaseolus vulgaris* L.) cultivars of Andean origin. Leafy petals (phyllody) and aborted seed pods resembling thin, twisted, and curved leaf-like structures were the symptoms of DBPh that appeared during mid-to-late pod development. Phylogenetic analysis of amplified 16S rDNA sequences and RFLP was utilized to identify this phytoplasma. As a result of these analyses, it was confirmed that the phytoplasma belonged to the clover proliferation group (16SrVI) subgroup A (16SrVI-A) (Lee et al., 2004).

One disease of common bean was characterized by the most distinctive symptom of long elongation of stems in El Salvador. It was revealed that the pathogenic agent of this phytoplasma was identified as a phytoplasma of the aster yellows group by PCR of 16S rDNA (Schwartz et al., 2005).

Another phytoplasma disease which is known as witches'-broom was observed on common bean plants in Japan and the symptoms were characterized by chlorosis, phyllody of floral organs, shoot proliferation, and reduced leaf size. The visualization of the causal phytoplasma has been made with electron microscopy, yet its identity and vector have not been determined. It was well-understood that the symptoms become

more visible after the leafhopper vector, *Orosius orientalis* feeds on bean plants for one month (Schwartz et al., 2005).

A disease known locally as amachamiento has been observed in the main dry bean production areas of Costa Rica. The primary symptoms include significant loss of bean pods owing to blooming reduction or abortion, interveinal chlorosis, and leaf deformation with corrugated midribs. The amachamiento phytoplasma was identified as a member of group 16SrI, '*Ca. Phytoplasma asteris*', subgroup 16SrI-B using RFLP patterns with *AluI*, *BfaI*, *RsaI*, *HaeIII*, *HhaI*, *HpaII*, *KpnI*, and *MseI* (Moreira et al., 2010).

Chlorosis of the crown leaves, short internodes, stunting, shoot proliferation, and yellowing has been documented in the vast majority of plants throughout Cuba's key common bean growing areas during the 2009–2010 survey. Nested PCR assay with universal primers has been applied with R16mF2/R1 primers for the first PCR amplification followed by fU5/rU3 primers for the nested amplification. The restriction profiles obtained from the digestion of PCR amplicons with *HpaII*, *HaeIII*, and *Sau3AI* endonucleases revealed that all of the fragments belonged to the same species, '*Ca. Phytoplasma asteris*' (16SrI group). As many as 99% of the bases in the five representative amplicons sequenced were identical to those in '*Ca. Phytoplasma asteris*' (16SrI group) phytoplasmas (Zamora et al., 2012).

In Serbia, '*Ca. Phytoplasma solani*' was detected on common bean by PCR with P1/P7 and R16F2n/R16R2 primers, and then confirmed through RFLP with *MseI* (Lee et al., 1998). StampF/R0 primers for direct, StampF1/R1 for nested PCR were used to characterize stolbur phytoplasma based on the *stamp* gene, and sequenced data were deposited in GenBank under Accession No. KM977907. The *stamp* gene sequences were 100 percent identical to the reference strain Rqg50 (KC703019), which has previously been found in cixiid species *Reptalus quinquecostatus*, and *R. panzeri*, on vineyards (Cvrkovic et al., 2014). From all stolbur-positive samples, a *vmp1* gene fragment with the use of the stolbur-specific primers StolH10F1/R1 and TYPH10F/R was amplified and then digested the resulting amplicon with *RsaI*. All six isolates matched the V14 *vmp1* type, a reference profile of stolbur phytoplasma linked to grapevine in Serbia, in terms of the restriction patterns in their *vmp1* genes (Mitrovic et

al., 2015). Table 2.6 shows some of the detected phytoplasma diseases infecting common bean worldwide.

Table 2.6. The studies related to the detection of some phytoplasma diseases infecting common bean

Disease/Casual Agent Name	Country	Symptom	Group	Detection Method	Reference
Dry bean phyllody (DBPh)	Columbia Basin of Washington	Leafy petals (phyllody) and aborted seed pods resembling thin, twisted, and curved leaf-like structures	The clover proliferation group (16SrVI) subgroup A (16SrVI-A)	Direct and Nested-PCR and RFLP	Lee et al., 2004
Long Stem	El Salvador	Long elongation of stems	The aster yellows group	PCR	Schwartz et al., 2005
Witches'-broom	Japan	Chlorosis, phyllody of floral organs, shoot proliferation, and reduced leaf size.	—	Electron microscopy	Schwartz et al., 2005
Amachamiento	Costa Rica	Significant loss of bean pods owing to blooming reduction or abortion, interveinal chlorosis, and leaf deformation with corrugated midribs	16SrI, ' <i>Candidatus</i> Phytoplasma asteris', subgroup 16SrI-B	Direct and Nested-PCR and RFLP	Moreira et al., 2010
<i>Candidatus</i> Phytoplasma asteris	Cuba	Chlorosis of the crown leaves, short internodes, stunting, shoot proliferation, and yellowing	<i>Candidatus</i> Phytoplasma asteris' (16SrI group)	Direct and Nested-PCR and RFLP	Zamora et al., 2012
<i>Candidatus</i> Phytoplasma solani	Serbia	Discoloration, yellowing, and downward rolling of the leaves	—	Direct and Nested-PCR and RFLP	Mitrovic et al., 2015

CHAPTER III

MATERIALS AND METHODS

3.1 Collection of Symptomatic Common Bean Plant Samples

In this research, common bean fields in Niğde province were visited twice a month for 3 months in the summer season of 2021. Sampling was made in the Central District of Niğde province; the villages (Ağcaşar, Çayırılı, Elmalı, Hasaköy, Ovacık, Yarhisar, Yeşilova) and the towns (Amas Bağları, Alay, Kiledere, Konaklı, Yeşilgölcük) (Figure 3.1). From the selected common bean fields, the leaves of plants showing virus and phytoplasma-associated symptoms were collected, labeled, and then placed in polyethylene bags. The number of plant samples collected from each visited field was given in Table 3.1. The samples collected were named with the numbers considering the collection date and they were maintained in a deep freezer at -80°C for further analysis.



Figure 3.1. Villages and towns where symptomatic common bean plant samples were collected within the scope of the research. Site 1 represents Alay, Site 2 Gölcük, Site 3 Konaklı, Site 4 Kiledere, and Site 5 Amas Bağları. In contrast, Site 6 represents Ağcaşar, Site 7 Çayırılı, Site 8 Elmalı, Site 9 Hasaköy, Site 10 Ovacık, Site 11 Yarhisar, and Site 12 Yeşilova

Table 3.1. Collection sites, field numbers visited and the number of common bean plant samples studied in this research

Province	Villages and Towns	Sample Number	Field Numbers Visited
Niğde	Ağcaşar	25	5
	Alay	18	4
	Amas Bağları	10	1
	Çayırılı	2	1
	Elmalı	15	4
	Hasaköy	4	1
	Kiledere	6	1
	Konaklı	64	8
	Ovacık	44	7
	Yarhisar	1	1
	Yeşilgölcük	29	6
	Yeşilova	10	1
General Total		228	41

3.2 Detection of Viruses in Common Bean Samples Using Potyvirus-Specific Antiserum

The leaves of collected all common bean plants were tested by PTA-ELISA using a specific commercial monoclonal antibody against *Potyvirus* genera (Bioreba, Reinach, Switzerland). The PTA-ELISA was applied based on the standard procedure supplied by the antibody's manufacturer (Bioreba) as follows:

Adding plant extracts

According to the recommendation of the company producing the antiserum, plant samples were homogenized at ratios 1:50 (w/v) with freshly diluted extraction buffer. Also, the lyophilized controls were resuspended in 2.5 ml of the same extraction buffer. Then, 100 µl of extracted plant samples and controls were distributed into the wells of a microtiter plate in a way of each sample was duplicated for the reliability of the test. After the plate was covered with a lid, incubated overnight at 4°C.

Washing

The plate was washed carefully 3 times with PBST buffer supplied by the company.

Adding IgG

The diluted IgG at ratios of 1:1000 in conjugate buffer was dispersed as 100 µl per well and the covered plate was incubated for 2 h at 37°C. After the incubation period, the plate was washed again with PBST as described in the washing step above.

Adding Goat AntiMouse alkaline phosphatase (AP)

The diluted goat anti-mouse AP at ratios of 1:1000 in conjugate buffer was dispersed as 100 µl in each well. Then, the plate was incubated for 2 h at 37°C again.

Adding Substrate

The plate washed with PBST as described above was subsequently loaded with 100 µl pNPP solution per well and incubated for 60-120 min at room temperature under dark conditions.

The results were read at 405 nm. According to the absorbance values that are read in 405 nm wavelength, the samples having optical density (OD) that exceeded two times the mean OD of negative controls were considered as the positive samples (Strausbaugh et al., 2003a). While positive samples were included in the PCR tests, negative samples were stored for other studies. The total nucleic acids were isolated from positive samples according to the below-mentioned method. RT-PCR tests were used to check virus species in the samples using *Potyvirus* species-specific primers as described below.

3.3 Total Nucleic Acid Isolation From Common Bean Samples

Total nucleic acid (TNA) from plant samples for virus and phytoplasma diagnosis was isolated with some modifications according to the Doyle and Doyle (1990) method. According to this method, 200 mg of fresh plant material (leaves including basal midribs) was crushed with liquid nitrogen and mixed with 2 µl CTAB buffer (2% CTAB, 1,4 M NaCl, 20 mM EDTA, 500 mM Tris, 0,2% β-mercaptoethanol pH 8.0). The crushed tissues in the tubes were incubated for 30 min at 70°C and then centrifuged

at 15,000 rpm for 10 min. The 850 µl supernatant was transferred to new 2 ml tubes and an equal volume of chloroform: isoamyl alcohol (24: 1) was added into the tubes. Tubes were mixed gently for 2 min and centrifuged at 15,000 rpm for 10 min. After centrifugation, 750 µl of the upper phase including DNA was transferred to new Eppendorf tubes. Later on, chloroform: isoamyl alcohol (24: 1) was added into the tubes and centrifuged again at 15,000 rpm for 10 min. The supernatant (600 µl) was then transferred to new tubes and added to a volume of 450 µl of cold isopropanol and let to stay overnight at -20°C. The tubes were again centrifuged at +4°C at 15,000 rpm for 10 minutes.

After the centrifugation, the supernatant was poured and the TNA pellet was collected at the bottom and washed with 70% cold ethanol. After drying, the pellet was suspended with 70 µl TE buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA). The concentration of extracted TNAs was determined using a spectrophotometer (NanoDrop ND-2000c, Thermo, USA). Then, TNA samples were stored at -20°C until use.

3.4 One-Step RT-PCR Method for the Detection of *Potyvirus* Species

Out of 132 samples, 60 confirmed as potyvirus-positive by ELISA were selected based on their different symptom characteristics to be used in RT-PCR (Table 3.2). These selected samples were tested for *Potyvirus* species (BCMV, BCMNV, BYMV, CABMV, CIYVV, SMV, and PeMoV).

The diagnosis of selected 60 potyvirus-infected plant samples was performed based on the One-Step RT-PCR method by using TNAs obtained in the previous section. The amplification was carried out in a thermal cycler using specific primers for each potyvirus included in this research (Table 3.3). Since the initial concentration of TNAs was high at about 2500 ng of DNA µl⁻¹, TNAs were diluted at a 1:50 ratio (equivalent to 0.4 µl of DNA; 49.6 µl nuclease-free water) corresponding to 50 ng/µl template DNA per 1 µl used in RT-PCR reaction. For PCR reactions, 6 µl 2X DreamTaq master mix (Thermo Fisher Scientific), 0.5 µl forward primer (10 mM), 0.8 µl reverse primer (10 mM), 0.05 µl Reverse Transcriptase (Thermo Fisher Scientific), 0.1 µl RNase inhibitor (2 u/µl) (Thermo Fisher Scientific), 1 µl diluted TNA at a ratio of 1:50, and 3.55 µl nuclease-free water (Thermo Fisher Scientific) was mixed in a final volume of 12 µl

(Table 3.4). PCR cycling conditions for each potyvirus were set up the same with only different annealing temperatures (Table 3.5).

Table 3.2. PTA-ELISA positive samples used in One-Step RT-PCR reactions

Collection Date	Collection Site	Field No	ELISA Positive Sample Codes Used in One-Step RT-PCR Reactions	Symptoms
24.06.2021	Ovacık	2	F18	Dwarfing, yellowing, mottling
07.07.2021	Ovacık	1	F7	Dwarfing
			F12	Mosaic
	Ovacık	2	F23	Dwarfing
	Ovacık	3	F37	Dwarfing
			F38	Dwarfing
13.07.2021	Yeşilgölcük	1	F2	Mosaic
03.08.2021	Konaklı	1	F4	Mosaic, dwarfing
	Konaklı	2	F9	Mosaic
	Konaklı	3	F11	Mosaic, mottling
	Konaklı	4	F22	Mosaic, mottling
	Alay	1	F25	Mosaic
			F29	Mottling
			F32	Mosaic, mottling
	Alay	2	F38	Mosaic
			F40	Mosaic
	Alay	3	F41	Mosaic, mottling
			F43	Mottling
	Ovacık	1	F46	Mosaic
			F47	Mosaic, mottling
	Ovacık	2	F51	Mosaic, reduced size of internodes
	Yeşilova	1	F53	Mosaic, mottling
19.08.2021	Elmalı	1	F56	Mosaic
			F58	Chlorotic lesion
	Elmalı	2	F61	Mosaic, leaf distortion
			F63	Mosaic
	Konaklı	1	F4	Mosaic, vein banding
			F7	Mosaic
			F11	Mosaic, mottling, leaf distortion
	Konaklı	2	F19	Mosaic
			F21	Mottling
	Yeşilgölcük	1	F22	Mosaic
24.08.2021			F23	Mosaic
	Hasaköy	1	F28	Mosaic, yellowing
			F29	Mosaic
	Yeşilgölcük	1	F3	Mosaic
			F5	Mosaic, necrotic lesion
	Yeşilgölcük	2	F9	Mosaic
			F11	Mosaic, mottling
	Yeşilgölcük	3	F15	Mosaic
			F16	Mosaic
	Alay	1	F21	Mosaic
			F23	Yellow mosaic
	Ağcaşar	1	F25	Mosaic, mottling, reduced size of internodes
			F26	Chlorotic lesion
	Kiledere	1	F29	Mosaic, mottling
			F30	Mosaic, mottling
14.09.2021	Ağcaşar	2	F35	Mosaic, necrotic lesion
			F36	Mosaic
	Ağcaşar	3	F39	Mosaic, mottling, dwarfing
			F42	Mosaic, dwarfing
			F44	Mosaic
	Ağcaşar	4	F47	Mosaic, leaf distortion
			F49	Mosaic, leaf distortion
			F52	Mosaic
20.09.2021	Elmalı	1	F1	Mosaic, dwarfing
			F2	Mosaic, mottling
			F2	Mosaic, necrotic lesion
20.09.2021	Amas Bağları	1	F6	Mosaic, leaf distortion
			F9	Yellowing, leaf distortion, reduced size of internodes
Total		32	60	

Table 3.3. Primer pairs used in One-Step RT-PCR amplification of *Potyvirus* species

Virus	Primer Name	Nucleotide sequence 5'–3'direction	Genomic region	Expected Product size (bp)	References
BCMV	CpF CpR	F-5'-CCAATGGTTGAAAATGCAAAGCC-3' R-5'-CGACGCGAGATGCTAACTG-3'	CP and 3'UTR	407 bp	Traykova et al., 2008
BCMNV	BCMNVF1 BCMNV R1	F-5'-CAAAGGCCCAGCGGATAAA-3' R-5'-GGTGGTATAACCACACTGGAATTG-3'	CP and 3'UTR	823 bp	Mwaipopo et al., 2018
BYMV	BYMV-pnsF BYMV-pnsR	F-5'-TCAGATCAAGAGCAACTCAATGCA-3' R-5'-GACGGATACTCTAAATACGAACA-3'	CP	832 bp	Sharma et al., 2015
	BYMV-pnsF1*	F-5'-TCHGAHCAAGARMAACTCAATGC-3'		830 bp	This study
SMV	SMV-CPf SMV-CPr	F-5'-CAAGCAGCAAAG ATGTAAATG-3' R-5'-GTCCATATCTAGGCATA TACG-3'	CP	469 bp	Wang and Ghabrial, 2002
CABMV	CABMV-F CABMV-R	F-5'-CGCTCAAACCCATTGTAGAA-3' R-5'-TATTGCTTCCCTTGCTCTTTC-3'	CP	221 bp	Gillaspie et al., 2001
PeMoV	PeMo 17 PeMo 14	F-5'-ACAATGATGAAGTTCGTTAC-3' R-5'-GCTGTGAATTGTGTTGAGAA-3'	Nib CP	327 bp	Dietzgen et al., 2001
CIYVV	CIYVV-F CIYVV-R	F-5'-TTGATGACAGCCAGATG-3' R-5'-GAATCGTGCTCCAGCAATG-3'	Nib CP	835 bp	Larsen et al., 2008

* BYMV-pnsF1; New designed degenerate forward primer for the amplification of BYMV.

Table 3.4. The reaction mixture used in One-Step RT-PCR amplification of each potyvirus

Preparation of PCR Mixture for 1 Reaction	
Reaction Buffers	Quantity (μl)
d ₂ H ₂ O	3.55
2X DreamTaq Master Mix	6
Forward Primer (10 μM)	0.5
Reverse Primer (10 μM)	0.8
Reverse Transcriptase (200 u/ μl)	0.05
RNase inhibitor (2 u/μl)	0.1
1:50 Diluted TNA	1
Total Volume	12

Table 3.5. Amplification conditions for each *Potyvirus* species used in PCR tests

Temperature (°C)	Time (Duration)	Cycle
42	30 min	1
95	2 min	
95	30 sec	35
52, 55, 60*	30 sec	
72	45 sec	
72	10 minute	
		1

*: Annealing temperature (52°C) was used for the amplification of CIYVV, PeMoV, SMV, whereas (55°C) for BCMNV, BYMV, CABMV and (60°C) for only BCMV amplification.

3.5 Agarose Gel Electrophoresis

The PCR products were separated in a 1.5% agarose gel in TAE Buffer and visualized on UV illumination using an electrophoresis gel documentation system (Bio-Rad Laboratories, Inc.).

3.6 Nucleotide Sequence Analysis of BYMV Isolate

The viral amplicons acquired by PCR amplification were subjected to sequence analysis. The sequence data were obtained from the sequencing company (Medsantek, İstanbul/Türkiye) with the help of the Sanger Method, and the sequences of PCR products were assembled and edited by Geneious Prime software (Biomatters Ltd., Auckland, New Zealand). The Basic Local Alignment Search Tool (BLASTn) analysis was applied to search for sequence similarity in the National Center for Biotechnology Information (NCBI) databases.

3.7 Phylogenetic Analysis of BYMV Isolate

For phylogenetic analysis, the sequences of BYMV isolates were retrieved from NCBI database taking into account the country and host criteria of an isolate deposited in GenBank (Table 3.6). Then, multiple alignments of the sequences of BYMV retrieved from the GenBank and BYMV isolate obtained in this study were performed by CLUSTALW Method (Thompson et al., 1994) in Mega-X Software. Bootstrap value was adjusted to (1000 replicates) which shows the percentage of replicate trees in which the associated taxa clustered together. The Maximum-likelihood algorithm was used to infer the initial tree (Tamura and Nei, 1993).

Table 3.6. BYMV isolates used in phylogenetic analysis

Accession Number	Isolate/Strain/Gene Name	Origin	Host
AB439731	Strain: 90-2	Japan	<i>Vicia faba</i> (Faba bean or broad bean)
AB439732	Strain: 92-1	Japan	<i>Trifolium pratense</i> (Red clover)
AF185960	Isolate Pterostylis	Australia	<i>Pterostylis curta</i> (Blunt greenhood)
AM884180	Genomic RNA	Taiwan	<i>Eustoma russellianum</i> (Texas bluebell)
AY845011	Isolate VM-0	India	<i>Vanilla fragrans</i> (Vanilla)
EU144223	Polyprotein gene	USA	<i>Lupinus luteus</i> (Yellow lupin)
FJ618532	Polyprotein gene	New Zealand	<i>Freesia</i> sp.
In this study	Isolate 186	Türkiye	<i>Phaseolus vulgaris</i> (Common bean)
GU126690	Alaska polyprotein gene	USA	<i>Trifolium</i> sp. (Clover)
HG970855	Isolate LMBNN	Australia	<i>Lupinus angustifolius</i> (Blue lupin)
HG970859	Isolate ES11A	Australia	<i>Lupinus angustifolius</i> (Blue lupin)
HG970867	Isolate FB	Australia	<i>Vicia faba</i> (Faba bean or broad bean)
JQ026001	Iraq6 partial cds	Iraq	<i>Vicia faba</i> (Faba bean or broad bean)
JX156423	SW3.2	Australia	<i>Diuris</i> sp. (Donkey orchids)
KC731531	Isolate Arg	Argentina	<i>Glycine max</i> (Soybean)
KY595423	BYM-Ore	Russia	<i>Canna</i> sp. (Canna lily)
LC500882	Aus14BY RNA	Australia	<i>Lens culinaris</i> (Lentil)
MG002647	Isolate Saffron2	China	<i>Crocus sativus</i> (Saffron)
MG600297	Isolate BYMV-PV2	Czech Republic	<i>Trifolium pratense</i> (Red clover)
MK131270	Isolate OV65	India	<i>Gladiolus</i>
MK649741	Isolate ARGbb	Argentina	<i>Vicia faba</i> (Faba bean or broad bean)
MN017306	Isolate E2	Poland	<i>Vicia faba</i> (Faba bean or broad bean)
MN399729	Isolate Salzlandkreis-2_18	Germany	<i>Pisum sativum</i> (Pea)
MN509831	Isolate BYMV-ALP	India	<i>Alpinia galanga</i> (Blue ginger)
MT787571	Isolate BJTt1	China	<i>Therioaphis trifolii</i> (Yellow clover aphid)
MZ405650	Isolate DSMZ PV-0717	Germany	<i>Phaseolus vulgaris</i> (Common bean)
MW052328	Isolate n11	Türkiye	<i>Cicer arietinum</i> (Chickpea)
MW188030	Isolate BYMV-Troja	Czech Republic	<i>Dracula astuta</i> (Dracula plant)
MW411034	Isolate BIQ83	Iraq	<i>Vicia faba</i> (Faba bean or broad bean)
OK030755	Isolate The Deepings BYMV	United Kingdom	<i>Pisum sativum</i> (Pea)
OK558657	Isolate Pea Mosaic	Canada	<i>Vicia faba</i> (Broad bean)
OL584370	Isolate DSMZ PV-1213	Germany	<i>Mardevallia schroederiana</i> (Orchid species)
OP525293	Isolate DSMZ PV-0946	Germany	<i>Vicia faba</i> (Faba bean or broad bean)

3.8 Investigation of Reactions of Different Common Bean Cultivars to BCMV

3.8.1 Common bean seed sources used in the reaction studies to BCMV and in resistance gene analysis

The seeds of registered commercial common bean cultivars were obtained from different governmental agricultural research institutes and trading enterprises. BCMV positive-isolate 154 obtained from the site known as Konaklı was selected as an inoculum source to be used in virus reaction studies based on the criteria which is the most virulent isolate among all the acquired BCMV positive-isolates.

Different ten common bean cultivars obtained from distinctive establishments in Türkiye (Adabeyazı, Akbulut, Akman-98, Borsa, Canıpek, Cihan, Ergan, Güngör, Sururbey, and Zirve) were used in virus reaction studies (Table 3.7). Additionally, these described common bean cultivars were also tested with markers for their resistance genes to BCMV. For control, the susceptible bean cultivar (Host Group 1) Stringless Green Refugee (SGR) having resistance genes (*i*, *bc-u*), kindly provided from Prof. Dr. Miray Sökmen (Ondokuz Mayıs University, Türkiye) with the code number 22, was used in the virus reaction studies. On the other hand, the common bean cvs. Adayakut, Altın, Akın, Arslan, Batallı, Gündoğan, Göynük-98, Karbeyazı, Nirvana, Noyanbey-98, and Topçu were also used in BCMV resistance analyses (Table 3.7).

Table 3.7. The common bean cultivars with codes from one to ten are used in virus reaction studies as well as BCMV resistance analyses and also the common bean cultivars with codes from eleven to twenty-one are included in BCMV resistance analyses

Dry Bean Cultivar Name	BCMV Inoculation and Resistance Analysis Method	Code	Establishments from which bean seeds were procured
Adabeyazı	Inoculation and marker	1	Republic of Türkiye Ministry of Agriculture and Forestry Maize Research Institute, Sakarya
Akbulut	Inoculation and marker	2	Alp Agriculture, Konya
Akman-98	Inoculation and marker	3	Republic of Türkiye Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute, Eskişehir
Borsa	Inoculation and marker	4	Mersin Commodity Exchange, Mersin
Canıpek	Inoculation and marker	5	Republic of Türkiye Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute, Eskişehir
Cihan	Inoculation and marker	6	Republic of Türkiye Ministry of Agriculture and Forestry Aegean Agricultural Research Institute, İzmir
Ergan	Inoculation and marker	7	Republic of Türkiye Ministry of Agriculture and Forestry Erzincan Horticultural Research Institute, Erzincan
Güngör	Inoculation and marker	8	Republic of Türkiye Ministry of Agriculture and Forestry East Mediterranean Transitional Zone Agricultural Research of Institute, Kahramanmaraş
Sururbey	Inoculation and marker	9	Mersin Commodity Exchange, Mersin
Zirve	Inoculation and marker	10	Taşpınar Agriculture, Konya
Adayakut	Marker	11	Republic of Türkiye Ministry of Agriculture and Forestry Maize Research Institute, Sakarya
Altın	Marker	12	Beta Agriculture, Konya
Akın	Marker	13	Republic of Türkiye Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute, Eskişehir
Arslan	Marker	14	Mersin Commodity Exchange, Mersin
Batallı	Marker	15	Mersin Commodity Exchange, Mersin
Göynük-98	Marker	16	Republic of Türkiye Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute, Eskişehir
Gündoğan	Marker	17	Republic of Türkiye Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute, Eskişehir
Karbeyazı	Marker	18	Mersin Commodity Exchange, Mersin
Nirvana	Marker	19	Taşpınar Agriculture, Konya
Noyanbey-98	Marker	20	Republic of Türkiye Ministry of Agriculture and Forestry Aegean Agricultural Research Institute, İzmir
Topçu	Marker	21	Republic of Türkiye Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute, Eskişehir

3.8.2 Inoculum preparation and mechanical virus inoculation to common beans

As the inoculum source, BCMV-positive isolate 154 confirmed by ELISA and RT-PCR which didn't show any mixed infections was used in virus inoculation trials. This isolate was chosen based on its high level of virulence trait. For this reason, this isolate was propagated on cv. Great Northern which was determined as susceptible to BCMV in previous virus inoculation trials in order to get more inoculum sources to be used in the main virus inoculation trials.

For mechanical inoculations, the leaves were ground in a sterile mortar and pestle with 1% phosphate solution (K_2HPO_4) containing 0.1% sodium sulfite (Na_2SO_3) [100 mg of infected leaf: 1 ml of inoculation buffer (pH 7.5)] (Sengooba et al., 1997). The germinated common bean seedlings were sprinkled with a 400-mesh carborandum powder when the primary leaves of these plants reached at least a size of $\frac{3}{4}$ whole leaf. Then, the ground-infected plant sap was spread onto the primary leaves of bean plants using plastic gloves. As negative control plants, mock control plants were applied with only inoculation buffer, whilst untreated plants were just left without any implementation to check any seed-transmitted virus presence. After approximately five minutes, the plants were washed with sterilized distilled water. Later, the plants were kept in a climate room at temperatures of 20°C (dark) and 25°C (light) for 14 hours of photoperiod.

Seeds of each common bean cultivar (Adabeyazı, Akbulut, Akman-98, Borsa, Canipek, Cihan, Ergan, Güngör, Sururbey, and Zirve) indicated in Table 3.7 were planted in small plastic pots filled with a torf-perlite mixture. For control, one plant was applied with only inoculation buffer, whereas one plant was just left without any implementation. Once the primary leaves of bean plants at about 10 days old have come out, a piece of leaf parts approx. 0.5 cm have been taken by sterilized scissors from each cultivar including the control ones to test them through PTA-ELISA in order to check whether potyviruses have been transmitted by the seeds for each bean cultivar. The sampling of the plant materials from each cultivar for seed-transmitted potyvirus was carried out based on the composite sampling method. According to this method, the plant samples of each cultivar were categorized into three groups, each having 8 plant

samples in one group comprising 24 plant samples in total. Potyvirus-infected plantlets were discarded for further inoculation studies.

The primary leaves of those common bean plants were mechanically inoculated with propagated BCMV isolate on the cv. Great Northern based on the method which was applied as mentioned above (Figure 3.2).



Figure 3.2. The virus inoculation trial on common bean cultivars

The inoculated plants were observed at weekly intervals for evaluation of local and systemic symptoms. The plants were tested for virus transmission with PTA-ELISA. For accuracy, the trial was implemented twice with the same method on the same cultivars.

3.9 Determination of Resistance Level of Common Bean Cultivars to BCMV

3.9.1 Determination of resistance level and resistance genes of common bean cultivars based on the symptoms

After inoculation of common bean cultivars with BCMV including the Host Group 1, the symptoms of the plants were observed and weekly recorded. Cultivars that developed systemic infection 7-28 days after inoculation were classified as phenotypically susceptible (S), whereas those that did not develop systemic symptoms were classified as resistant (R).

3.9.2 Determination of resistance level of common bean cultivars based on PTA-ELISA results

Inoculated plants and the controls were subjected to ELISA test to determine whether they were systemically infected with the virus. For this purpose, the top non-inoculated trifoliolate leaves of plants exhibiting no visible virus symptoms or mild symptoms were analyzed by PTA-ELISA at the third and four week of inoculations. The ELISA test was performed as described in section 3.2 using potyvirus-specific antiserum.

3.9.3 Screening resistance genes on common bean cultivars by PCR method

First of all, TNAs were isolated from trifoliolate leaves of three-week-old common bean plants according to CTAB method following the protocol described by Doyle and Doyle (1990) with some modifications as expressed in section 3.3. For this purpose, TNAs were isolated from two plants of each cultivar. The 22 common bean cultivars along with the Host Group 1 cultivar (Stringless Green Refugee) (Table 3.7) were analyzed by PCR method using SCAR and CAPS markers which are specific markers used in the determination of whether the dominant *I* gene and the recessive *bc-1*² and *bc-3* genes are present.

While the presence of the dominant *I* gene and recessive *bc-1*² gene were investigated by SCAR markers (SW-13 and SBD-5) respectively, the presence of the recessive *bc-3* gene was scrutinized using the SCAR and CAPS markers (ROC11 and eIFE4), separately (Table 3.8). Since the initial concentration of TNAs was high at about 2000 ng of DNA μl^{-1} , TNAs were diluted at a 1:100 ratio (equivalent to 1.00 μl of DNA; 99 μl nuclease-free water) corresponding to 20 ng/ μl template DNA per 1 μl used in RT-PCR reaction. For PCR reactions with all markers, 6 μl 2X DreamTaq master mix (Thermo Scientific), 0.5 μl forward primer, 0.8 μl reverse primer, 1 μl diluted total nucleic acid at a ratio of 1:100, and 3.7 μl nuclease-free water (Thermo Fisher Scientific) was mixed in a final volume of 12 μl . PCR cycling conditions for each marker were set up according to the displayed conditions in Table 3.9. PCR products were analyzed as described in 3.5 section.

3.9.3.1 Digestion of PCR products obtained by eIFE4 Marker

Besides, 12 µl PCR product obtained by eIFE4 marker was digested with 0.5 µl *RsaI* enzyme (Fast digest *RsaI*, Fermentas) and 1.5 µl 10X enzyme buffer (Fermentas) and 1 µl nuclease free-water in a final volume of 15 µl. Amplification was obtained in a thermal cycler at 37°C overnight. Amplified PCR products were analyzed as described in 3.5 section.

Table 3.8. Markers used in PCR amplification of resistance genes

Gene	Marker	Primer sequence 5'–3'direction	Expected Product size (bp)	References
<i>I</i>	SW-13	F-5'-CACAGCGACATTAATTTTCCTTTC-3' R-5'-CACAGCGACAGGAGGAGCTTATTA-3'	690 bp	Melotto et al., 1996
<i>bc-1²</i>	SBD-5	F-5'-GTGCGGGAGAGGCCATCCATTGGTG-3' R-5'-GTGCGGAGAGTTTCAGTGTGACA-3'	1300 bp	Miklas et al., 2000
<i>bc-3</i>	ROC11	F-5'-CCAATTCTCTTTCACCTGTAACC-3' 5'- GCATGTTCCAGCAAACC-3' R-5'-GCATGTTCCAGCAAACC-3'	420 bp	Johnson et al., 1997
<i>bc-3</i>	eIFE4	F-5'-ACCGATGAGCAAAACCCTA-3' R-5'-CAACCAACTGGTATCGGATT-3'	541/(<i>RsaI</i> digestion 381/160) bp	Naderpour et al., 2010

Table 3.9. Amplification conditions for each marker used in PCR

PCR Conditions for Markers								
SW-13 and SBD-5			ROC11			eIFE4		
Temperature (°C)	Time (Minutes)	Cycle	Temperature (°C)	Time (Minutes)	Cycle	Temperature (°C)	Time (Minutes)	Cycle
94	2	1	95	4	1	95	3	1
94	10 s	34	94	10 s	35	94	20 s	40
66	40 s		65	10 s		58	20 s	
72	2		72	30 s		72	20 s	
72	5	1	72	5	1	72	5	1

3.10 Detection of Phytoplasma in Common Bean Plants

The TNA products above-mentioned in section 3.3 were used in phytoplasma detection analyses.

3.10.1 Phytoplasma DNA-enriched isolation method

Additionally, 13 common bean samples (isolate no 44-56) collected on the dates of 14.09.2021 and 20.09.2021 (Table 3.10) were isolated with some modifications according to Lee et al. (1994) to get more phytoplasma-enriched DNAs in the samples. Depending on this method, leaf midribs, and petioles were taken from healthy and phytoplasma-infected plant samples and they were divided into small pieces with the help of scissors. Then, 6 ml phytoplasma grinding buffer [Phytoplasma grinding buffer: 125 mM potassium phosphate, 10% sucrose, 0.15% bovine serum albumin (BSA), 2% polyvinylpyrrolidone (PVP-40), 30 mM ascorbic acid, pH 7.6] was added into the mortars and kept on ice for 10 minutes. The ground tissues were again subjected to a crushing procedure adding 8 ml phytoplasma grinding buffer into the mortars. Hereafter, the ground tissues were taken into 2 ml Eppendorf tubes and centrifuged at +4°C, 1100 rpm for 5 min. The supernatant was decanted and the tissues left inside the tubes were centrifuged at +4°C, 1100 rpm for 25 min. Thereafter, the pellets were resuspended by adding 1.5 ml CTAB buffer (2% CTAB, 1,4 M NaCl, 20 mM EDTA, 500 mM Tris, 0,2% β -mercaptoethanol pH 8.0) based on the method as described in isolation of TNA above.

Fifty-six of 228 total collected bean samples showing distinctive phytoplasma-associated symptoms were included in PCR tests for phytoplasma detection (Table 3.10).

DNA samples provided from *Ca. P. pyri*, *Ca. P. mali* infected periwinkle samples were used as a phytoplasma-infected positive control in PCR reactions.

Table 3.10. Selected common bean samples for phytoplasma detection in both direct and nested-PCR analyses

Collection Date	Collection Site	Field No	Sample Codes Used in Direct and Nested-PCR Tests	Phytoplasma Test Code	Symptom
24.06.2021	Yeşilova	1	F6	1	Dwarfing, abnormal leaf development
			F7	2	Dwarfing, abnormal leaf development
			F8	3	Dwarfing, abnormal leaf development
			F9	4	Dwarfing, abnormal leaf development
	Ovacık	1	F10	5	Dwarfing, reddening of the leaves
07.07.2021	Ovacık	1	F4	6	Abnormal leaf development
			F7	7	Dwarfing, abnormal leaf development, yellowing
			F8	8	Dwarfing, abnormal leaf development, yellowing
	Ovacık	2	F23	9	Dwarfing, abnormal leaf development
			F29	10	Dwarfing, abnormal leaf development
	Yarhisar	1	F36	11	Dwarfing, abnormal leaf development
	Ovacık	3	F37	12	Dwarfing, abnormal leaf development
13.07.2021	Yeşilgölcük	1	F1	13	Dwarfing, abnormal leaf development, yellowing
	Konaklı	1	F8	14	Dwarfing, abnormal leaf development, yellowing, reddening of the leaves
	Konaklı	3	F18	15	Shortened internodes, yellowing
			F19	16	Dwarfing, abnormal leaf development
			F20	17	Dwarfing, abnormal leaf development
			F21	18	Dwarfing, abnormal leaf development
03.08.2021	Konaklı	1	F2	19	Yellowing
	Alay	1	F33	20	Shortened internodes, yellowing
			F34	21	Shortened internodes, yellowing
			F50	22	Shortened internodes, yellowing
	Elmalı	1	F54	23	Shortened internodes, phyllody like
			F61	24	Shortened internodes, phyllody like
	Elmalı	2	F63	25	Yellowing
			F64	26	Shortened internodes
19.08.2021	Konaklı	1	F5	27	Shortened internodes, yellowing, phyllody like
			F11	28	Dwarfing, yellowing
			F12	29	Shortened internodes, phyllody like
			F13	30	Shortened internodes, phyllody like
			F15	31	Yellowing
	Yeşilgölcük	1	F24	32	Shortened internodes, phyllody like
			F25	33	Shortened internodes
24.08.2021	Ağcaşar	2	F26	34	Shortened internodes, yellowing
	Kiledere	1	F-27	35	Shortened internodes, phyllody like
			F29	36	Shortened internodes, phyllody like
			F32	37	Dwarfing, shortened internodes, reddening of the leaves
	Ağcaşar	3	F36	38	Shortened internodes, yellowing
	Ağcaşar	4	F39	39	Shortened internodes, phyllody like
			F44	40	Shortened internodes, yellowing, phyllody like
	Ağcaşar	5	F47	41	Shortened internodes, yellowing
			F49	42	Shortened internodes, phyllody like
F51			43	Dwarfing, abnormal leaf development, phyllody like	
14.09.2021	Elmalı	1	F1	44	Shortened internodes, yellowing
			F2	45	Shortened internodes, yellowing
			F3	46	Shortened internodes, yellowing
			F1	47	Dwarfing, abnormal leaf development
20.09.2021	Amas Bağları	1	F2	48	Yellowing
			F3	49	Shortened internodes, yellowing, phyllody like
			F4	50	Shortened internodes, yellowing
			F5	51	Shortened internodes, yellowing, phyllody like
			F6	52	Shortened internodes, yellowing, phyllody like
			F7	53	Shortened internodes, yellowing, phyllody like
			F8	54	Shortened internodes, phyllody like
			F9	55	Shortened internodes, yellowing, phyllody like
			F10	56	Shortened internodes, yellowing
Toplam		23	56		

Direct and nested-PCR methods were utilized to perform PCR procedures on isolated DNA samples (Lee et al., 1992). Isolated DNA samples were diluted at the ratio of 1:50

and 2 µl DNA (corresponding to 100 ng/µl template DNA per PCR reaction) was added to the reaction mixture of the direct-PCR procedure. In addition, universal primers P1/P7 (Deng and Hiruki, 1991) were utilized in the direct-PCR method. Then, the products of the direct-PCR procedure were diluted at the ratio of 1:30 and 2 µl added to the reaction mixture of the nested-PCR procedure. In nested-PCR procedures, R16F2n/R16R2 (Lee et al., 2002), F1/B6 (Lee et al., 1995; Padovan et al., 1995), M1/M2 (Gibb et al., 1995), and U5/U3 (Lorenz et al., 1995) were assayed. Nucleotide sequences of the primers (Table 3.11), preparation of the reaction mixtures of direct and nested-PCR (Table 3.12), and programs of thermocycler for direct and nested-PCR procedures were shown at Table 3.13. Figure 3.3 shows a schematic diagram of used primers in direct and nested-PCR procedures respectively for the detection of phytoplasma in common bean plant samples. Primer pairs (P1/P7- R16F2n/R16R2) were tried first for four selected common bean samples with the isolate names 27, 28, 29, and 30, then the other primer pairs were used for the rest of the common bean samples in one reaction of direct and nested-PCR tests based on the gel result, respectively. The obtained products from nested-PCR procedures were analyzed as described in 3.5 section.

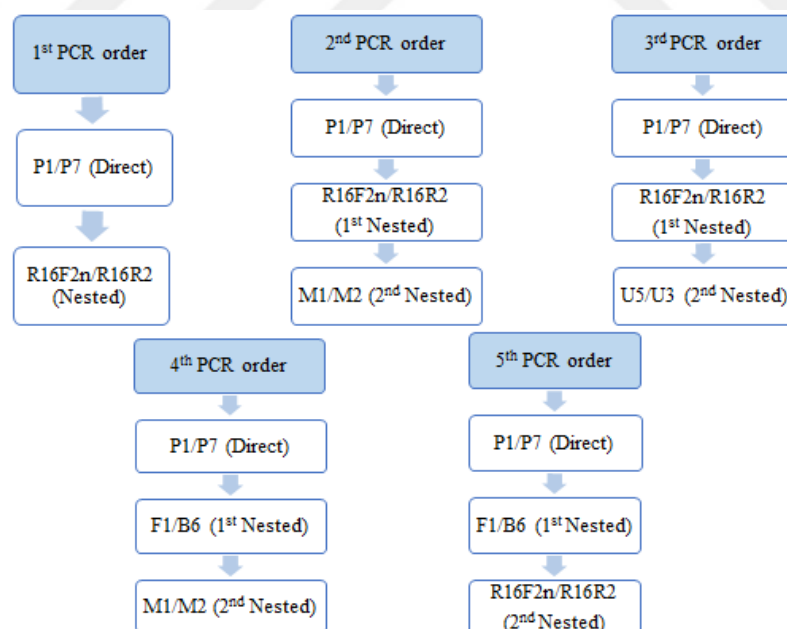


Figure 3.3. Schematic diagram of used primers sequentially in direct and nested-PCR procedures for the detection of phytoplasma in common bean plant samples. Primer pairs (P1/P7- R16F2n/R16R2) were tried first for four selected common bean samples with the isolate names 27, 28, 29, and 30, then the other primer pairs were used for the rest of the common bean samples in one reaction of direct and nested-PCR tests based on the gel result, respectively

Table 3.11. Nucleotide sequences of universal and specific primers used in direct and nested-PCR procedures for phytoplasma detection in common bean plants

PCR Method	Fragment Length (bp)	Primer	Primer Sequence (5'-3')	Reference
Direct-PCR	1800	P1	AGAGTTTGATCCTGGCTCAGGA	Deng and Hiruki, 1991
		P7	CGTCCTTCATCGGCTCTT	Smart et al., 1996
Nested-PCR	1200	R16F2n	GAAACGACTGCTAAGACTGG	Gundersen and Lee, 1996
		R16R2	TGACGGGCGGTGTGTACAAACCCCG	
Nested-PCR	1050	F1	AAGACGAGGATAACAGTTGG	Lee et al., 1995
		B6	TAGTGCCAAGGCATCCACTGTG	Padovan et al., 1995
Nested-PCR	509	M1	GTCTTTACTGACGCTGAGGC	Gibb et al., 1995
		M2	CTTCAGCTACCCTTTGTAAC	
Nested-PCR	860	U5	CGGCAATGCAGGAAACT	Lorenz et al., 1995
		U3	TTCAGCTACTCTTTGTAACA	

Table 3.12. Quantities of the components for preparation of the reaction mixtures of direct and nested-PCR procedures

PCR Mixture			
Direct-PCR		Nested-PCR	
Reaction buffer	Quantity (μl)	Reaction buffer	Quantity (μl)
d ₂ H ₂ O	11	d ₂ H ₂ O	11
10X Buffer	2	10X Buffer	2
MgCl ₂ (25 mM)	2	MgCl ₂ (25 mM)	2
dNTP (10 mM)	1	dNTP (10 mM)	1
Primer (Forward) P1 (10 μM)	1	Primer (Forward) F1 / M1 / R16F2n / U5 (10 μM)	1
Primer (Reverse) P7 (10 μM)	1	Primer (Reverse) B6 / M2 / R16R2 / U3 (10 μM)	1
Taq Polymerase (2U)	0,15	Taq Polymerase (2U)	0,15
1:50 Diluted DNA	2	1:30 Diluted DNA	2
Total volume	20	Total volume	20

Table 3.13. Amplification conditions used in PCR tests for the detection of phytoplasma

PCR Conditions					
Direct-PCR			Nested-PCR		
P1/P7			F1/B6 - M1/M2 - R16F2n/R16R2 - U5/U3		
Temperature (°C)	Time (Minutes)	Cycle	Temperature (°C)	Time (Minutes)	Cycle
95	5	1	95	5	1
94	1	35	94	1	35
58	2		55, 57, 58, 60*	1	
72	3		72	1	
72	10	1	72	10	1

*: Annealing temperature (58°C) was used for the amplification of F1/B6, whereas (55°C) was used for M1/M2, (57°C) for U5/U3, and (60°C) used for only the amplification of R16F2n/R16R2.

CHAPTER IV

RESULTS

4.1 Detection of Viruses in Common Bean Samples Using Potyvirus-Specific Antiserum

The common bean samples collected from the Central District of Niğde province; the villages (Ağcaşar, Çayırılı, Elmalı, Hasaköy, Ovacık, Yarhisar, Yeşilova) and the towns (Amas Bağları, Alay, Kiledere, Konaklı, Yeşilgölcük) showed mainly the symptoms of mosaic, mottling, chlorotic and necrotic lesions, vein banding, leaf distortion, dwarfing, yellowing, reduced size of internodes (Figure 4.1 and 4.2). A total of 228 bean samples collected from 41 fields were subjected to PTA-ELISA using potyvirus-specific antiserum to check whether the plants were infected with potyvirus. According to ELISA results, 132 samples out of 228 have been found infected with potyvirus (Table 4.1). The infection rates of collected bean leaf samples based on months surveyed in Niğde province were indicated in Table 4.1. According to the infection rates of collected bean samples obtained monthly, higher infections have been acquired towards the end of August. The analyzed bean leaves had an overall infection rate of 57.8%, whereas the overall infection rate of visited bean fields had 78% (Table 4.2). It was concluded that the samples collected in August and September were almost all positive in terms of potyviruses.

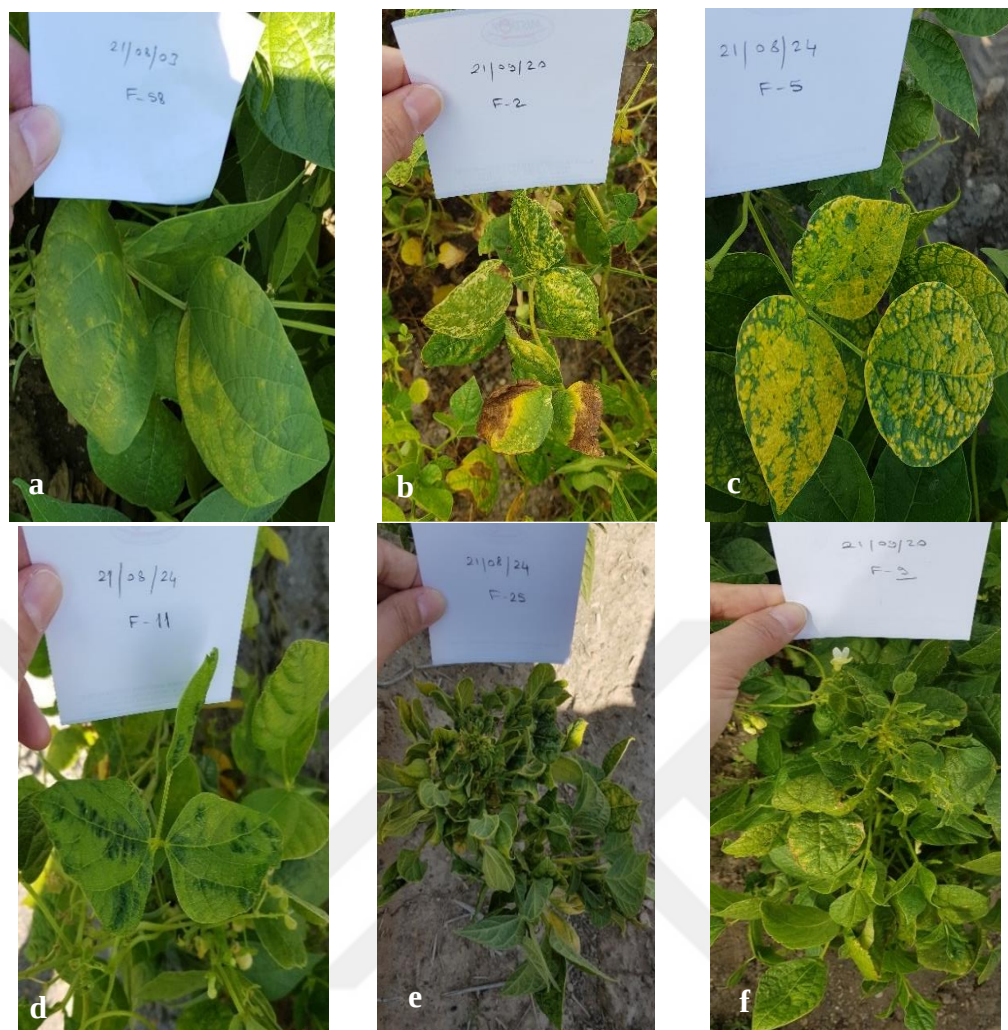


Figure 4.1. Some of the principal symptoms belonging to common bean plants surveyed in the fields that were confirmed as potyvirus (BCMV) infected; chlorotic and necrotic lesions (a, b), mosaic and vein banding (b, c), mottling (d), leaf distortion and reduced size of internodes (e), dwarfing and yellowing (f)

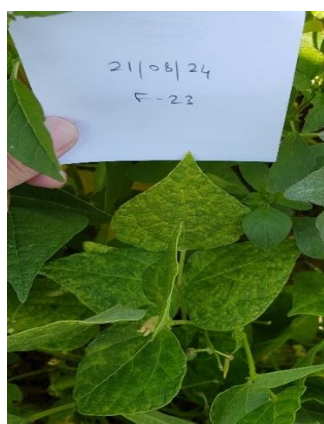


Figure 4.2. Some of the principal symptoms belonging to common bean plants surveyed in the fields that were confirmed as potyvirus (BYMV) infected; yellow mosaic caused by BYMV

Table 4.1. Potyvirus-specific PTA-ELISA results of common bean leaf samples collected from different places

Collection Date	Collection Sites	Field No	Sample Code	Virus Test Code	PTA-ELISA Result	Infection Rate
24.06.2021	Yeşilova	1	F1	1	n	0%
			F2	2	n	
			F3	3	n	
			F4	4	n	
			F5	5	n	
			F6	6	n	
			F7	7	n	
			F8	8	n	
			F9	9	n	
	Ovacık	1	F11	10	n	0%
			F12	11	n	
			F13	12	n	
	Ovacık	2	F14	13	p	40%
			F15	14	n	
			F16	15	n	
			F17	16	n	
			F18	17	p	
7.07.2021	Ovacık	1	F1	18	p	100%
			F2	19	p	
			F3	20	p	
			F4	21	p	
			F5	22	p	
			F6	23	p	
			F7	24	p	
			F8	25	p	
			F9	26	p	
			F10	27	p	
			F11	28	p	
			F12	29	p	
			F13	30	p	
			F14	31	p	
			F15	32	p	
			F16	33	p	
			F17	34	p	
			F-18	35	p	
			F19	36	p	
			F20	37	p	
			F21	38	p	
			F22	39	p	
	Ovacık	2	F23	40	p	50%
			F24	41	n	
	Elmalı	1	F25	42	n	0%
	Çayırılı	1	F26	43	n	0%
			F27	44	n	
	Yarhisar	2	F36	45	n	0%

Table 4.1. (Continued) Potyvirus-specific PTA-ELISA results of common bean leaf samples collected from different places

Collection Date	Collection Sites	Field No	Sample Code	Virus Test Code	PTA-ELISA Result	Infection Rate
7.07.2021	Ovacık	3	F37	46	p	75%
			F38	47	p	
			F39	48	p	
			F40	49	n	
13.07.2021	Yeşilgölcük	1	F1	50	n	20%
			F2	51	p	
			F3	52	n	
			F4	53	n	
			F5	54	n	
	Konaklı	1	F6	55	n	0%
			F7	56	n	
			F8	57	n	
			F9	58	n	
			F10	59	n	
			F11	60	n	
			F12	61	n	
			F13	62	n	
	Konaklı	2	F14	63	n	0%
			F15	64	n	
			F16	65	n	
			F17	66	n	
			F18	67	n	
3.08.2021	Konaklı	1	F19	68	n	25%
			F20	69	n	
			F1	70	n	
			F2	71	n	
	Konaklı	2	F3	72	n	67%
			F4	73	p	
			F5	74	p	
			F6	75	p	
			F7	76	n	
			F8	77	n	
			F9	78	p	
			F10	79	p	
	Konaklı	3	F11	80	p	11%
			F12	81	n	
			F13	82	p	
			F14	83	n	
			F15	84	n	
			F16	85	n	
			F17	86	n	
			F18	87	n	
	Konaklı	4	F19	88	n	33%
			F20	89	n	
			F21	90	n	
			F22	91	p	
			F23	92	n	
			F24	93	p	
	Alay	1	F25	94	p	29%
			F26	95	n	
			F27	96	n	
			F28	97	n	
			F29	98	p	
			F30	99	n	
			F31	100	n	
			F32	101	p	
			F33	102	n	
			F34	103	n	

Table 4.1. (Continued) Potyvirus-specific PTA-ELISA results of common bean leaf samples collected from different places

Collection Date	Collection Sites	Field No	Sample Code	Virus Test Code	PTA-ELISA Result	Infection Rate
3.08.21	Alay	1	F35	104	n	
	Alay	2	F36	105	n	40%
			F37	106	n	
			F38	107	p	
			F39	108	n	
			F40	109	p	
	Alay	3	F41	110	p	67%
			F42	111	n	
			F43	112	p	
	Yeşilgölcük	1	F44	113	n	0%
	Ovacık	1	F45	114	p	80%
			F46	115	p	
			F47	116	p	
			F48	117	p	
			F49	118	n	
	Ovacık	2	F50	119	n	67%
			F51	120	p	
			F52	121	p	
	Yeşilova	1	F53	122	p	100%
	Elmalı	1	F54	123	n	50%
			F55	124	n	
			F56	125	p	
			F57	126	n	
			F58	127	p	
	Elmalı	2	F59	128	p	80%
			F60	129	p	
			F61	130	p	
			F62	131	n	
			F63	132	p	
19.08.2021	Konaklı	1	F64	133	p	93%
			F1	134	p	
			F2	135	p	
			F3	136	p	
			F4	137	p	
			F5	138	p	
			F6	139	p	
			F7	140	p	
			F8	141	p	
			F9	142	p	
			F10	143	n	
			F11	144	p	
			F12	145	p	
			F13	146	p	
			F14	147	p	
	Konaklı	2	F15	147	p	33%
			F16	149	n	
			F17	150	n	
			F18	151	n	
			F19	152	p	
			F20	153	n	

Table 4.1. (Continued) Potyvirus-specific PTA-ELISA results of common bean leaf samples collected from different places

Collection Date	Collection Sites	Field No	Sample Code	Virus Test Code	PTA-ELISA Result	Infection Rate
19.08.21	Konaklı	2	F21	154	p	80%
	Yeşilgölcük	1	F22	155	p	
			F23	156	p	
			F24	157	n	
			F25	158	p	
			F26	159	p	100%
	Hasaköy	1	F27	160	p	
			F28	161	p	
			F29	162	p	
			F30	163	p	
24.08.2021	Yeşilgölcük	1	F1	164	p	100%
			F2	165	p	
			F3	166	p	
			F4	167	p	
			F5	168	p	
			F6	169	p	100%
	Yeşilgölcük	2	F7	170	p	
			F8	171	p	
			F9	172	p	
			F10	173	p	
			F11	174	p	
			F12	175	p	
	Yeşilgölcük	3	F13	176	p	100%
			F14	177	p	
			F15	178	p	
			F16	179	p	
			F17	180	p	
			F18	181	p	33%
	Alay	1	F19	182	n	
			F20	183	n	
			F21	184	p	67%
	Ağcaşar	1	F22	185	n	
			F23	186	p	
			F-4	187	p	100%
	Ağcaşar	2	F25	188	p	
			F26	189	p	
			F27	190	n	50%
	Kiledere	1	F28	191	n	
			F29	192	p	
			F30	193	p	
			F31	194	p	
			F32	195	n	67%
	Ağcaşar	3	F33	196	n	
			F34	197	n	
			F35	198	p	
			F36	199	p	
			F37	200	p	
			F38	201	p	71%
	Ağcaşar	4	F39	202	p	
			F40	203	p	
			F41	204	n	
			F42	205	p	
			F43	206	n	
			F44	207	p	
			F45	208	p	

Table 4.1. (Continued) Potyvirus-specific PTA-ELISA results of common bean leaf samples collected from different places

Collection Date	Collection Sites	Field No	Sample Code	Virus Test Code	PTA-ELISA Result	Infection Rate
24.08.21	Ağcaşar	5	F46	209	p	86%
			F47	210	p	
			F48	211	p	
			F49	212	p	
			F50	213	p	
			F51	214	n	
			F52	215	p	
14.09.2021	Elmalı	1	F1	216	p	67%
			F2	217	p	
			F3	218	n	
20.09.2021	Amas Bağları	1	F1	219	p	70%
			F2	220	p	
			F3	221	p	
			F4	222	n	
			F5	223	n	
			F6	224	p	
			F7	225	p	
			F8	226	n	
			F9	227	p	
			F10	228	p	
General Total		41	132/228			

*n: negative, p: positive

Table 4.2. Potyvirus infection rates estimated by PTA-ELISA of the collected common bean samples in Niğde province

Collection Sites	No. of fields infected/visited (%)	No. of samples infected/collected (%)
Yeşilova	1/2 (50)	1/10 (10)
Çayırılı	0/1 (0)	0/2 (0)
Yarhisar	0/1 (83.3)	0/1 (0)
Ovacık	5/7 (71.4)	33/44 (75)
Elmalı	3/4 (75)	9/15 (60)
Konaklı	6/8 (75)	26/64 (40.6)
Yeşilgölcük	5/6 (83.3)	23/29 (79.3)
Alay	4/4 (100)	7/18 (38.8)
Ağcaşar	5/5 (100)	19/25 (76)
Kiledere	1/1 (100)	3/6 (50)
Hasaköy	1/1 (100)	4/4 (100)
Amas Bağları	1/1 (100)	7/10 (70)
General Total	32/41 (78)	132/228 (57.8)

4.2 One-Step RT-PCR Method for the Detection of *Potyvirus* Species

The presence of *Potyvirus* species on common bean samples collected was investigated by the One-Step RT-PCR method using species-specific primers for each *Potyvirus* included in this study. Sixty potyvirus-detected samples were involved in PCR reactions depending on their distinctive symptoms. The results were demonstrated sequentially for each virus tested in PCR reactions. The sample number was shown only with virus test codes not with the letter F as it was shown in Table 4.1 which depicts the samples involved in PCR tests.

4.2.1 PCR results for BCMV

In the first attempt, BCMV was tried to amplify in six selected samples depending on their distinct symptoms associated with BCMV using the primers CpF/CpR, yielding 407 bp whose efficiency was declared in previous studies (Figure 4.3). In these PCR tests, no positive controls were used.

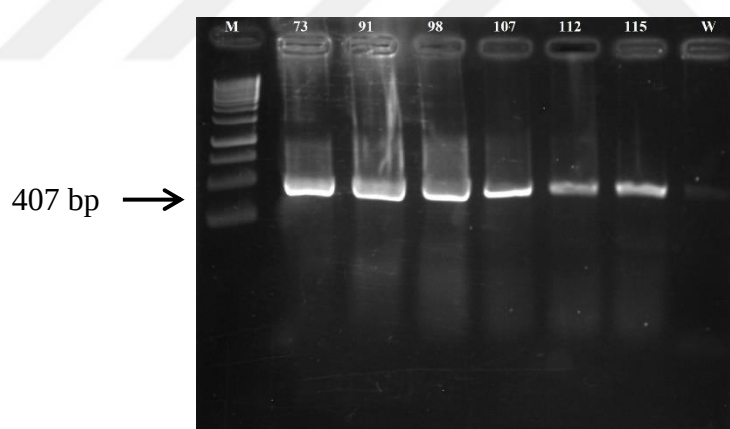


Figure 4.3. The PCR result of common bean samples analyzed for BCMV in One-Step RT-PCR method. M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). W: Water control

Owing to the primer pairs having worked very well in PCR reactions in the samples with the virus test codes (73, 91, 98, 107, 112, and 115), the other 54 samples left have been tested by the same PCR method (Figure 4.4). The samples detected as positive for BCMV at the initial test were also included in the next tests as the positive control.

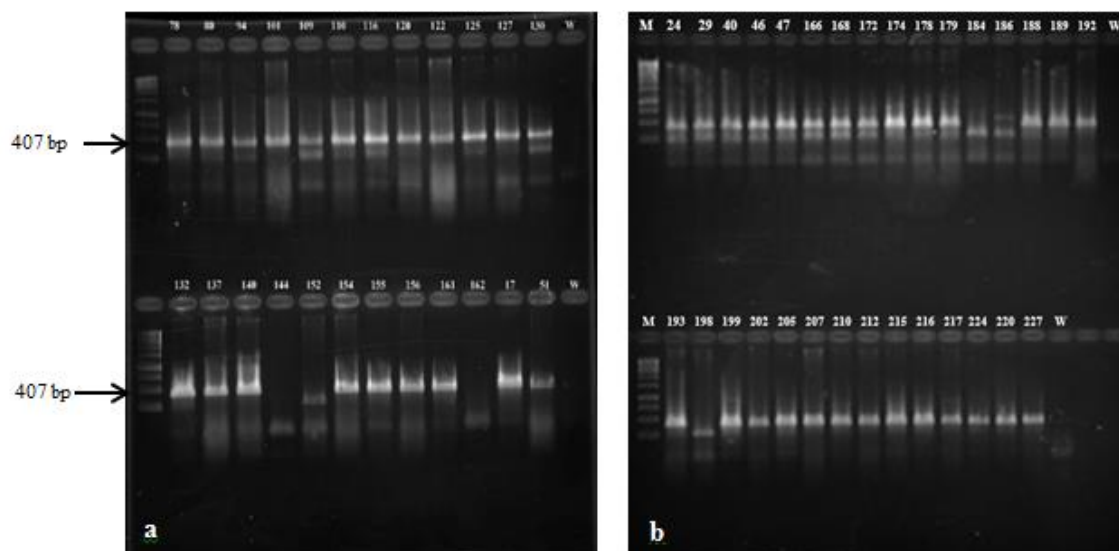


Figure 4.4. Gel results of 24 infected common bean samples (a) and the last 30 common bean samples for BCMV (b). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). W: Water control

Consequently, it was seen that the dilution of TNAs belonging to 54 samples tested by RT-PCR produced the predicted amount of BCMV PCR products as 407 bp. However, the isolates 144, 152, 162, 184, 186, and 198 did not result expected size of amplicons. While the amount of primer-dimer formation was observed highly in some samples, the amount of primer-dimer was barely noticed in the samples with the diluted form of TNAs (Figure 4.4).

4.2.2 PCR results for BCMV negative isolates tested against the other *Potyvirus* species

The samples that were determined as BCMV-negative by RT-PCR were checked instantly for the presence of other potyviruses using the specific primers since these samples gave the unknown fragment of bands. These isolates (144, 152, 162, 184, 186, 198) were tested against BCMNV, BYMV, and CABMV which had the same annealing temperature (55°C). Besides, BCMV-positive isolates (110, 116, 120) were included in PCR tests for the accuracy of the test (Figure 4.5).

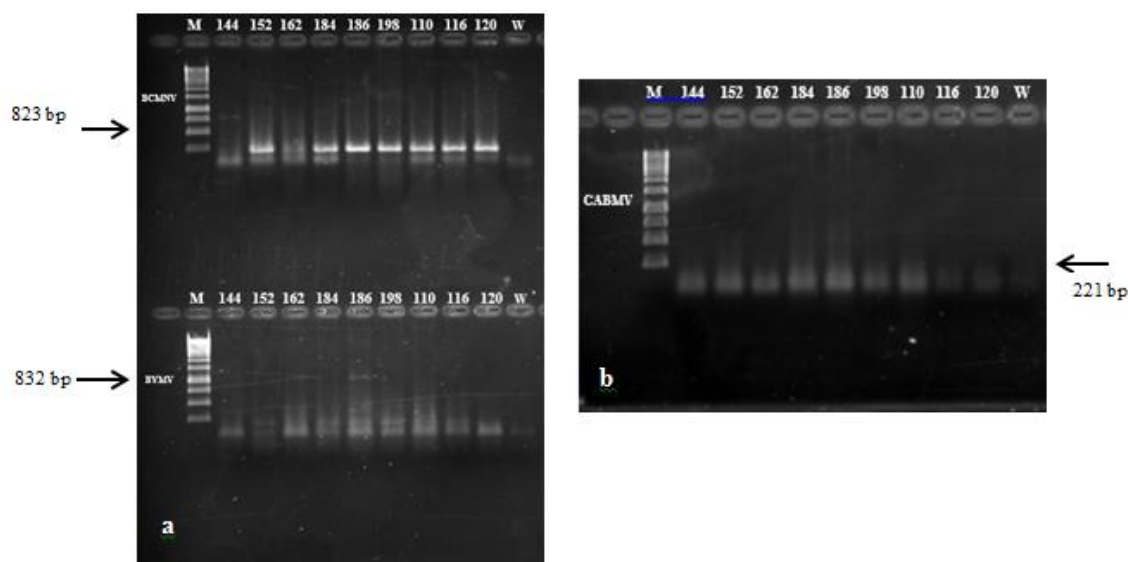


Figure 4.5. Agarose gel results of nine symptomatic common bean samples for BCMNV and BYMV (a) and the results for CABMV (b). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). W: Water control

According to the results, it seemed that diluted isolate 144 generated a reasonable amount of PCR products for BCMNV between 750 and 1000 bp (Figure 4.5a). For this reason, this isolate was retested again with the remaining samples to be tested later on to check if it would produce the same expected product as in this gel result. Also, no other isolates produced the expected amount of product for BCMNV. On the other hand, isolate 186 produced a reasonable amount of fragment for BYMV above 750 near 1000 bp (Figure 4.5a). Then, this positive PCR product was subjected to sequencing. Besides, no expected fragment length was obtained when the isolates mentioned-above were tested for CABMV (Figure 4.5b).

The same BCMV-negative isolates were checked for the presence of SMV, PeMoV, and CIYVV using the specific primers which had the same annealing temperature (52°C). In addition to this, BCMV-positive isolates (110, 116, 120) were included in PCR tests for PCR accuracy again (Figure 4.6).

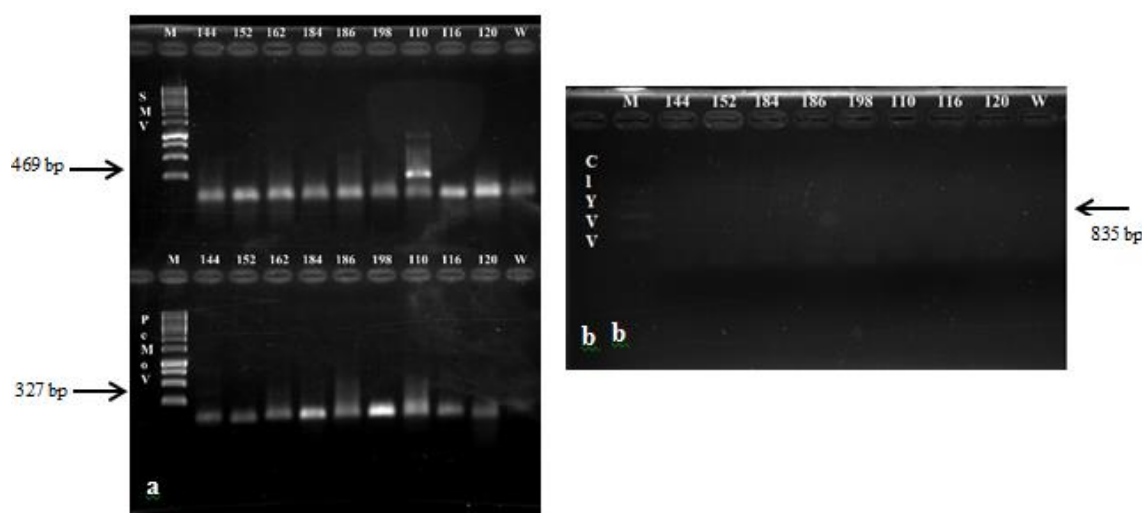


Figure 4.6. Gel results of nine infected common bean samples for SMV and PeMoV (a) and the results for CIYVV (b). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). W: Water control

Based on these gel results, there was no specific amplification for SMV, PeMoV, and CIYVV (Figure 4.6). The amount of primer-dimer was highly observed in the gel of SMV and PeMoV even if the RNA templates were diluted at ratios of 1:100 (Figure 4.6a).

These isolates (144, 152, 162, 184, 3, 198) were inoculated to both cvs. Arslan and Batallı. Although some of the inoculated plants were showing clear symptoms, only the isolate 152 inoculated on cv. Batallı was found to be positive for PTA-ELISA in terms of potyvirus 35 days after post-inoculation. Hereby, the other inoculated plants were found to be negative in terms of potyviruses. The isolate 152 inoculated on cv. Batallı was stored as the inoculum source for further studies.

4.2.3 PCR results for BYMV

When the isolates were tested by RT-PCR, multiple bands have been obtained for some bean isolates (Figure 4.7b). Also, positive control (isolate number 186) confirmed in this study gave an unexpected PCR product above 1000 bp, although it should have given an 832 bp fragment length. For this reason, on the basis of there could be some differences in nucleotide sequences between the other genomes of BYMV isolates, degenerate primer BYMV-pnsF1 was designed to test the samples again (Figure 4.8).

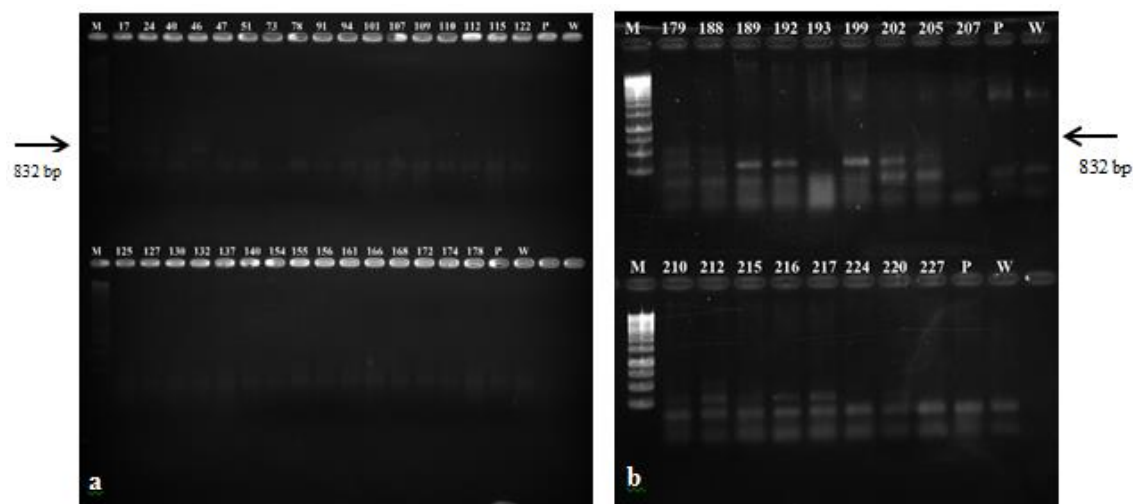


Figure 4.7. Gel results of 34 infected bean plants for BYMV (a) and the results for the remaining 17 samples with BYMV (b). M; GeneRuler 1kb DNA Ladder (Thermo Scientific). P: Positive control confirmed in this study (Isolate number 186) W: Water control

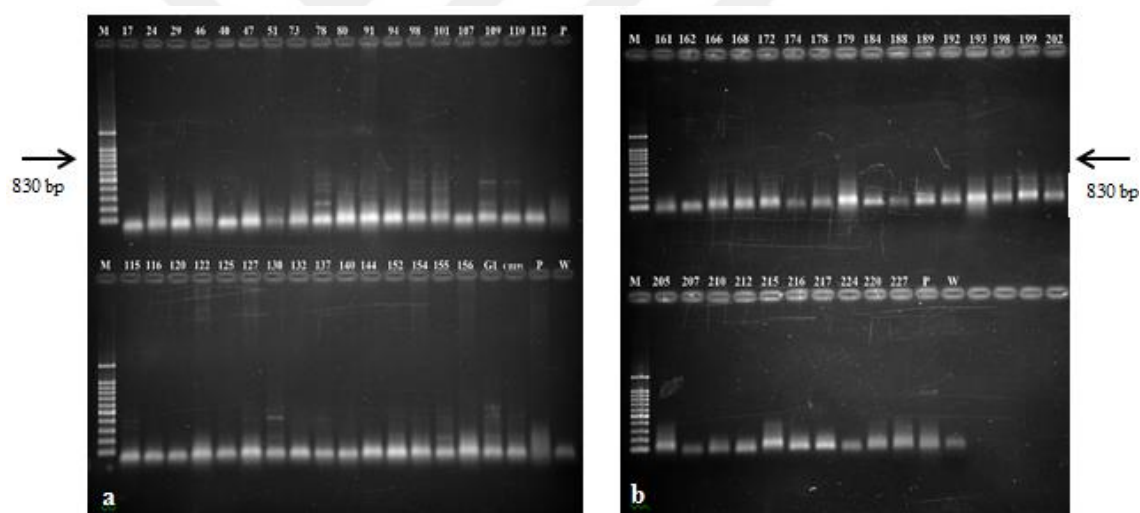


Figure 4.8. Gel results of 33 infected bean plants for BYMV (a) and the results for the remaining 27 samples for BYMV with new designed degenerate BYMV-pnsF1 primer (b). M; Hibrigen 100 bp DNA Ladder. P: Positive control confirmed in this study (Isolate number 186) W: Water control

4.2.4 PCR results for BCMNV

When all isolates were tested against BCMNV, no expected product size (823 bp) was acquired. Also, when isolate number 144 which gave a band above 750 bp before was tested again, it didn't produce the same PCR product (Row 2, Column 11 in Figure

4.9a). Moreover, the isolates 172, 178, 179, and 202 gave a band that was not specific to BCMNV (Figure 4.9b).

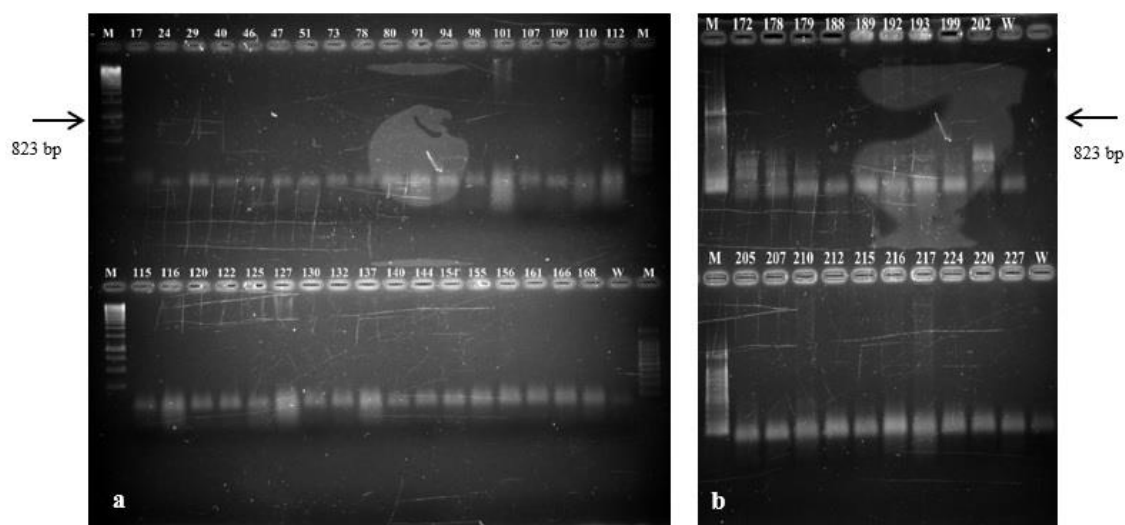


Figure 4.9. Gel results of 35 infected bean plants for BCMNV (a) and the results for the remaining 19 samples with BCMNV (b). M; GeneRuler 1kb DNA Ladder (Thermo Scientific) in the first column of the gel and Hibrigen 100 bp DNA Ladder in the last column of the gel. W: Water control

4.2.5 PCR results for CABMV and PeMoV

According to the results, the isolates (132, 137, 166, 193, 202, 205, 207, 210, and 212) produced PCR products above 400 bp which were not the expected size of the product (221 bp) for CABMV (Figure 4.10). Also, the isolates (192, 193, 199, 202, 205, 210, 212, 215, 216, and 217) produced PCR products above 400 bp which were not an expected size of the product (327 bp) for PeMoV (Figure 4.11).

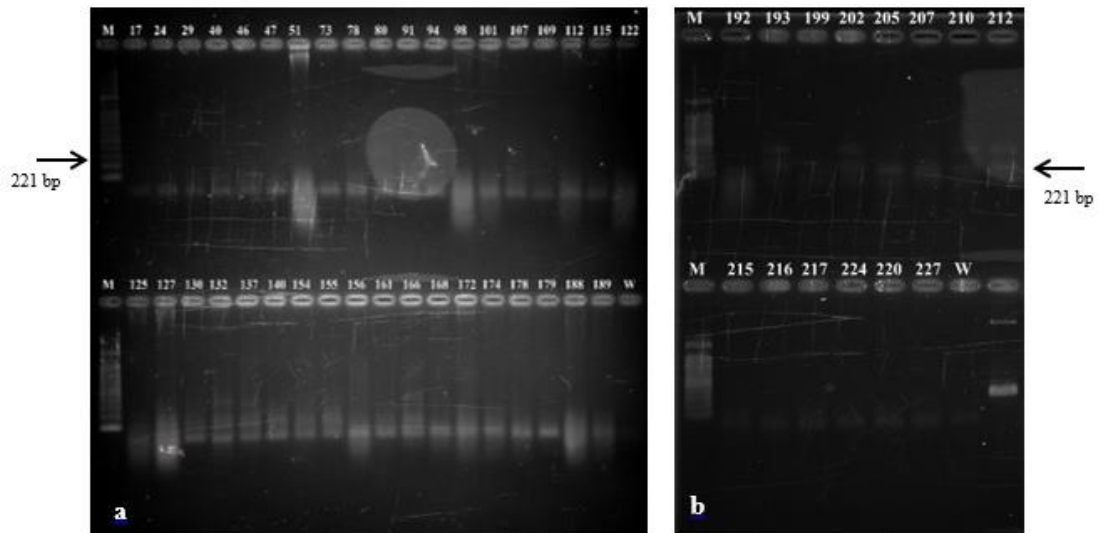


Figure 4.10. Gel results of 37 infected bean plants for CABMV (a) and the results for the remaining 14 samples with CABMV (b). M: Hibrigen 100 bp DNA Ladder. W: Water control. *: The band in the last column in Figure 4.10b does not belong to CABMV, it belongs to another study

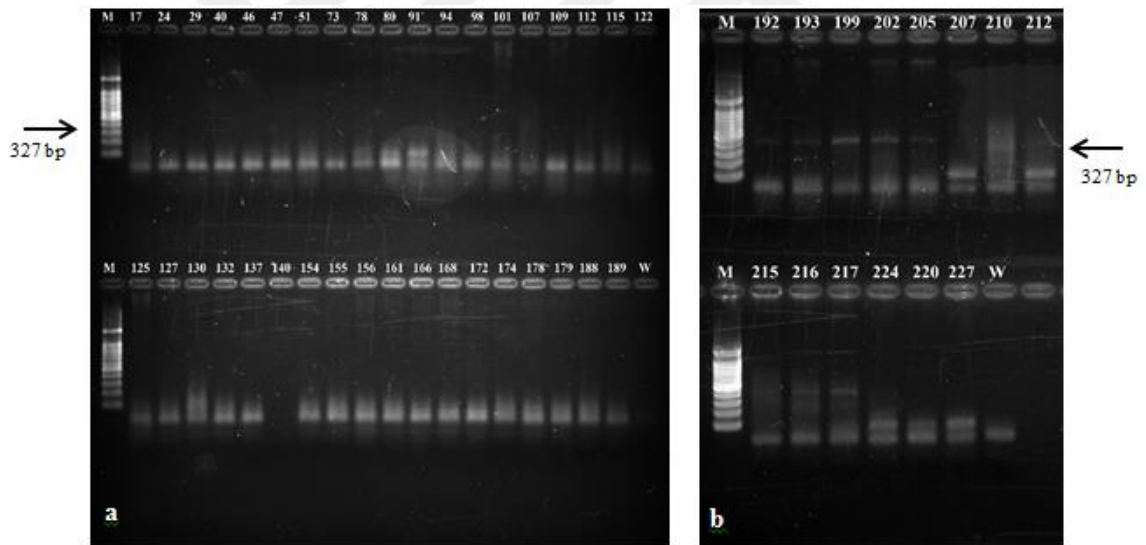


Figure 4.11. Gel results of 37 infected bean plants for PeMoV (a) and the results for the remaining 14 samples with PeMoV (b). M: Hibrigen 100 bp DNA Ladder. W: Water control

Later on, the two randomly selected isolates (202, 212) for CABMV and the two isolates (199, 202) which gave the bands for PeMoV respectively in the previous PCR tests were tested again with different annealing temperatures (Figure 4.12).

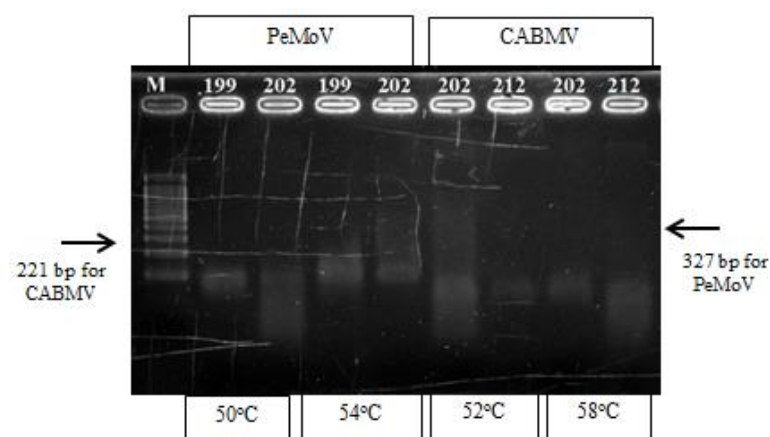


Figure 4.12. Gel results of four infected bean plants for PeMoV and CABMV at different annealing temperatures (50°C, 52°C, 54°C, and 58°C). M: Hibrigen 100 bp DNA Ladder

The results showed that the expected size of products for both PeMoV and CABMV couldn't have been obtained from PCR tests using different annealing temperatures (Figure 4.12).

4.2.6 PCR results for SMV

Based on the results, no expected size of products (469 bp) was acquired for SMV. As seen in Figure 4.13a, there were some bands around 100 and 200 bp, yet these would be because of the primer-dimer formations even if the TNAs were diluted appropriately.

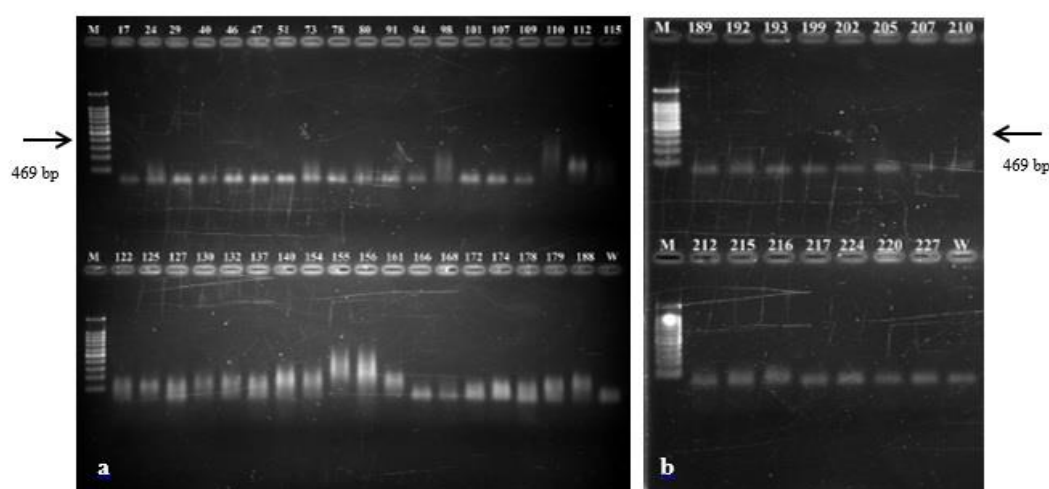


Figure 4.13. Gel results of 37 infected bean plants for SMV (a) and the results for the remaining 15 samples with SMV (b). M: Hibrigen 100 bp DNA Ladder. W: Water control

4.2.7 PCR results for CIYVV

Based on the results, no expected size of products (835 bp) was acquired for CIYVV (Figure 4.14). As seen in Figure 4.14b, there were some bands for the isolates (193 and 227) around 500 bp, yet these products were not the expected fragment lengths.

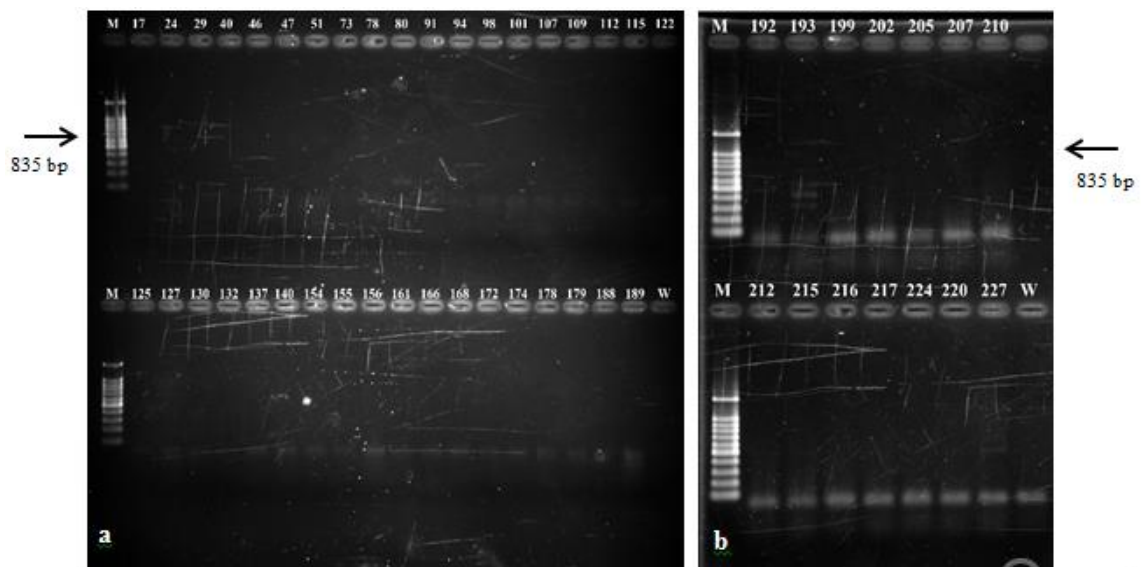


Figure 4.14. Gel results of 37 infected bean plants for CIYVV (a) and the results for the remaining 14 samples with CIYVV (b). M: Hibrigen 100 bp DNA Ladder. W: Water control

4.3 Nucleotide Sequence Analysis of BYMV Isolate

The amplicon belonging to isolate 186 was sequenced with the Sanger Method. A nucleotide sequence of 980 bp obtained after editing the sequence of this isolate (Appendix-5) was used in this study. The provided 980 nt length sequence showed 89% identity to a BYMV isolate deposited in NCBI with 100% query coverage (Table 4.3).

Table 4.3. BLASTn analysis of BYMV isolate obtained from this study (Isolate 186)*

Isolate of This Study	NCBI Accession Number	Isolate Name	Strain Name	Country	Query Coverage (%)	Identity (%)
Isolate 186	MN399729	Salzlandkreis-2_18	-	Germany	100.00	89.09
	MW411034	BIQ83	-	Iraq	100.00	87.59
	MK649741	ARGbb	-	Argentina	100.00	87.21
	OK030755	The Deepings BYMV	-	United Kingdom	99	83.93
	MG600297	BYMV-PV2	-	Czech Republic	100.00	83.33
	AB032023	Large nuclear inclusion protein and coat protein	-	Japan	100.00	83.23
	GU126690	Alaska polyprotein gene	-	USA	100.00	83.05
	AY520092	Verbena polyprotein gene	-	USA	100.00	82.95
	AB373203	-	Strain CS	Japan	100.00	82.74
	D00604	Capsid Protein Region	-	Japan	100.00	82.74
	OL584370	DSMZ PV-1213	-	Germany	99	82.76
	HG970869	NG1	-	Australia	99	82.74

-: Not available, *: The first twelve BYMV isolates with the highest nucleotide identities were given.

4.4 Phylogenetic Analysis of BYMV Isolate

To infer the evolutionary history, the Maximum Likelihood method based on the General Time Reversible model was applied. Three significant clusters emerged from the phylogenetic tree of BYMV isolates. According to the phylogenetic tree, the host of the plant virus appears to have a crucial role in the creation of clustering, since viruses with the same host plant were generally clustered in the same sub-group. In addition, our isolate 186, was discovered to be in the sub-group with the BYMV isolate from a pea in Germany that was deposited in GenBank (MN399729) (Figure 4.15).

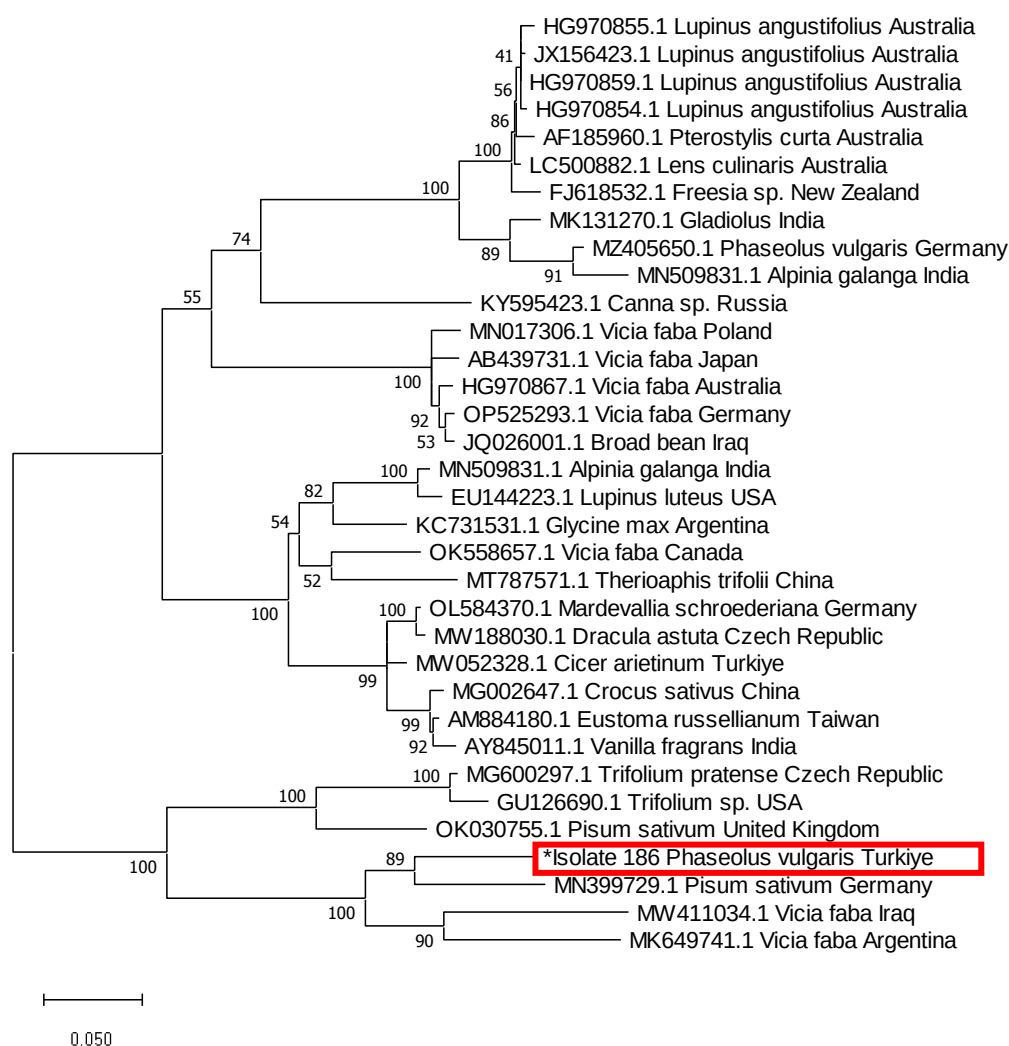


Figure 4.15. Phylogenetic tree of the Bean yellow mosaic virus isolate 186 constructed in MEGA-X. Bootstrap value was adjusted to (1000 replicates) which shows the percentage of replicate trees in which the associated taxa clustered together

4.5 Investigation of Reactions of Different Common Bean Cultivars to BCMV

4.5.1 Determination of resistance level and resistance genes of common bean cultivars based on the symptoms

Before the inoculation of each common bean cultivar with BCMV isolate, the plant samples belonging to cvs. Adabeyazı, Akbulut, Akman-98, Borsa, Canipek, Cihan, Ergen, Güngör, Sururbey, and Zirve which were used in virus reaction studies were tested by PTA-ELISA in order to check whether potyviruses have been transmitted by the seeds or not. The PTA-ELISA results for each group of common bean cultivars described above were summarized in Table 4.4.

Table 4.4. The PTA-ELISA results for each bean cultivar having 3 different groups before BCMV inoculation to evaluate seed-transmitted potyviruses

Cultivars	Results of PTA-ELISA		
	Groups (Comprised of 8 plants)		
	1	2	3
Adabeyazı	N	N	N
Akbulut	N	N	N
Akman-98	N	N	N
Borsa	N	N	N
Canipek	N	N	N
Cihan	N	N	N
Ergan	N	N	N
Güngör	N	P	P
Sururbey	N	N	N
Zirve	N	N	N

P: Positive, **N:** Negative

As seen in Table 4.4, only group 2 and group 3 belonging to the cv. Güngör was found to be potyvirus-positive by ELISA testing. This result has revealed that the cv. Güngör has been already infected by any type of potyvirus. So those plants were not used in BCMV inoculation studies.

According to the result of testing plants for seed-borne potyvirus, the ELISA-negative plants were inoculated with BCMV isolate later on and checked for their symptoms from 7 to 35 days after virus inoculation. Seven days after post-inoculation, severe vein clearing and leaf curling were observed on inoculated leaves of the cv. Güngör. On the other hand, mild mosaic and leaf curling symptoms started to come up on non-inoculated leaves of the cv. Güngör two weeks after inoculation and also mosaic symptoms became more prevalent at three weeks after post-inoculation. Besides, two weeks after post-inoculation, leaf curling symptoms were observed on non-inoculated leaves of the cv. Akbulut and these symptoms became more pronounced in the third week following inoculation. (Figure 4.16). Additionally, systemic symptoms on non-inoculated leaves of the cvs. Akman-98 and Sururbey at three weeks after post-inoculation were quite different from those detected on non-inoculated leaves of cvs. Güngör and Akbulut (Figure 4.17). Moreover, mild mosaic and leaf curling symptoms on non-inoculated leaves of the cvs. Cihan and Ergan were detected at three weeks after post-inoculation with BCMV (Figure 4.18). Furthermore, while chlorotic symptoms on non-inoculated leaves of the cvs. Canipek and Borsa were detected at three weeks after post-inoculation with BCMV (Figure 4.19), vein clearing symptoms were noticed on

non-inoculated leaves of the cvs. Adabeyazı and Zirve (Figure 4.20). The same symptoms were observed on the control plant of each cultivar. It was thought that these cultivars showing distinctive symptoms were infected with another virus except potyvirus. The symptoms that occurred on each common bean cultivar used in the virus inoculation trial were summarized in Appendix-1.

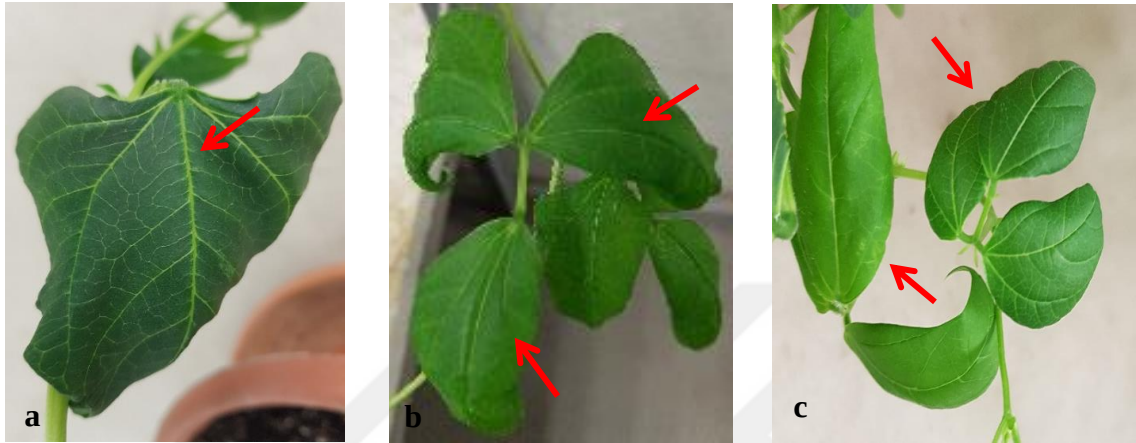


Figure 4.16. Symptoms belonging to the first inoculation trial. Vein clearing and leaf curling symptoms on inoculated leaves of cv. Güngör after 7 days of inoculation (a), mosaic symptoms with leaf curling three weeks after post-inoculation on the cv. Güngör (b), leaf curling symptoms on non-inoculated leaves of the cv. Akbulut (c)

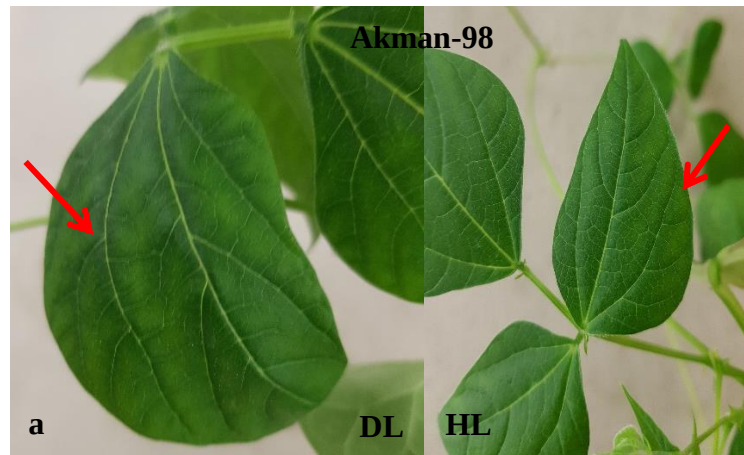


Figure 4.17. Systemic mosaic symptoms on non-inoculated leaves of the cvs. Akman-98 and Sururbey three weeks after post-inoculation with BCMV (a, b), DL: Diseased Leaf; HL: Healthy leaf

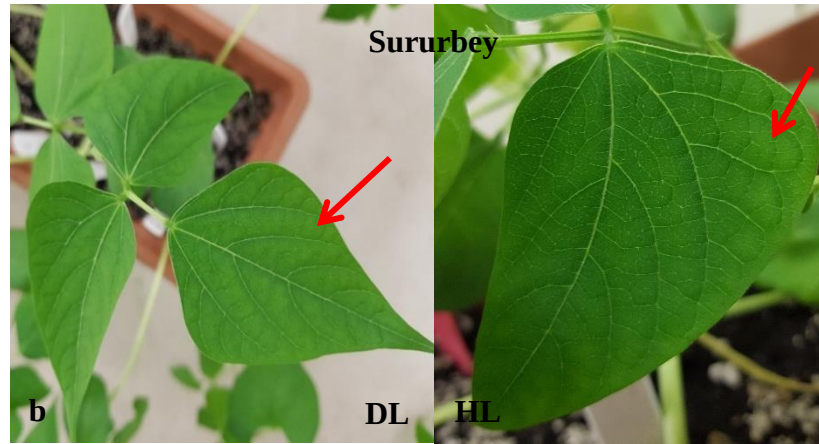


Figure 4.17. (Continued) Systemic mosaic symptoms on non-inoculated leaves of the cvs. Akman-98 and Sururbey three weeks after post-inoculation with BCMV (a, b), DL: Diseased Leaf; HL: Healthy leaf

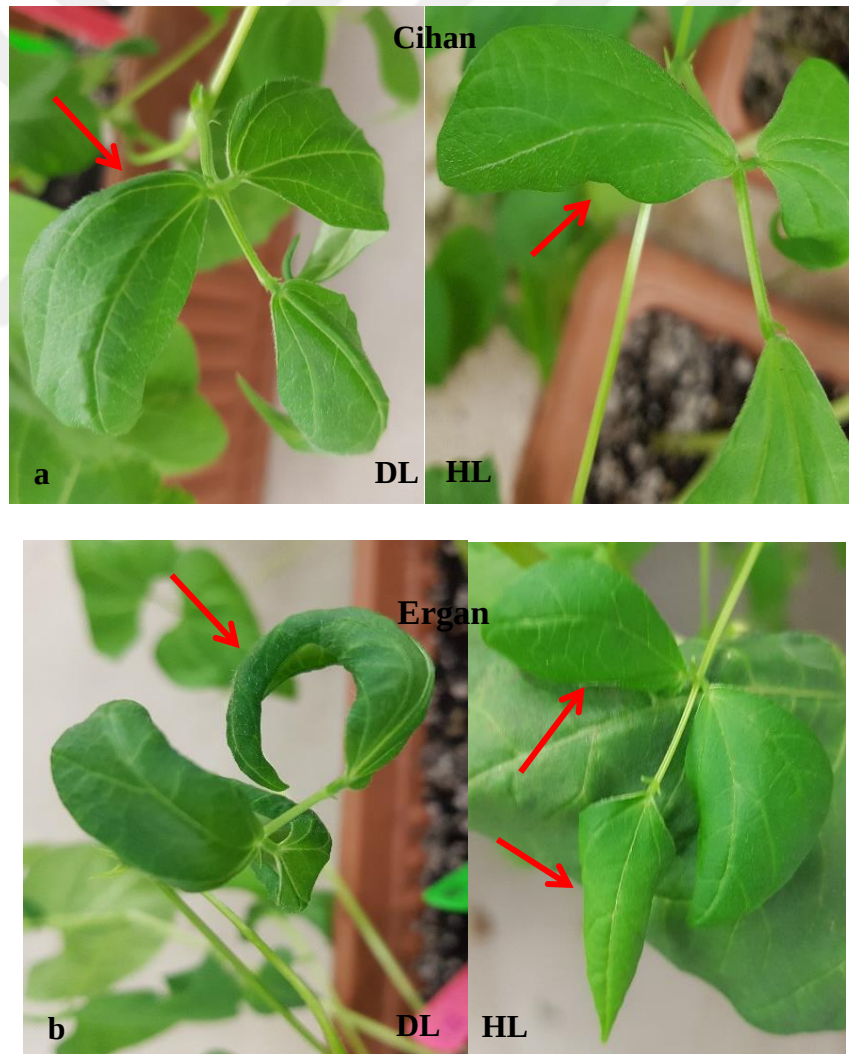


Figure 4.18. Mild mosaic and leaf curling symptoms on non-inoculated leaves of the cvs. Cihan and Ergen three weeks after post-inoculation with BCMV (a, b), DL: Diseased Leaf; HL: Healthy leaf

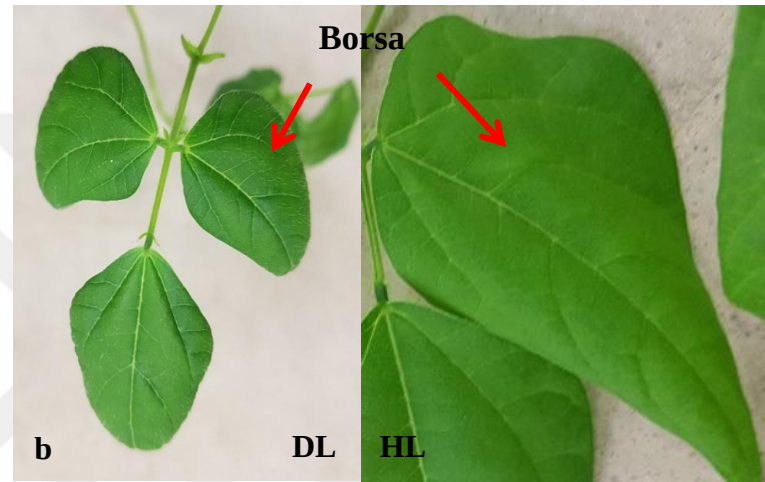
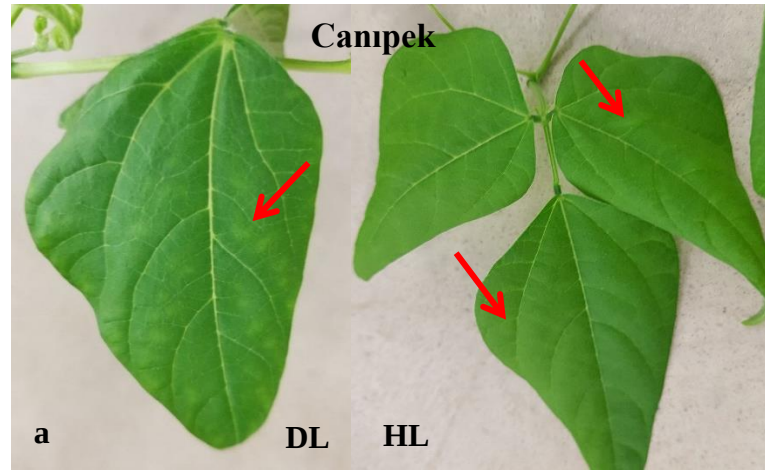


Figure 4.19. Chlorotic symptoms on non-inoculated leaves of the cvs. Canipek and Borsa three weeks after post-inoculation with BCMV (a, b), DL: Diseased Leaf; HL: Healthy leaf

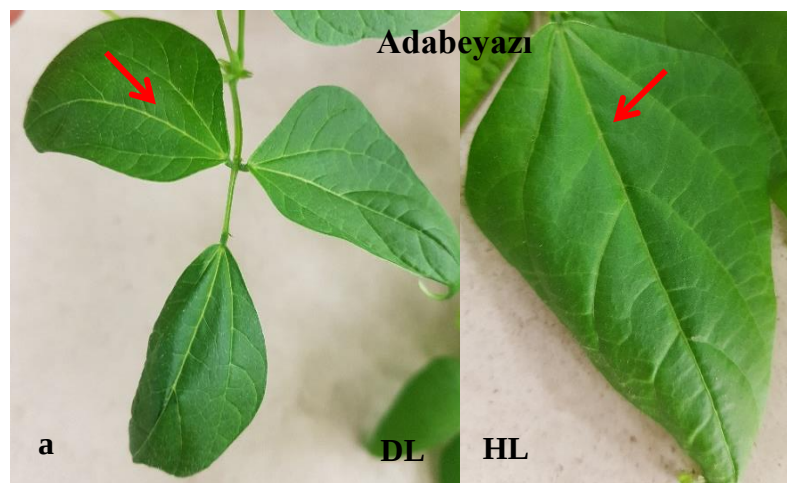


Figure 4.20. Vein clearing symptoms on non-inoculated leaves of the cvs. Adabeyazi and Zirve at three weeks after post-inoculation with BCMV (a, b), DL: Diseased Leaf; HL: Healthy leaf

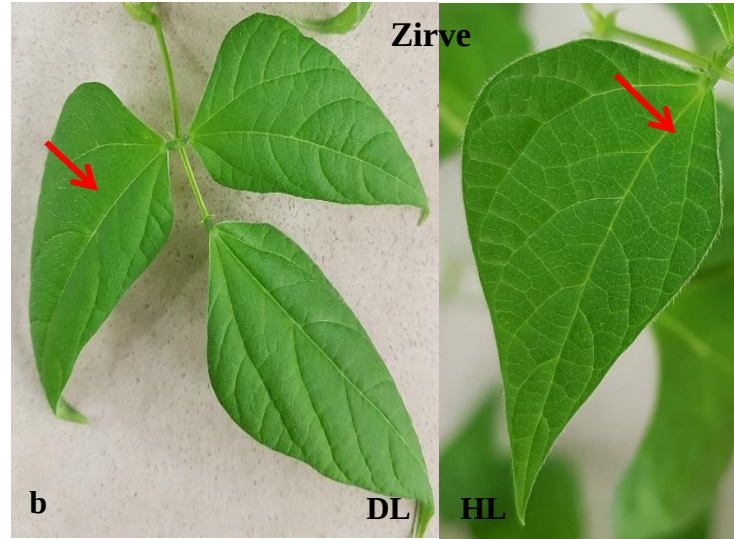


Figure 4.20. (Continued) Vein clearing symptoms on non-inoculated leaves of the cvs. Adabeyazı and Zirve at three weeks after post-inoculation with BCMV (a, b), DL: Diseased Leaf; HL: Healthy leaf

When the same cultivars were inoculated with BCMV isolate in another trial, severe vein clearing and leaf curling were again observed on inoculated leaves of the cv. Güngör three weeks after post-inoculation. On the other hand, mild mosaic and leaf curling symptoms started to come up again on non-inoculated leaves of the cv. Güngör three weeks after inoculation, and also mosaic symptoms became more prevalent four weeks after post-inoculation. Besides, three weeks after post-inoculation, leaf curling symptoms were also observed on non-inoculated leaves of the cv. Akbulut and this symptom became more pronounced on non-inoculated leaves in the four week of following inoculation (Figure 4.21). Meanwhile, the same symptoms in the previous inoculation trial were also observed on the cvs. Adabeyazı, Akman-98, Borsa, Canıpek, Cihan, Ergan, and Zirve as described above for each cultivar. The same symptoms were also observed in their control plants. Besides, no clear symptoms belonging to BCMV were detected in the susceptible control plant (HG1) in two virus inoculation studies. The symptoms that occurred on each common bean cultivar used in the virus inoculation trial were outlined in Appendix-2.

These results proved that another virus except potyvirus could be transmitted via seed for the cultivars. Because of that molecular markers identifying resistance genes in common bean for BCMV decided to be applied to the cultivars.

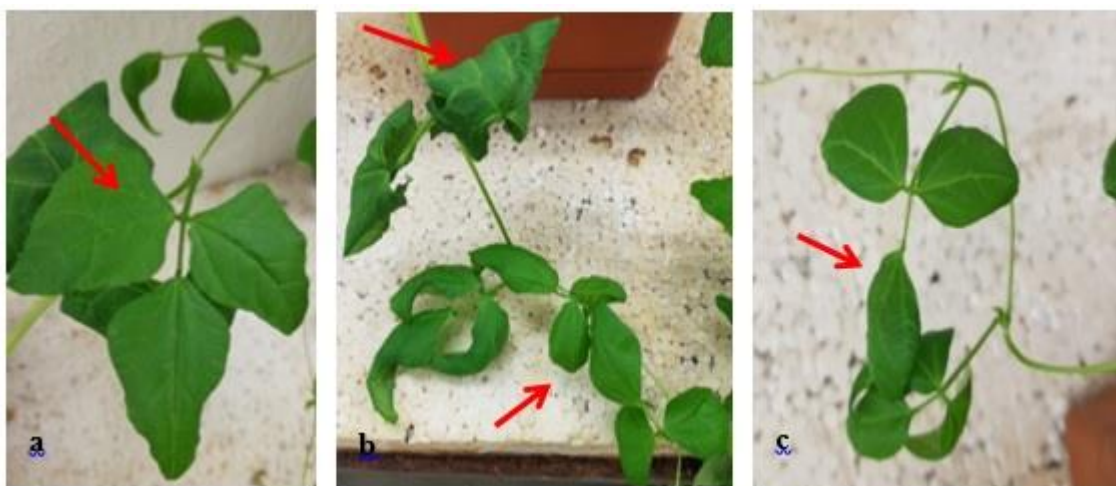


Figure 4.21. Symptoms belonging to the second inoculation trial. Mild mosaic and leaf curling symptoms on non-inoculated leaves of GÜNGÖR and vein clearing and leaf curling symptoms on inoculated leaves of cv. GÜNGÖR three weeks after inoculation (a), systemic mosaic with leaf curling symptoms four weeks after post-inoculation on the cv. GÜNGÖR (b), leaf curling symptom on non-inoculated leaves of cv. Akbulut at three weeks after post-inoculation (c)

4.5.2 Determination of resistance level of common bean cultivars to BCMV based on PTA-ELISA results

Eight inoculated and one control common bean plant belonging to each variety at three-weeks after post-inoculation were tested by PTA-ELISA. Although bean cvs. Akman-98 and Sururbey demonstrate virus-like symptoms (systemic mosaic), Cihan and Ergen (mild mosaic and leaf curling), Canipek and Borsa (chlorotic spots) Adabeyazı and Zirve (vein clearing), these cultivars were found to be negative at the third week's testing of ELISA (Table 4.5). Only the cvs. GÜNGÖR and Akbulut were found to be positive at the third week's according to the ELISA. As a result of phenotypic evaluations and ELISA-based screening (Table 4.5), out of ten bean cultivars, only two cultivars (GÜNGÖR and Akbulut) were found to be susceptible to BCMV, while eight cultivars (Akman-98, Sururbey, Cihan, Ergen, Canipek, Borsa, Adabeyazı, and Zirve) were found to be resistant to BCMV. Table 4.5 summarizes the complete findings of biological and serological testings for each replication of common bean cultivars. The absorbance values for each replication of bean cultivars were specified in Appendix-3.

Table 4.5. The results of systemic symptoms and PTA-ELISA results of inoculated common bean cultivars from the first inoculation trial

Cultivar	Systemic Symptoms									Results of PTA-ELISA									Reaction
	1	2	3	4	5	6	7	8	9*	1	2	3	4	5	6	7	8	9*	
Güngör	-	-	-	-	-	+	+	-	+	N	N	N	N	N	P	P	N	N	S
Akman-98	+	+	-	+	-	+	-	-	+	N	N	N	N	N	N	N	N	N	R
Cihan	+	-	+	+	-	-	-	-	+	N	N	N	N	N	N	N	N	N	R
Ergan	-	-	-	-	-	-	+	-	+	N	N	N	N	N	N	N	N	N	R
Akbulut	-	+	-	+	+	+	-	-	+	N	P	N	N	N	N	N	N	N	S
Sururbey	-	-	-	-	+	+	-	+	+	N	N	N	N	N	N	N	N	N	R
Canipek	-	+	+	-	-	-	+	+	+	N	N	N	N	N	N	N	N	N	R
Adabeyazı	+	-	-	-	-	-	-	-	+	N	N	N	N	N	N	N	N	N	R
Zirve	-	-	+	-	-	-	-	-	+	N	N	N	N	N	N	N	N	N	R
Borsa	-	-	-	-	-	-	+	-	+	N	N	N	N	N	N	N	N	N	R

*: Control plants, +: Present -: Absent P: Positive, N: Negative, R: Resistant, S: Susceptible. Pink areas show positivity.

In the second virus inoculation trial, four inoculated and one control bean plant showing distinctive symptoms at four-weeks after post-inoculation were tested by PTA-ELISA. Only the cvs. Güngör and Akbulut were found to be positive at the four week's ELISA. The previous result obtained from the first inoculation trial was proved with this repeated virus inoculation study. Hence, out of ten common bean cultivars used in virus inoculation studies, only two cultivars (Güngör and Akbulut) were found to be susceptible to BCMV, while eight cultivars (Akman-98, Sururbey, Cihan, Ergan, Canipek, Borsa, Adabeyazı, and Zirve) were found to be resistant to BCMV again. Table 4.6 summarizes the complete findings of biological and serological testings for each replication of common bean cultivars. The absorbance values for each replication of common bean cultivars were specified in Appendix-4.

Table 4.6. The results of systemic symptoms and ELISA results of inoculated bean cultivars from second inoculation trial

Cultivar	Systemic symptoms					Results of PTA-ELISA					Reaction
	1	2	3	4	5*	1	2	3	4	5*	
Güngör	+	+	+	+	+	P	P	P	N	P	S
Akman-98	+	+	+	+	+	N	N	N	N	N	R
Cihan	+	+	-	+	+	N	N	N	N	N	R
Ergan	+	+	-	-	+	N	N	N	N	N	R
Akbulut	+	+	-	-	+	P	P	N	N	N	S
Sururbey	+	+	+	+	+	N	N	N	N	N	R
Canipek	+	-	+	+	+	N	N	N	N	N	R
Adabeyazı	-	+	-	-	+	N	N	N	N	N	R
Zirve	-	+	-	-	+	N	N	N	N	N	R
Borsa	+	-	+	+	+	N	N	N	N	N	R

*: Control plants, +: Present -: Absent P: Positive, N: Negative, R: Resistant, S: Susceptible. Pink areas show positivity.

Moreover, although the susceptible cultivar (HG1) used as a control in the virus inoculation studies showed mosaic symptoms, this cultivar was found to be negative at the third and four week's testing of ELISA in terms of potyvirus.

4.5.3 Screening resistance genes on common bean cultivars by markers

While the presence of the dominant *I* gene and recessive *bc-1*² gene were investigated by SCAR markers (SW-13 and SBD-5) respectively, the presence of the recessive *bc-3* gene was scrutinized using the SCAR (ROC11) and CAPS markers (eIFE4), separately. The results of this study were shown in Table 4.7.

When the cultivars were tested with SW-13 marker which is specific for the dominant *I* gene, the results showed that the expected size of products (690 bp) was obtained for the cvs. Akın, Akman-98, Batallı, Canipek, Cihan, Göynük-98, Gündoğan, Karbeyazı, Nirvana, Noyanbey-98, and Sururbey. The results showed that these cultivars carry the dominant *I* gene (Figure 4.22).

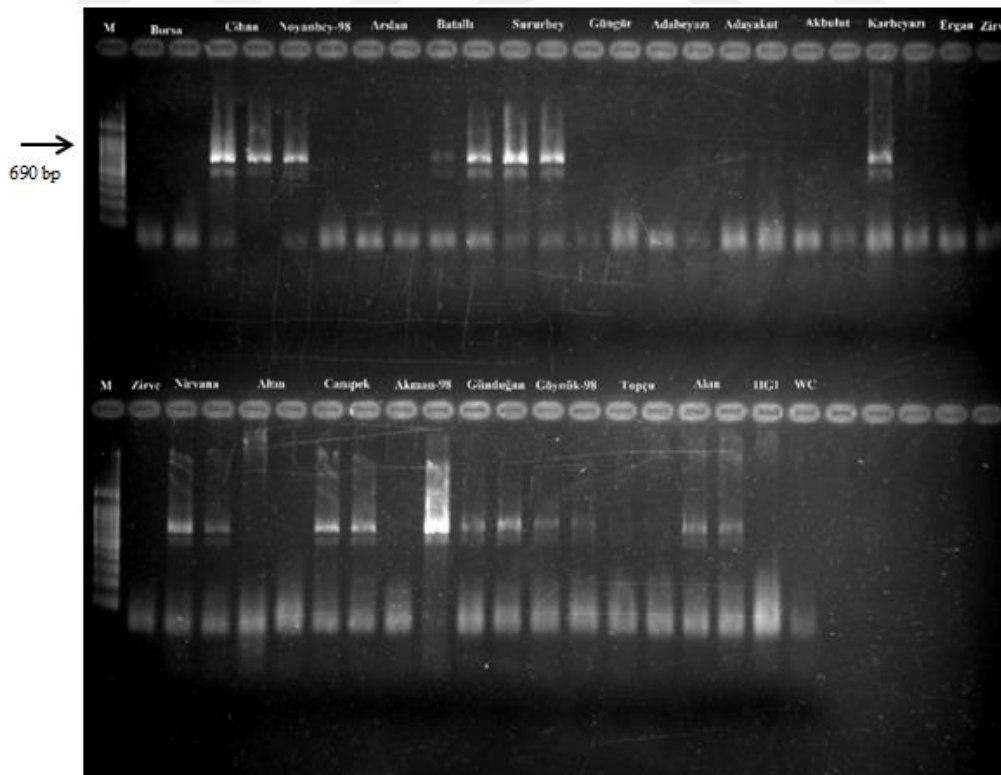


Figure 4.22. Gel results of common bean cultivars by using the SCAR marker SW-13 which is specific for the dominant *I* gene. Each cultivar has two rows except cv. Ergen. M: Hibrigen 100 bp DNA Ladder, HG1: Host Group 1, WC: Water control

Also, it was obtained that Adayakut, Akın, Batallı, Cihan, Göynük-98, Gündoğan, Güngör, Karbeyazı, Nirvana, Noyanbey-98, Sururbey, Topçu, and Zirve carry the recessive *bc-1²* gene when these cultivars were tested with SBD-5 marker which is specific for this gene as they gave an expected size of products (1300 bp). Even though the results showed that Host Control (HG1) carries the *bc-1²* gene, this was the false positive result with this marker, because this host group cultivar doesn't contain *bc-1²* gene (Figure 4.23).

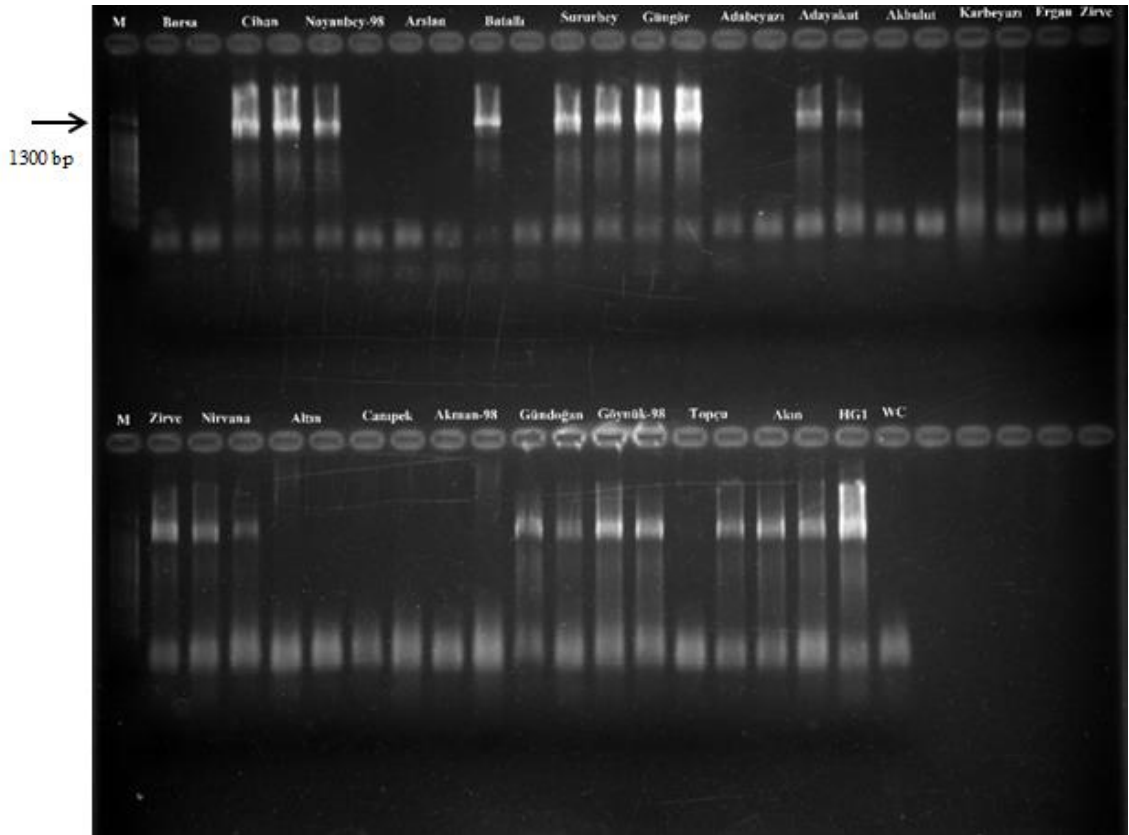


Figure 4.23. Gel results of common bean cultivars by using the SCAR marker SBD-5 which is specific for *bc-1²* gene. Each cultivar has two rows except cv. Ergen. M: Hibrigen 100 bp DNA Ladder, HG1: Host Group 1, WC: Water control

According to the results, no amplification (420 bp) was obtained for the 22 cultivars tested in resistance gene analyses, when these cultivars were tested with the SCAR marker ROC11 which is specific for *bc-3* gene. The absence of band indicates the presence of the recessive *bc-3* gene on the tested common bean cultivars (Figure 4.24).



Figure 4.24. Agarose gel results of common bean cultivars by using the SCAR marker ROC11 which is specific for *bc-3* gene. Absence of signal indicates the presence of *bc-3* gene. Each cultivar has two rows except cv. Ergon. M: Hibridgen 100 bp DNA Ladder, HG1: Host Group 1, WC: Water control

Moreover, all the cultivars tested with eIFE4 (CAPS marker) which is specific for *bc-3* gene produced the expected size of products (541 bp) (Figure 4.25).

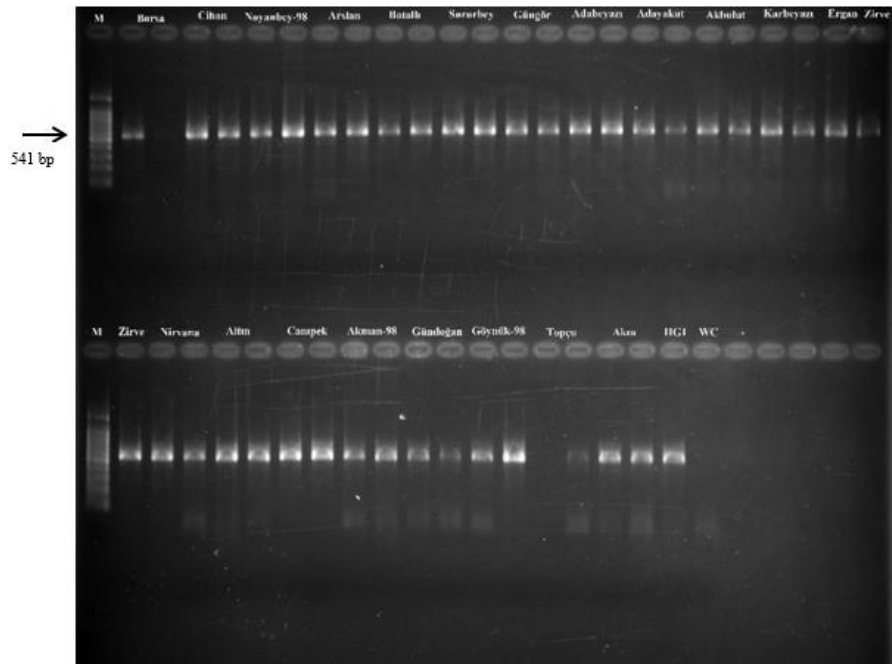


Figure 4.25. Gel results of common bean cultivars by using the CAPS marker eIFE4 which is specific for *bc-3* gene. Each cultivar has two rows except cv. Ergon. M: Hibridgen 100 bp DNA Ladder, HG1: Host Group 1, WC: Water control

Furthermore, *RsaI* enzyme was used for the digestion of amplified PCR products obtained by eIFE4 marker belonging to common bean cultivars (shown in Figure 4.25), yet no enzyme restriction (381/160 bp) was acquired for the cultivars indicating that *bc-3* resistance gene is not present (Figure 4.26).

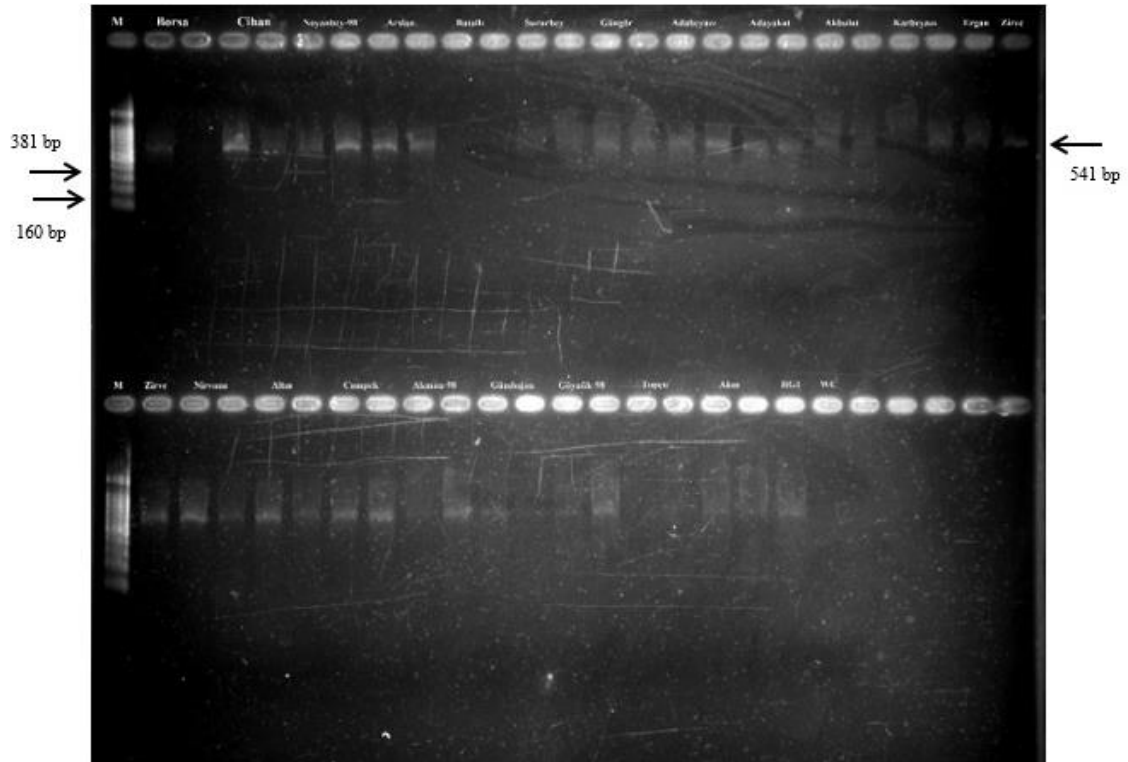


Figure 4.26. Gel results of digestion by using *RsaI* enzyme for common bean cultivars. Each cultivar has two rows except cv. Ergen. Presence of two signals (381 and 160 bp) indicates the presence of *bc-3* allele. Presence of 541-bp fragment indicates the absence of *bc-3* allele. M: Hibrigen 100 bp DNA Ladder, HG1: Host Group 1, WC: Water control

Table 4.7. The results of dominant *I*, recessive *bc-1²* and *bc-3* genes by DNA markers in common bean cultivars

Common Bean Cultivars	Code	SCAR Markers			CAPS Marker
		SW-13 (<i>I</i>)	SBD-5 (<i>bc-1²</i>)	ROC11(<i>bc-3</i>)	eIFE4 (<i>bc-3</i>)
Adabeyazı	1	-	-	+	-
Akbulut	2	-	-	+	-
Akman-98	3	+	-	+	-
Borsa	4	-	-	+	-
Canipek	5	+	-	+	-
Cihan	6	+	+	+	-
Ergan	7	-	-	+	-
Güngör	8	-	+	+	-
Sururbey	9	+	+	+	-
Zirve	10	-	+	+	-
Adayakut	11	-	+	+	-
Altın	12	-	-	+	-
Akın	13	+	+	+	-
Arslan	14	-	-	+	-
Bataklı	15	+	+	+	-
Göynük-98	16	+	+	+	-
Gündoğan	17	+	+	+	-
Karbeyazı	18	+	+	+	-
Nirvana	19	+	+	+	-
Noyanbey-98	20	+	+	+	-
Topçu	21	-	+	+	-
Stringless Green Refugee (HG1)	22	-	+	+	-

+: Marker present, -: Marker absent

As seen in Table 4.7, the recessive genes (*bc-1²* and *bc-3*) was obtained in the control cultivar Stringless Green Refugee (HG1), yet this finding shows a false positive result for this host control plant, since this cultivar is known to have *ii+bc-u* genotype. SCAR marker ROC-11 developed for the *bc-3* gene gave false positive in cv. Stringless Green Refugee, therefore the results of eIFE4 (CAPS marker) have been considered in this study.

When resistance genes were evaluated on common bean cultivars, due to the fact that the cv. Güngör showed a susceptible reaction to BCMV and produced mild mosaic on non-inoculated leaves, it was concluded that this cultivar has the recessive *ii* and may contain *bc-1²* genes. Also, it was concluded that the cultivar Akbulut has the recessive *ii* since this cultivar showed a susceptible reaction to BCMV. Even though symptoms have been seen on the cvs. Akman-98 and Sururbey, these symptoms were not specific to BCMV. Because the cvs. Akman-98 and Sururbey showed resistance reaction to BCMV, it was concluded that the cv. Akman-98 has the dominant *I* gene, while

Sururbey has the dominant *I* gene and may contain the recessive *bc-1²* gene. Since the cvs. Cihan and Ergan showed resistance reaction to BCMV, it was concluded that the cv. Cihan has the dominant *I* gene and may have the recessive *bc-1²* gene, while Ergan has *ii* gene with undetected resistance gene (s). Moreover, the cvs. Canipek and Borsa displayed resistance to BCMV. It was seen that Canipek has the dominant *I* gene, whereas Borsa has *ii* gene with undetected resistance gene(s). Additionally, the cvs. Adabeyazı and Zirve displayed resistance to BCMV even though they showed vein clearing symptoms. It was seen that Zirve may carry the recessive *bc-1²* gene, whereas Adabeyazı has *ii* gene with undetected resistance gene (s) (Table 4.7).

4.6 Detection of Phytoplasma in Common Bean Plant Samples

In the first attempt to detect phytoplasma on infected common bean samples, four isolates (27, 28, 29, and 30) having more pronounced phytoplasma-associated symptoms were selected and tested by direct-PCR using P1/P7 primers and subsequently by nested-PCR using R16F2n/R16R2. These samples were tested with diluted DNA products at the ratios of 1:50 and 1:100. The result of agarose gel electrophoresis belonging to nested-PCR products with R16F2n/R16R2 primers showed that the diluted all PCR products produced a smear pattern of genomic DNAs (Figure 4.27).

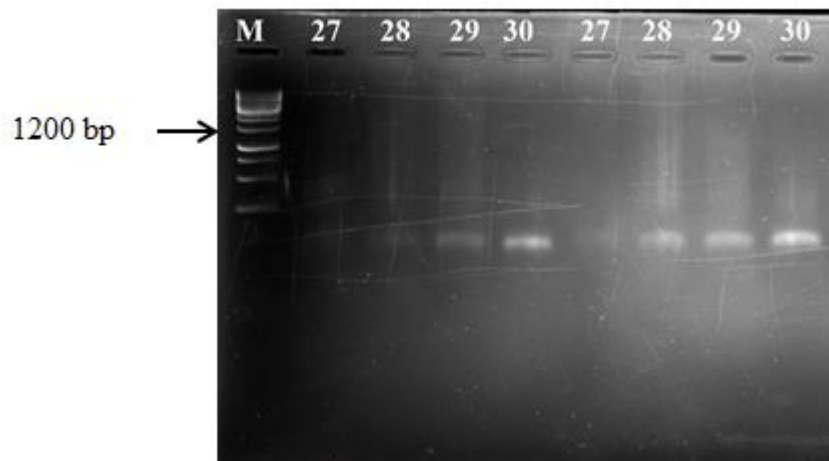


Figure 4.27. Gel images of nested-PCR products with R16F2n/R16R2 at the ratios of 1:50 (the first four samples on the left side) and 1:100 (the last four samples on the right side). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific)

Then, the same isolates were tested by the primer pairs P1/P7 - R16F2n/R16R2 - M1/M2, respectively. The gel results obtained by using M1/M2 nested-PCR products revealed that isolate 30 formed the same band profile with the positive controls of AP at levels of around 509 bp (Figure 4.28). However, it was understood that after sequencing those bands belonged to apple proliferation due to the presence of contamination for the amplified PCR product.

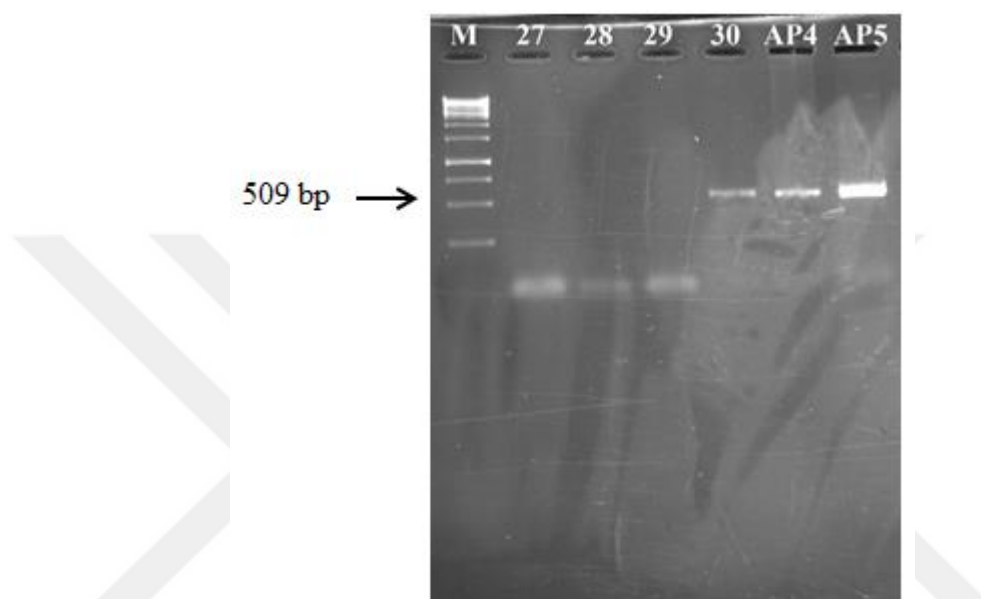


Figure 4.28. Gel images of nested-PCR products with M1/M2 primer pairs at the ratios of 1:50. M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). AP4 and AP5: Positive controls

The same isolates were tested by the primer pairs P1/P7 - R16F2n/R16R2 - U5/U3, respectively. The gel results obtained by using U5/U3 nested-PCR products revealed that isolate 30 couldn't be able to produce the same band profile as in the positive controls of AP at the levels of around 860 bp (Figure 4.29).

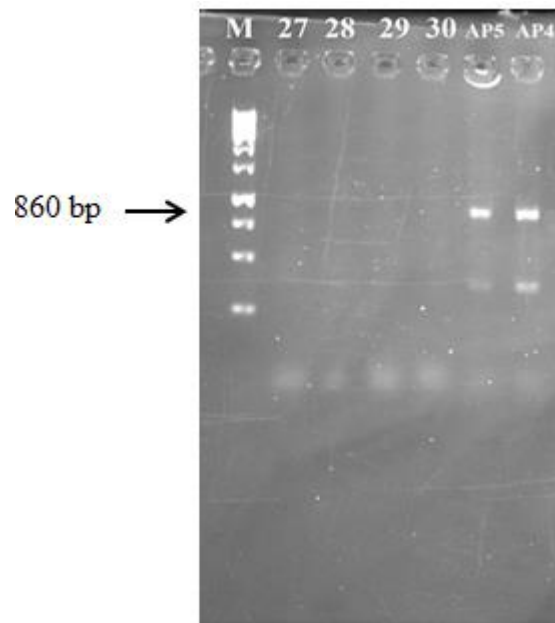


Figure 4.29. Gel images of nested-PCR products with U5/U3 primer pairs at the ratios of 1:50. M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). AP5 and AP4: Positive controls

Consequently, 56 samples that were selected for the PCR assays based on their clear symptoms were tested by the primer pairs P1/P7 - F1/B6 - M1/M2, respectively (Figure 4.30). According to the gel results obtained by using M1/M2 nested-PCR products, some isolates indicated in Figure 4.30a produced the expected size of the fragment lengths (509 bp).

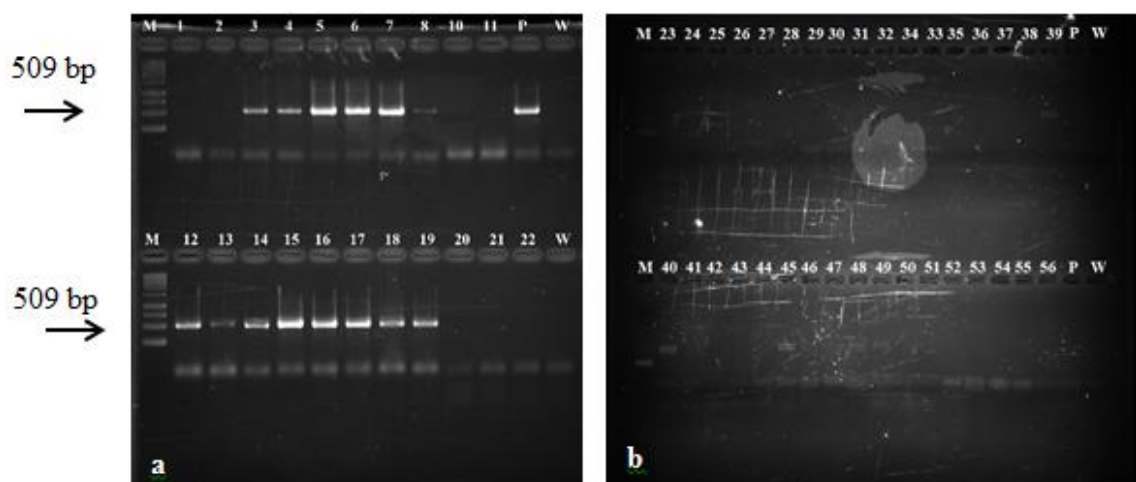


Figure 4.30. Gel images of nested-PCR products with M1/M2 primer pairs at the ratios of 1:50 (a, b). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). P: Positive control (AP3). W: Water control

When 56 samples were tested with P1/P7 - F1/B6 - M1/M2 primers, the isolates (3, 4, 5, 6, 7, 8, 12, 13, 14, 15, 16, 17, 18, and 19) gave expected positive bands (Figure 4.30a). For this reason, the isolates chosen randomly among those amplified isolates (4, 5, 6, 7, 15, 16, and 19) were tested with the primer pairs P1/P7 - F1/B6 - R16F2n/R16R2. However, no tested isolates produced the expected size of the fragment lengths (1200 bp) (Figure 4.31).

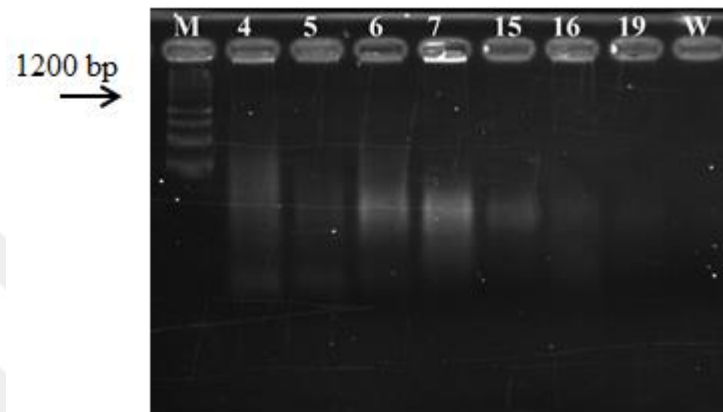


Figure 4.31. Gel images of nested-PCR products with R16F2n/R16R2 primer pairs at the ratios of 1:50 (a, b). M; GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific). W: Water control

CHAPTER V

DISCUSSION

During surveys conducted in different common bean-producing areas of Niğde province, it was determined that viruses generally showed the symptoms of mosaic, mottling, chlorotic and necrotic lesions, vein banding, leaf distortion, dwarfing, yellowing, and reduced size of internodes in common bean plants. When the symptomatic common bean leaves were screened for potyvirus infection by PTA-ELISA, 132 (58%) samples out of 228 have been found to be infected with potyvirus. According to the infection rates of collected bean samples obtained monthly, higher infections have been acquired towards the end of August. The analyzed bean leaves had an overall infection rate of 58%, whereas the overall infection rate of the visited bean fields had 78%. It was concluded that the samples collected in August and September were almost all positive in terms of potyviruses. Our data showed that the higher infection rate on bean plants in these months could be attributable to the increased aphid population which transmits the potyviruses. Because the increase in temperature gives rise to the development of insects in a shorter time with accelerated physiology and the increase in the number of offspring (generation) per year by causing an increase in reproductive capacity. For example; for aphids (*Hemiptera: Aphididae*) with a temperature increase of 2°C, it is estimated that the number of offspring per year will be 4 or 5 more (Harrington et al., 2001).

In addition, global climate change impacts insect populations by causing water stress in plants and causing differences in plant quality, both of which influence insect reproductive and feeding habits. As temperatures rise, plant leaves may begin to yellow, which could lead to pests becoming more specialized in the selection of their host and ultimately expanding their host range. As a result, a wider variety of hosts presents an opportunity to enrich and spread insect-feeding behavior over great distances (Mattson and Haack, 1987).

For the detection of potyvirus, the importance of rapid, accurate, reliable, and affordable methods such as ELISA and PCR is becoming more apparent since these viruses spread to new geographic areas and are thus considered potential epidemic pathogens

worldwide (Inoue-Nagata et al. 2002; Fuji et al. 2003). When 60 ELISA-positive samples involved in RT-PCR reactions depending on their distinctive symptoms were tested for potyviruses with the species-specific primer pairs, Fifty-four samples out of 60 were found to be infected with BCMV. BCMV and BCMNV are the most prevalent viruses in common bean areas worldwide (Galvez and Morales, 1989; Gilbertson et al., 2001). Both *Aphididae* vectors and infected seeds can transmit and spread these potyviruses (Galvez and Morales, 1989; Spence and Walkey, 1995). These viruses pose a potential threat to Türkiye as well as to Niğde province, which is a major producer of common beans in the country. BCMV was detected to be the most prevalent virus in common bean-producing areas of Türkiye with high infection rates among all other discovered viruses such as AMV, BCMNV, BYMV, and CMV in previous studies. Similarly, the results of RT-PCR tests in this study showed that BCMV was the most prominent virus in common bean-growing areas of Niğde province. In the previous study conducted by Arli-Sokmen et al. (2016), the results of ELISA testing for nine bean seed samples obtained from Niğde province revealed a high prevalence of BCMV with a 44% of infection rate in the area.

Additionally, when the selected 60 ELISA-positive samples were tested for BYMV with gene-specific primers in RT-PCR reactions, only one sample gave the expected size of a fragment around 830 bp. After sequencing was accomplished for this isolate, the provided 980 nt length sequence of BYMV isolate was subjected to BLAST analysis, and the provided sequence showed 89% identity to a BYMV isolate with 100% query coverage (MN399729) from *Pisum sativum* L. in Germany deposited in NCBI. The provided BYMV isolate was further characterized for a genetic relationship using Tamura-Nei method by MEGA-X software. Phylogenetic analysis of the BYMV isolate showed that this isolate was clustered in the same group with the BYMV isolate that was deposited in GenBank (MN399729) supported by bootstrap scores of a thousand (Figure 4.15). The Spread of BYMV is facilitated by the contaminated seeds and the virus can be transported over great distances, and this is a situation in which the presence of viral infection would be difficult, if not impossible, to detect visually. Sequence comparisons show that the isolate found in Niğde shares a lot in common with those found in Germany. This was the first study in obtaining the first partial sequence data of BYMV isolate in Türkiye.

All of these viruses can do substantial damage to plants in the presence of aphid vectors on bean plants, which is one of the most essential elements for the propagation of the virus in the field (Spence and Walkey, 1995). Key determinants in the severity of viral disease in beans include the susceptibility of the bean cultivar, the plant development stage at which infection occurs, environmental parameters, and the effect of these factors on vector activity (Sutic et al., 1999).

The six potyvirus-infected samples (144, 152, 162, 184, 186, and 198) that were tested for BCMV and determined to be negative were not found to be infected with the viruses included in this study, except isolate 186 which was infected with BYMV. Based on the results, the negativity of the samples tested by PTA-ELISA and RT-PCR might be because the virus titre could be very low in the plant samples or the primers used in the tests were not appropriate for these isolates. Accordingly, BYMV forward primer was designed according to the sequence data in this study and the sequences of GeneBank isolates, as a degenerate primer. In addition to this, except for BCMV and BYMV, no other viruses were detected among the samples tested in the study.

When ten bean cultivars included in the virus inoculation study (Adabeyazı, Akbulut, Akman-98, Borsa, Canipek, Cihan, Ergen, Güngör, Sururbey, and Zirve) were evaluated for any potyvirus infection transmitted through seed by PTA-ELISA, only the cv. Güngör was found to be a seed-transmitted potyvirus. Besides, during screening common bean cultivars by the virus sap-inoculation study performed by BCMV-positive isolate, only the cvs. Güngör and Akbulut displayed typical symptoms such as mosaic and leaf rolling on the trifoliate leaves. Also, these cultivars showed the same symptoms in the second virus inoculation study. These distinctive symptoms agreed with the findings of the study by Kapil et al. (2011) and Sharma et al. (2016). Udayashankar et al. (2012) reported similar symptoms such as mosaic, vein clearing, and vein banding on the primary leaves followed by mosaic and leaf curling on the trifoliate leaves. Kılıç et al. (2020) stated that the characteristic symptoms associated with BCMV such as mosaic, deformations, leaf tortion, and stunting were observed on inoculated common bean test plants. Moreover, these two cultivars were found to be positive at the third and four week's ELISA. Based on phenotypic evaluations and ELISA-based screening, the cvs. Güngör and Akbulut were evaluated to be susceptible to BCMV. Even though the other eight common bean cultivars showed particular

symptoms, they were found to be ELISA-negative in terms of potyvirus. Hence, they were evaluated to be resistant to BCMV.

As a result of the molecular detection of resistance genes, the SCAR marker ROC11 showed the presence of the recessive *bc-3* gene in all common bean cultivars tested, CAPS marker eIFE4 indicated the absence of *bc-3* gene. For the dominant *I* gene, PCR testing using the SW-13 marker revealed a positive result in the cultivars Akman-98, Akın, Batallı, Canıpek, Cihan, Göynük-98, Gündoğan, Nirvana, Noyanbey-98, Karbeyazı, and Sururbey with a 690 fragment band. According to these results, Haley et al. (1994); Melotto et al. (1996); Pasev et al. (2014) obtained the same size of the band fragments. Besides, when Deligöz et al. (2022) evaluated the dominant *I* gene with SW-13 marker for cvs. Akman-98, Göynük-98, and Noyanbey-98, it was understood that their results were positive for those cultivars, revealing that our results were completely in line with the findings of their study. Nevertheless, even though the dominant *I* gene was obtained for cv. Akman-98 with SW-13 marker in this study, the virus-like symptoms did not probably belong to BCMV since it gave negative results in PTA-ELISA tests. In addition to this, the negative result obtained with SW-13 primer for the cv. Güngör in this study showed that this cultivar has the recessive *ii* and may contain *bc-1*² gene. The findings for the cv. Güngör were consistent with those obtained by Deligöz et al. (2022). However, dry bean cultivars, Arslan and Zirve, are responsible for our disputed findings. Analysis of phenotypic observations and molecular marker (SW-13) used to screen for resistance genes in the present study indicated that the dominant *I* gene was absent in cvs. Arslan and Zirve. When Yeken et al. (2018) tested these cultivars with the same marker, they identified the dominant *I* gene in both cultivars. Considering the results of PTA-ELISA and molecular marker analyses, the dominant *I* gene was obtained only in cvs. Akman-98, Canıpek, Cihan, and Sururbey among the tested ten common bean cultivars used in virus inoculation studies (Adabeyazı, Akbulut, Akman-98, Borsa, Canıpek, Cihan, Ergan, Güngör, Sururbey, and Zirve). Therefore, those cultivars should be tested with different *bc-1*² and *bc-3* gene markers and also inoculated with the NL-4 strain of BCMV belonging to PG VIII and the NL-3 strain of BCMNV belonging to PG VI in future studies.

Besides, it was shown with SBD marker that the cvs. Adayakut, Akın, Batallı, Cihan, Göynük-98, Gündoğan, Güngör, Karbeyazı, Nirvana, Noyanbey-98, Sururbey, Topçu,

and Zirve may contain the recessive *bc-1²* gene. In our country, a study using SW-13 and SBD-15 markers revealed the presence of the *I* and *bc-1²* genes in five bean cultivars and one breeding line with the help of the multiplex PCR method. According to the result of this study, the product with a size of 690 bp was obtained with the SW-13 marker in the cultivars Özeren Şeker, 4F-3260, and Zülbiye (Deligöz and Sökmen, 2013). Moreover, the existence of the *I* and *bc-1²* genes was evaluated in 58 different bean genotypes, including both registered green bean cultivars and dry bean cultivars, as well as local genotypes and breeding lines (Deligöz et al., 2022). Though only three genotypes were found to involve the *bc-1²* gene based on phenotypic testing, 44 genotypes were found to have *bc-1²* with the SBD-5 marker. As revealed by Yeken et al. (2018) and Palacioglu et al. (2020), the SBD-5 marker yielded highly positive results when tested by polymerase chain reaction. Deligöz et al. (2021); Miklas et al. (2000); Pasev et al. (2014); Strausbaugh et al. (2003) all found considerable inconsistencies between SBD-5 marker results and phenotypic data. Possible explanations include variations in the genetic background of the genotypes involved. Phenotypic assays are thus required to validate molecular analysis findings. For this reason, those dry bean cultivars should be inoculated with the NL-4 strain of BCMV and NL-3 strain of BCMNV, involving all pathogenicity genes so as to validate the results of the molecular analysis with SBD-5 marker.

The recessive *bc-3* gene was attained by ROC11 marker, whereas this gene was not obtained with eIFE4 marker in all common bean cultivars used in this study, even though the amplified products obtained with eIFE4 marker were digested with *RsaI* enzyme. Besides, the presence of the recessive *bc-1²* gene shows a false positive result in the susceptible host group cultivar (Stringless Green Refugee), since the reliability of SBD-5 marker is not only sufficient to comment on the presence of *bc-1²* gene in the tested cultivars. Additionally, the presence of the recessive *bc-3* gene in the host group cultivar and the bean cultivars tested in this study shows a false positive result as well.

In the last part of the study, phytoplasma diseases were investigated on collected common bean samples from different growing areas of Niğde province. Since phytoplasmas are not uniformly distributed and are only found in low concentrations in the phloem tissues of infected hosts, a highly sensitive detection technology is even more crucial. Thus, the classification of the phytoplasmas is accomplished based on the

nucleotide sequence and RFLP of the 16S rRNA gene which is a conserved region in all prokaryotes. PCR amplification of 16S rDNA of phytoplasmas is a more accurate and reliable method in the detection of phytoplasma diseases. Nested-PCR assay, which is more specific in the detection of phytoplasmas, is done by reamplifying the DNA fragments obtained from the direct-PCR using group-specific primer pairs (Lee et al., 1994; 1995).

In Türkiye, the first detection of phytoplasma diseases was started in 2002 by using molecular methods (PCR-RFLP), DAPI (DNA staining with 4'-6- diamidino-2-phenylindole), and electron microscope (TEM) in the East Mediterranean Region of Türkiye (Sertkaya et al., 2005). Later on, the studies towards the detection of phytoplasma diseases and their vectors have gained importance in especially stone and pome fruits (Sertkaya et al., 2004; Çağlayan et al., 2004; Ulubaş Serçe et al., 2006; 2011). Concurrently, studies on the detection of phytoplasmas were expanded throughout the country. So far, no studies have been conducted in the detection of phytoplasma diseases of common beans in Türkiye. As far as our knowledge, this is the first study in the attempt at molecular identification of phytoplasma diseases in Türkiye. However, although collected bean samples were tested for phytoplasmas using a different kind of group-specific primers to increase the accuracy of the tests, no phytoplasma was detected in tested common bean samples. This might be because the phytoplasma occurs in a low titer in their hosts or we didn't catch the diseases due to seasonal fluctuations.

CHAPTER VI

CONCLUSION AND FUTURE ASPECTS

As a result of this study, significant data was obtained about the detection of potyviruses and phytoplasmas on collected common bean samples using appropriate serological and molecular methods, and the reactions of different common bean cultivars to BCMV were determined in this study. Seeing how the high temperatures and highest aphid populations occurred in August and September, it was determined that this time of year would be ideal for collecting common bean samples.

Considering the virus inoculation studies, some of the common bean cultivars induced the same symptoms against BCMV isolate that were similar to those previously documented for BCMV. Ten common bean cultivars (Adabeyazı, Akbulut, Akman-98, Borsa, Canipek, Cihan, Ergan, Güngör, Sururbey, and Zirve) included in the virus inoculation studies were first evaluated for their seed-transmission of the potyvirus by PTA-ELISA, only the seeds of one cultivar (Güngör) were found to be infected by a seed-transmitted potyvirus or potyviruses. Besides, two cultivars (Güngör and Akbulut) were determined to be susceptible to BCMV in the two virus inoculation studies, while eight cultivars (Adabeyazı, Akman-98, Borsa, Canipek, Cihan, Ergan, Sururbey, and Zirve) were resistant. Even though these eight cultivars were displaying some typical symptoms including mosaic symptoms, chlorotic and necrotic lesions, leaf curling, and vein clearing, they were found to be negative in PTA-ELISA. Thus, the presence of potyvirus infection is not inferred from these symptoms.

It was determined that Akman-98, Canipek, Cihan, and Sururbey used in the study had the dominant *I* gene. Dominant *I* gene provides resistance against NL-1, US-7, US-5, US-2, and NL-4 strains of BCMV at all temperatures, and NL-6, NL-2, and RU-1 strains of BCMV above 30°C. The findings of this study are important in terms of gaining the conserved *I* gene resistance in future breeding studies on these cultivars.

These results suggest that there could be resistance genes in other dry bean populations and registered bean cultivars in Türkiye. For this reason, it is necessary to investigate whether there are genes providing resistance to BCMV in commercial bean cultivars

and local bean populations registered in our country with a more comprehensive study. Also, pathogenicity groups of BCMV isolates identified in Niğde province and used in phenotypic evaluations need to be determined in future studies.

Moreover, although the collected common bean samples were tested for phytoplasmas using different kinds of group-specific primers to increase the accuracy of the tests, no phytoplasma was detected in the tested common bean samples. Phytoplasmas could be present in low concentrations in their hosts, or our timing of sampling may have been out of the critical detection period due to seasonal changes.

In Türkiye, there are limited studies on the determination of viruses in beans by molecular methods. When the results obtained from PTA-ELISA and RT-PCR tests are evaluated, the detection of potyviruses in this study provides useful information to bean breeders in Türkiye and around the world to seek virus-resistant bean cultivars and increase crop yield. Aside from these accomplishments, the study opens up new possibilities for the investigation of virus and phytoplasma diseases for researchers throughout the world. Furthermore, more research is needed to establish the epidemiology of common viral diseases, which will aid in the development of management approaches.

REFERENCES

Açıkgöz, S., Erzincan ve Erzurum yörelerinde *Phaseolus vulgaris* üzerinde virüslerin tanınması, yayılışları ve zararları üzerinde araştırmalar, **Doktora Tezi, Atatürk Üniversitesi Ziraat Fakültesi**, Erzurum, 75, 1984.

Ahrens, U. and Seemüller, R.E., “Detection of DNA of plant pathogenic mycoplasma-like organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene”, **Phytopathology**, 82, 828-832, 1992.

Akhtar, K. P., Shah, T. M., Atta, B. M., Dickinson, M., Jamil, F. F., Haq, M. A. and Iqbal, M. J., “Natural occurrence of phytoplasma associated with chickpea phyllody disease in Pakistan-a new record”, **Plant Pathology**, 57(4), 771, 2008.

Akhtar, K. P., Dickinson, M., Asghar, M. J., Abbas, G. and Sarwar, N., “Association of 16SrII-C phytoplasma with lentil phyllody disease in Pakistan”, **Tropical Plant Pathology**, 41(3), 198-202, 2016.

Albrechtsen, S. E., “Testing methods for seed-transmitted viruses: principles and protocols”, **CABI**, 2006.

Ali, M. A., “Genetics of resistance to the common bean mosaic virus (bean virus 1) in the bean (*Phaseolus vulgaris* L.)”, **Phytopathology**, 40(1), 1950.

Al-Saady, N. A., Khan, A. J., Calari, A., Al-Subhi, A. M. and Bertaccini, A., “*Candidatus* Phytoplasma omanense, associated with witches'-broom of *Cassia italica* (Mill.) Spreng. in Oman”, **International Journal of Systematic and Evolutionary Microbiology**, 58(2), 461-466, 2008.

Angioi, S. A., Rau, D., Attene, G., Nanni, L., Bellucci, E., Logozzo, G. and Papa, R., “Beans in Europe: origin and structure of the European landraces of *Phaseolus vulgaris* L”, **Theoretical and Applied Genetics**, 121(5), 829-843, 2010.

Anonymous., “Crop production statistics in 2021”, Turkey Statistical Institute, <https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1>, 18 December 2021.

Anonymous., “The major total common bean producers in the world”, Food and Agriculture Organization of the United Nations, <http://www.fao.org/faostat/en/#home>, 20 December 2021.

Anonymous., “Potyvirus genome organization”, ViralZone, https://viralzone.expasy.org/50?outline=all_by_species, 20 December 2022.

Ariyaratne, H. M., Coyne, D. P., Jung, G., Skroch, P. W., Vidaver, A. K., Steadman, J. R. and Bassett, M. J., “Molecular mapping of disease resistance genes for halo blight, common bacterial blight, and bean common mosaic virus in a segregating population of common bean”, *Journal of the American Society for Horticultural Science*, 124(6), 654-662, 1999.

Arli-Sökmen, M., Deligöz, I. and Kutluk-Yılmaz, N.D., “Türkiye’de Bean common mosaic virus (BCMV) ve Bean common mosaic necrosis virus izolatlarının streyn düzeyinde ayrımı ve BCMV’nin yeni nekrotik izolatlarının belirlenmesi”, *Türkiye IV. Bitki Koruma Kongresi*, 54, 28-30, 2011.

Arli-Sokmen, M., Deligoz, I. and Kutluk-Yilmaz, N. D., “Characterization of Bean common mosaic virus and Bean common mosaic necrosis virus isolates in common bean growing areas in Turkey”, *European Journal of Plant Pathology*, 146(1), 1-16, 2016.

Arocha, Y., Lopez, M., Pinol, B., Fernandez, M., Picornell, B., Almeida, R. and Jones, P., “*Candidatus* Phytoplasma graminis and *Candidatus* Phytoplasma caricae, two novel phytoplasmas associated with diseases of sugarcane, weeds and papaya in Cuba”, *International Journal of Systematic and Evolutionary Microbiology*, 55(6), 2451-2463, 2005.

Arocha, Y., Piñol, B., Picornell, B., Almeida, R. and Jones, P., “Broad bean and sweet pepper: two new hosts associated with *Candidatus* phytoplasma asteris (16SrI phytoplasma group) in Cuba”, ***Plant Pathology***, 56(2), 2007.

Beaver, J. S., Rosas, J. C., Myers, J., Acosta, J., Kelly, J. D., Nchimbi-Msolla, S. and Coyne, D. P., “Contributions of the Bean/Cowpea CRSP to cultivar and germplasm development in common bean”, ***Field Crops Research***, 82(2-3), 87-102, 2003.

Beebe, S. E., Rao, I. M., Mukankusi, C. M. and Buruchara, R. A., “Improving resource use efficiency and reducing risk of common bean production in Africa”, ***Latin America, and the Caribbean***, 2012.

Bellucci, E., Bitocchi, E., Rau, D., Rodriguez, M., Biagetti, E., Giardini, A. and Papa, R., “Genomics of origin, domestication and evolution of *Phaseolus vulgaris*”, ***Genomics of plant genetic resources***, 483-507, 2014a.

Berger, P. H., Wyatt, S. D., Shiel, P. J., Silbernagel, M. J., Druffel, K. and Mink, G. I., “Phylogenetic analysis of the Potyviridae with emphasis on legume-infecting potyviruses”, ***Archives of Virology***, 142(10), 1979-1999, 1997.

Bertaccini, A., and Martini, M., “Ribosomal and non-ribosomal primers for sensitive detection and identification of phytoplasmas [plant diseases]”, ***Petria (Italy)***, 1999.

Bertaccini, A., “Phytoplasmas: diversity, taxonomy, and epidemiology”, ***Frontiers in Bioscience-Landmark***, 12(2), 673-689, 2007.

Bertaccini, A., and Duduk, B., “Phytoplasma and phytoplasma diseases: a review of recent research”, ***Phytopathologia mediterranea***, 48(3), 355-378, 2009.

Bertaccini, A., Duduk, B., Paltrinieri, S. and Contaldo, N., “Phytoplasmas and phytoplasma diseases: a severe threat to agriculture”, ***American Journal of Plant Sciences***, 2014.

Bitocchi, E., Nanni, L., Bellucci, E., Rossi, M., Giardini, A., Zeuli, P. S. and Papa, R., “Mesoamerican origin of the common bean (*Phaseolus vulgaris* L.) is revealed by sequence data”, ***Proceedings of the National Academy of Sciences***, 109(14), E788-E796, 2012.

Bourguet, D., and Guillemaud, T., “The hidden and external costs of pesticide use”, ***Sustainable agriculture reviews***, 35-120, 2016.

Brack, J., “Leafhopper and planthopper vectors of plant disease agents in central and southern Europe”, ***Leafhopper vectors and plant disease agents***, 97-154, 1979.

Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J. and Watson, L., “Viruses of plants”, ***CAB International***, Wallingford, UK, 1484pp, 1996.

Cabrera Mederos, D., Bejerman, N., Trucco, V., De Breuil, S., Lenardon, S. and Giolitti, F., “Complete genome sequence of sunflower ring blotch virus, a new potyvirus infecting sunflower in Argentina”, ***Archives of virology***, 162(6), 1787-1790, 2017.

Castano, M., and Morales, F. J., “Seed transmission of soybean mosaic virus in *Phaseolus vulgaris* L”, ***Fitopatologia Brasileira***, 8(1), 103-107, 1983.

Chew, M. Y. I., Velásquez, V. R., Mena, C. J. and Gaytán, M. A., “Virus de frijol en la Comarca Lagunera y Zacatecas”, ***Folleto Técnico Campo Experimental Zacatecas CIRNOC-INIFAP, Zacatecas***, 41, 2010.

Choi, S. K., Choi, J. K., Park, W. M. and Ryu, K. H., “RT-PCR detection and identification of three species of cucumoviruses with a genus-specific single pair of primers”, ***Journal of Virological Methods***, 83(1-2), 67-73, 1999.

Collins, M. B., Ndong’a Millicent, M. B. and Karakacha, W. H., “Evaluation of common bean cultivars resistance to the bean common mosaic necrosis virus in Western Kenya”, ***International Journal of Genetics and Genomics***, 7(4), 115, 2019.

Cordova, I., Jones, P., Harrison, N. A. and Oropeza, C., “In situ PCR detection of phytoplasma DNA in embryos from coconut palms with lethal yellowing disease”, **Molecular Plant Pathology**, 4(2), 99-108, 2003.

Coyne, D. P., Steadman, J. R., Godoy-Lutz, G., Gilbertson, R., Arnaud-Santana, E., Beaver, J. S. and Myers, J. R., “Contributions of the Bean/Cowpea CRSP to management of bean diseases”, **Field crops research**, 82(2-3), 155-168, 2003.

Crnov, R., and Gilbertson, R. L., “Outbreak of Clover yellow vein virus in a bean field in Colusa County, California”, **Plant Disease**, 85(4), 444-444, 2001.

Cvrkovic, T., Jovic, J., Mitrovic, M., Krstic, O. and Tosevski, I., “Experimental and molecular evidence of *R. eptalus panzeri* as a natural vector of bois noir”, **Plant Pathology**, 63(1), 42-53, 2014.

Çağlayan, K., Ulubaş Serçe, Ç., Gazel, M., Polat, A. and Voight, E., “Reaction of some of the Turkish plum and apricot cultivar to Plum pox virus”, **J. Turk. Phytopath**, 33(1-3), 19-24, 2004.

Davis, R. E., and Lee, I. M., “Cluster-specific polymerase chain reaction amplification of 16S rDNA sequences for detection and identification of mycoplasma-like organisms”, **Phytopathology (USA)**, 1993.

Debouck, D. G., Toro, O., Paredes, O. M., Johnson, W. C. and Gepts, P., “Genetic diversity and ecological distribution of *Phaseolus vulgaris* (Fabaceae) in northwestern South America”, **Economic botany**, 47(4), 408-423, 1993.

Deligöz, I., and Arlı Sökmen, M., “Bazı fasulye genotiplerinin Bean common mosaic virus (BCMV) ve Bean common mosaic necrosis virus (BCMNV)’a dayanıklılık durumlarının kalitatif, kantitatif ve moleküler yöntemlerle belirlenmesi”, **Bitki Koruma Bülteni**, 53(2), 101-113, 2013.

Deligöz, İ., Sevcan, S.A.R.I. and Karaağaç, O., “Karadeniz Tarımsal Araştırma Enstitüsü tarafından geliştirilen taze fasulye ıslah hatları ve bazı ticari çeşitlerin Bean

common mosaic virus (BCMV)’a dayanıklılık durumlarının araştırılması”, *Derim*, 32(1), 1-10, 2015.

Deligoz, I., Arlı-Sökmen, M. and Tekeoglu, M., “Phenotypic and molecular screening of dry bean (*Phaseolus vulgaris* L.) breeding lines for resistance to bean common mosaic virus and bean common mosaic necrosis virus”, *Acta Scientiarum Polonorum Hortorum Cultus*, 20(6), 7-18, 2021.

Deligöz, İ., Sokmen, M., Yılmaz, N. K., Özçelik, H. and Tekeoğlu, M., “Screening of snap and dry bean (*Phaseolus vulgaris* L.) genotypes for resistance to Bean common mosaic virus and Bean common mosaic necrosis virus”, *Plant Protection Bulletin*, 62(4), 5-13, 2022.

Deng, S., and Hiruki, C., “Amplification of 16S rRNA genes from culturable and nonculturable mollicutes”, *Journal of microbiological methods*, 14(1), 53-61, 1991.

Dickson, M. H., and Natti, J.J., “Inheritance of resistance of *Phaseolus vulgaris* to bean yellow mosaic virus”, *Phytopathology*, 58(1450), 846, 1968.

Dietzgen, R. G., Callaghan, B., Higgins, C. M., Birch, R. G., Chen, K. and Xu, Z., “Differentiation of peanut seedborne potyviruses and cucumoviruses by RT-PCR”, *Plant disease*, 85(9), 989-992, 2001.

Doyle, J.J., and Doyle, J.L., “Isolation of plant DNA from fresh tissue”, *Focus*, 12(13), 39-40, 1990.

Drijfhout, E., “Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance”, *Wageningen University and Research*, 1978.

Eastop, V. F., “The biology of the principal aphid virus vectors”. In “*Plant Virus Epidemiology*” (RT Plumb and JM Thresh, eds.), 1983.

Feng, X., Orellana, G. E., Myers, J. R. and Karasev, A. V., “Recessive resistance to Bean common mosaic virus conferred by the *bc-1* and *bc-2* genes in common bean (*Phaseolus vulgaris*) affects long-distance movement of the virus”, *Phytopathology*, 108(8), 1011-1018, 2018.

Fidan, Ü., and Yorgancı, Ü., “Investigation on the detection and seed transmission of the virus diseases occurring on pulse crops in Aegean Region. 2. Seed transmission of virus diseases by grower seeds and seeds of artificially infected pulse crops”, *Journal of Turkish Phytopathology*, 19(1), 1-5, 1990.

Fisher, M. L., and Kyle, M. M., “Inheritance of resistance to potyviruses in *Phaseolus vulgaris* L. III. Cosegregation of phenotypically similar dominant responses to nine potyviruses”, *Theoretical and Applied Genetics*, 89(7), 818-823, 1994.

Flores-Estévez, N., Acosta-Gallegos, J. A. and Silva-Rosales, L., “Bean common mosaic virus and Bean common mosaic necrosis virus in Mexico”, *Plant disease*, 87(1), 21-25, 2003.

Fuji, S., Yamamoto, H., Furuya, H. and Naito, H., “Characterization of a new potyvirus isolated from Chinese artichoke in Japan”, *Archives of virology*, 148(11), 2249-2255, 2003.

Galvez, G. E., and Morales, F. J., “Whitefly-transmitted viruses”, *Bean production problems in the tropics*, 2, 379-406, 1989.

Gepts, P., and Debouck, D., “Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris* L.)”, *Common beans: Research for crop improvement*, 7-53, 1991.

Gepts, P., “*Phaseolus vulgaris* (beans)”, *Encyclopedia of genetics*, 1444, 1445, 2001.

Ghorbani, S. G. M., Shahraeena, N. and Elahinia, S. A., “Distribution and impact of virus associated diseases of common bean (*Phaseolus vulgaris* L.) in northern Iran”, *Archives of Phytopathology and Plant Protection*, 43(12), 1183-1189, 2010.

Gibb, K. S., Padovan, A. C. and Mogen, B. D., “Studies on sweet potato little-leaf phytoplasma detected in sweet potato and other plant species growing in Northern Australia”, *Phytopathology*, 85(2), 169-174, 1995.

Gibbs, A. J., Hajizadeh, M., Ohshima, K. and Jones, R. A., “The potyviruses: an evolutionary synthesis is emerging”, *Viruses*, 12(2), 132, 2020.

Gilbertson, R. L., Guzman, P., Rojas, M., Crnov, R. and Mkandawire, A., “Detection of bean-infecting viruses in California with an emphasis on the CRSP-facilitated work”, *Bean/Cowpea Collaborative Research Support Program in East Africa Proceedings: January*, 12, 14, 2001.

Gillaspie A.G., Pio-Ribeiro J.G., Andrade G.P. and Pappu H.R., “RT-PCR detection of seedborne Cotypea aphid-borne mosaic virus in peanut”, *Plant Disease* 85, 1181, 2001.

Gioia, T., Logozzo, G., Attene, G., Bellucci, E., Benedettelli, S., Negri, V. and Spagnoletti Zeuli, P., “Evidence for introduction bottleneck and extensive inter-gene pool (Mesoamerica x Andes) hybridization in the European common bean (*Phaseolus vulgaris* L.) germplasm”, *PLoS One*, 8(10), e75974, 2013.

Graham, P. H., and Ranalli, P., “Common bean (*Phaseolus vulgaris* L.)”, *Field Crops Research*, 53(1-3), 131-146, 1997.

Grylls, N. E., “Leafhopper vectors and the plant disease agents they transmit in Australia”, 1979.

Gundersen, D. E., and Lee, I. M., “Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs”, *Phytopathologia mediterranea*, 144-151, 1996.

Gümüő, M., Erkan, S., Yorgancı, Ü. ve Duman, I., “Bazı sebzelerin tohumlarında bulunan viral etmenlerin saptanması üzerine araőtırmalar”, *Türkiye IX. Fitopatoloji Kongresi*, 190(197), 3-8, 2001.

Güzel, Ö., and Arlı-Sökmen, M., “Determination of some viruses infecting common bean (*Phaseolus vulgaris* L.) and their incidences in seed lots in Samsun Province”, *Journal of Turkish Phytopathology*, 32(2), 99-106, 2003.

Hall, R., “Compendium of bean diseases”, *St Paul (MN): APS Press Publishers*, 102p, 1991.

Hall, R., “Bean diseases, bean pathogens, bean disease control”, *Compendium of Bean Diseases*, 1994.

Hampton, R. O., “The nature of bean yield reduction by bean yellow and bean common mosaic virus”, *Phytopathology*, 65(12), 1342-1346, 1975.

Harrington, R., Fleming, R. A. and Woiwod, I. P., “Climate change impacts on insect management and conservation in temperate regions: can they be predicted?”, *Agricultural and Forest Entomology*, 3(4), 233-240, 2001.

Hart, J. P., and Griffiths, P. D., “A series of eIF4E alleles at the bc-3 locus are associated with recessive resistance to Clover yellow vein virus in common bean”, *Theoretical and applied genetics*, 126(11), 2849-2863, 2013.

Hart, J. P., and Griffiths, P. D., “Resistance to Clover yellow vein virus in common bean germplasm”. *Crop Science*, 54(6), 2609-2618, 2014.

Hart, J. P., and Griffiths, P. D., “Genotyping-by-sequencing enabled mapping and marker development for the by-2 potyvirus resistance allele in common bean”. *The plant genome*, 8(1), plantgenome2014-09, 2015.

Hosseini, A., and Hosseini, S., “Occurrence and distribution of Bean common mosaic virus and Bean yellow mosaic virus from common bean fields of Kerman province, Iran”, *Indian Journal of Fundamental and Applied Life Sciences*, 4(2), 528-535, 2014.

Inoue-Nagata, A. K., Fonseca, M. E. N., Resende, R. O., Boiteux, L. S., Monte, D. C., Dusi, A. N. and Van der Vlugt, R. A. A., “Pepper yellow mosaic virus, a new potyvirus in sweetpepper, *Capsicum annuum*”, ***Archives of virology***, 147(4), 849-855, 2002.

IRPCM., “*Candidatus* Phytoplasma, a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects”, ***International Journal of Systematic and Evolutionary Microbiology***, 54(Pt 4), 1243-1255, 2004.

Johnson, W. C., Guzmán, P., Mandala, D., Mkandawire, A. B., Temple, S., Gilbertson, R. L. and Gepts, P., “Molecular tagging of the bc-3 gene for introgression into Andean common bean”, ***Crop Science***, 37(1), 248-254, 1997.

Jomantiene, R., Davis, R. E., Maas, J. and Dally, E. L., “Classification of new phytoplasmas associated with diseases of strawberry in Florida, based on analysis of 16S rRNA and ribosomal protein gene operon sequences”, ***International Journal of Systematic and Evolutionary Microbiology***, 48(1), 269-277, 1998.

Jones, R. A., and Naidu, R. A., “Global dimensions of plant virus diseases: Current status and future perspectives”, ***Annual review of virology***, 6, 387-409, 2019.

Kami, J., Velásquez, V. B., Debouck, D. G. and Gepts, P., “Identification of presumed ancestral DNA sequences of phaseolin in *Phaseolus vulgaris*”. ***Proceedings of the National Academy of Sciences***, 92(4), 1101-1104, 1995.

Kanat, Z., Kuru Fasulye Ürün Raporu 2022, ***Tarımsal Ekonomi ve Politika Geliştirme Enstitüsü Müdürlüğü***, Türkiye, 2022.

Kapil, R., Sharma, P., Sharma, S. K., Sharma, O. P., Sharma, O. P., Dhar, J. B. and Sharma, P. N., “Pathogenic and molecular variability in Bean common mosaic virus infecting common bean in India”, ***Archives of Phytopathology and Plant Protection***, 44(11), 1081-1092, 2011.

Kaplan, L., “Archeology and domestication in American *Phaseolus* (beans)”, ***Economic Botany***, 19(4), 358-368, 1965.

Kelly, J. D., Afanador, L. and Haley, S. D., “Pyramiding genes for resistance to bean common mosaic virus”, *Euphytica*, 82, 207-212, 1995.

Kılıç, H. Ç., ve Yardımcı, N., “Burdur Çine Ovası fasulye alanlarında hıyar mozaik virüsü”, *Mehmet Akif Ersoy Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 3(2), 12-15, 2012.

Kılıç, H. Ç., Yardımcı, N. ve Ürgen, G., “Muğla ili Fethiye ilçesinde fasulye alanlarında önemli bazı virüs hastalıklarının araştırılması”, *Uludağ Üniversitesi Ziraat Fakültesi Dergisi*, 27(1), 1-8, 2013.

Kılıç, H. Ç., ve Yardımcı, N., “Burdur ili fasulye üretim alanlarında fasulye adi mozayik virüsü’nün serolojik ve moleküler yöntemlerle belirlenmesi”, *Türk Tarım ve Doğa Bilimleri Dergisi*, 1(2), 289-294, 2014.

Kılıç, H. Ç., Yardımcı, N., Açıkyürek, S. and Uzal, A., “Detection of BCMV, AMV and CMV by DAS-ELISA and immunocapture-RT-PCR in bean-growing areas in the West Mediterranean Region, Turkey”, *Feb-Editorial Board*, 1752, 2015.

Kılıç, H. Ç., Hesna, K. Ö. K. and Yardımcı, N., “Bean common mosaic virus and bean common mosaic necrosis virus infections in bean production areas in The Lakes Region of Turkey”, *Avrupa Bilim ve Teknoloji Dergisi*, (19), 386-392, 2020.

Kumar, C. A., Khetarpal, R. K., Parakh, D. B., Singh, S. and Nath, R., “Check list on seed transmitted viruses: Leguminous hosts”, *National Bureau of Plant Genetic Resources, New Delhi*, 110012, 1994.

Kumar, S., Singh, V. and Lakhanpaul, S., “A'Candidatus Phytoplasma asteris' isolate associated with bud proliferation disease of cowpea in India”, *New Disease Reports*, 25(1), 28-28, 2012.

Kutluk Yılmaz, N. D., Gümüş, M. ve Erkan, S., “Tokat ilinde fasulye tohumlarındaki viral etmenlerin saptanması üzerinde araştırmalar”, *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 39(3), 49-55, 2002.

Küzeci, U., Dağdemir, V. and Demir, O., “Türkiye’de kuru fasulye piyasasının ekonomik analizi ve pazarlama marjları”, *ÇOMÜ Ziraat Fakültesi Dergisi*, 7(2), 379-386, 2019.

Kyle, M. M., Dickson, M. H. and Provvidenti, R., “Linkage analysis of hypersensitive resistance to four viruses in *Phaseolus vulgaris* L”, 1986.

Kyle, M. M., and Provvidenti, R., “Inheritance of resistance to potato Y viruses in *Phaseolus vulgaris* L”, *Theoretical and Applied Genetics*, 74(5), 595-600, 1987.

Kyle, M. M., and Dickson, M. H., “Linkage of hypersensitivity to five viruses with the B locus in *Phaseolus vulgaris* L”, *Journal of Heredity*, 79(4), 308-311, 1988.

Kyle, M. M., and Provvidenti, R., “Inheritance of resistance to potyviruses in *Phaseolus vulgaris* L. II. Linkage relations and utility of a dominant gene for lethal systemic necrosis to soybean mosaic virus”, *Theoretical and applied genetics*, 86(2), 189-196, 1993.

Larsen, R. C., Miklas, P. N., Eastwell, K. C. and Grau, C. R., “A strain of Clover yellow vein virus that causes severe pod necrosis disease in snap bean”, *Plant disease*, 92(7), 1026-1032, 2008.

Larsen, R. C., and Miklas, P. N., “Mapping resistance to Peanut mottle virus in common bean”, *Annu. Rep. Bean Improv. Coop*, 53, 50-51, 2010.

Lee I. M. and Davis R. E., “Mycoplasmas which infect plants and insects. In Mycoplasmas”, *Molecular Biology and Pathogenesis*, 379-390, 1992.

Lee, I. M., Gundersen, D. E., Hammond, R. W. and Davis, R. E., “Use of mycoplasma-like organism (MLO) group-specific oligonucleotide primers for nested-PCR assays to detect mixed-MLO infections in a single host plant”, *Phytopathology*, 84(6), 559-566, 1994.

Lee, I. M., Bertaccini, A., Vibio, M. and Gundersen, D. E., “Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy”, ***Phytopathology***, 85(6), 728-735, 1995.

Lee, I. M., Gundersen-Rindal, D. E., Davis, R. E. and Bartoszyk, I. M., “Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences”, ***International Journal of Systematic and Evolutionary Microbiology***, 48(4), 1153-1169, 1998.

Lee, I. M., Davis, R. E. and Gundersen-Rindal, D. E., “Phytoplasma: phytopathogenic mollicutes”, ***Annual Reviews in Microbiology***, 54(1), 221-255, 2000.

Lee, M. E., Grau, C. R., Lukaesko, L. A. and Lee, I. M., “Identification of aster yellows phytoplasmas in soybean in Wisconsin based on RFLP analysis of PCR-amplified products (16S rDNAs)”, ***Canadian Journal of Plant Pathology***, 24(2), 125-130, 2002.

Lee, I. M., Bottner, K. D., Miklas, P. N. and Pastor-Corrales, M. A., “Clover proliferation group (16SrVI) subgroup A (16SrVI-A) phytoplasma is a probable causal agent of dry bean phyllody disease in Washington”, 2004.

Li, R. H., Zettler, F. W., Elliott, M. S., Petersen, M. A., Still, P. E., Baker, C. A. and Mink, G. I., “A strain of peanut mottle virus seedborne in bambarra groundnut”, ***Plant Dis***, 75, 130-133, 1991.

Lisa, V., Dellavalle, G., Vaira, A. M. and Morales, F. J., “Bean yellow mosaic and other viruses in bean (*Phaseolus vulgaris*) in western Asia, south-eastern Europe and northern China”, ***Bean Improvement Cooperative (USA)***, 1994.

Liu, J., Gopurenko, D., Fletcher, M. J., Johnson, A. C. and Gurr, G. M., “Phytoplasmas—the “crouching tiger” threat of Australian plant pathology”, ***Frontiers in Plant Science***, 8, 599, 2017.

Loebenstein, G., and Carr, J. P., “*Natural resistance mechanisms of plants to viruses*”, ***Springer Science & Business Media***, 2007.

Loebenstein, G., and Thottappilly, G., “Virus and virus-like diseases of major crops in developing countries”, **Springer Science & Business Media**, 2013.

Lorenz, K. H., Schneider, B., Ahrens, U. and Seemüller, E., “Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA”, **Phytopathology**, 85(7), 771-776, 1995.

Maejima, K., Oshima, K., and Namba, S., “Exploring the phytoplasmas, plant pathogenic bacteria”, **Journal of General Plant Pathology**, 80(3), 210-221, 2014.

Mangen, B. C., “Distribution and pathogenic characterization of Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) in western Kenya”, 2014.

Manjunatha, L., Basavaraja, T., Manjunatha, N., Srinivasa, N., Kumar, V., Chandora, R. and Singh, N. P., “Identification of stable resistant donors against necrosis inducing strain of bean common mosaic virus in common bean (*Phaseolus vulgaris* L.)”, **Archives of Phytopathology and Plant Protection**, 54(15-16), 1033-1046, 2021.

Marcone, C., “Molecular biology and pathogenicity of phytoplasmas”, **Annals of applied Biology**, 165(2), 199-221, 2014.

Mattson, W. J., and Haack, R. A., “The role of drought in outbreaks of plant-eating insects”, **Bioscience**, 37(2), 110-118, 1987.

McKern, N. M., Mink, G. I., Barnett, O. W., Mishra, A., Whittaker, L. A., Silbernagel, M. J. and Shukla, D. D., “Isolates of bean common mosaic virus comprising two distinct potyviruses”, **Phytopathology**, 82(9), 923-929, 1992.

Melotto, M., Afanador, L. and Kelly, J. D., “Development of a SCAR marker linked to the I gene in common bean”, **Genome**, 39(6), 1216-1219, 1996.

Meziadi, C., Richard, M. M., Derquennes, A., Thareau, V., Blanchet, S., Gratias, A. and Geffroy, V., “Development of molecular markers linked to disease resistance genes in common bean based on whole genome sequence”, ***Plant Science***, 242, 351-357, 2016.

Meziadi, C., Blanchet, S., Geffroy, V. and Pflieger, S., “Genetic resistance against viruses in *Phaseolus vulgaris* L.: State of the art and future prospects”, ***Plant Science***, 265, 39-50, 2017.

Miklas, P. N., Larsen, R. C., Riley, R. and Kelly, J. D., “Potential marker-assisted selection for bc-1 2 resistance to bean common mosaic potyvirus in common bean”, ***Euphytica***, 116(3), 211-219, 2000.

Miklas, P. N., Kelly, J. D., Beebe, S. E. and Blair, M. W., “Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding”, ***Euphytica***, 147(1), 105-131, 2006.

Mitrovic, M., Cvrkovic, T., Jovic, J., Krstic, O., Jakovljevic, M., Kosovac, A. and Tosevski, I., “First Report of ‘Candidatus Phytoplasma solani’ Infecting Garden Bean *Phaseolus vulgaris* in Serbia”, ***Plant Disease***, 99(4), 551-551, 2015.

Montano, H. G., Davis, R. E., Dally, E. L., Hogenhout, S., Pimentel, J. P. and Brioso, P. S., “*Candidatus* Phytoplasma brasiliense, a new phytoplasma taxon associated with hibiscus witches' broom disease”, ***International Journal of Systematic and Evolutionary Microbiology***, 51(3), 1109-1118, 2001.

Morales, F. J., “Common bean. In *Virus and virus-like diseases of major crops in developing countries*”, ***Springer***, 425-445, 2003.

Moreira, L., Villalobos, W., Saborío-R, G., Garita, L., Castro-Robleda, S., Romero-Cano, J. and Rivera, C., “A phytoplasma associated with 'amachamiento' disease of dry common bean in Costa Rica”, ***Plant Pathology***, 59(2), 2010.

Mukeshimana, G., Paneda, A., Rodríguez-Suárez, C., Ferreira, J. J., Giraldez, R. and Kelly, J. D., “Markers linked to the bc-3 gene conditioning resistance to bean common mosaic potyviruses in common bean”, *Euphytica*, 144, 291-299, 2005.

Mwaipopo, B., Nchimbi-Msolla, S., Njau, P., Tairo, F., William, M., Binagwa, P. and Mbanzibwa, D., “Viruses infecting common bean (*Phaseolus vulgaris* L.) in Tanzania: A review on molecular characterization, detection and disease management options”, *African Journal of Agricultural Research*, 12(18), 2017.

Mwaipopo, B., Nchimbi-Msolla, S., Njau, P. J., Mark, D. and Mbanzibwa, D. R., “Comprehensive surveys of Bean common mosaic virus and Bean common mosaic necrosis virus and molecular evidence for occurrence of other *Phaseolus vulgaris* viruses in Tanzania”, *Plant disease*, 102(11), 2361-2370, 2018.

Myers, J. R., and Kmiecik, K., “Common bean: Economic importance and relevance to biological science research. In *The common bean genome*”, *Springer*, 1-20, 2017.

Naderpour, M., Lund, O. S., Larsen, R. and Johansen, E., “Potyviral resistance derived from cultivars of *Phaseolus vulgaris* carrying bc-3 is associated with the homozygotic presence of a mutated eIF4E allele”, *Molecular plant pathology*, 11(2), 255-263, 2010.

Naidu, R. A., and Hughes, J. D. A., “Methods for the detection of plant virus diseases”, *Plant Virology in Sub Saharan Africa*, 233-253, 2003.

Neimark, H., and Kirkpatrick, B. C., “Isolation and characterization of full-length chromosomes from non-culturable plant-pathogenic *Mycoplasma*-like organisms”, *Molecular microbiology*, 7(1), 21-28, 1993.

Nielson, M. W., “Taxonomic relationships of leafhopper vectors of plant pathogens”, *US Agric Res Ser Reprints*, 1979.

Nyvall, R. F., “Field crop diseases handbook”, *Springer Science and Business Media*, 1989.

Okada, R., Yong, C. K., Valverde, R. A., Sabanadzovic, S., Aoki, N., Hotate, S. and Fukuhara, T., “Molecular characterization of two evolutionarily distinct endornaviruses co-infecting common bean (*Phaseolus vulgaris*)”, ***Journal of General Virology***, 94(1), 220-229, 2013.

Omar, A. F., “Detection and molecular characterization of phytoplasmas associated with vegetable and alfalfa crops in Qassim region”, ***Journal of Plant Interactions***, 12(1), 58-66, 2017.

Padovan, A. C., Gibb, K. S., Bertaccini, A., Vibio, M., Bonfiglioli, R. E., Magarey, P. A. and Sears, B. B., “Molecular detection of the Australian grapevine yellows phytoplasma and comparison with grapevine yellows phytoplasmas from Italy”, ***Australian Journal of Grape and Wine Research***, 1(1), 25-31, 1995.

Palacioğlu, G., Şanlı, İ., Bayraktar, H. and Özer, G., “Determination of resistance sources to BCMV and BCMNV in some common bean (*Phaseolus vulgaris* L.) cultivars grown in Turkey”, ***Uluslararası Tarım ve Yaban Hayati Bilimleri Dergisi***, 6(3), 453-460, 2020.

Pallavi, M. S., Ramappa, H. K., Shankarappa, K. S., Rangaswamy, K. T., Wickramaarachchi, W. A. R. T. and Maruthi, M. N., “Detection and molecular characterization of phytoplasma associated with chickpea phyllody disease in south India”, ***Phytoparasitica***, 40(3), 279-286, 2012.

Parizad, S., Dizadji, A., Habibi, M. K., Winter, S., Kalantari, S., García-Arenal, F. and Ayllón, M. A., “Prevalence of saffron latent virus (SaLV), a new Potyvirus species, in saffron fields of Iran”, ***Journal of Plant Pathology***, 99(3), 2017.

Park, S. J., and Tu, J. C., “Inheritance and allelism of resistance to a severe strain of Bean yellow mosaic virus in common bean”, ***Canadian Journal of plant Pathology***, 13(1), 7-10, 1991.

Pasev, G., Kostova, D. and Sofkova, S., “Identification of genes for resistance to Bean common mosaic virus and Bean common mosaic necrosis virus in snap bean (*Phaseolus*

vulgaris L.) breeding lines using conventional and molecular methods”, *Journal of Phytopathology*, 162(1), 19-25, 2014.

Petrovic, S., Osborne, M. and Lavrenko, V., “Streaming first story detection with application to twitter”, *In Human language technologies: The 2010 annual conference of the north american chapter of the association for computational linguistics*, pp. 181-189, 2010.

Provvidenti, R., and Schroeder, W. T., “Resistance in *Phaseolus vulgaris* to the severe strain of bean yellow mosaic virus”, *Phytopathology*, 63(1), 196-197, 1973.

Provvidenti, R., “Inheritance of resistance to watermelon mosaic virus 2 in *Phaseolus vulgaris*”, *Phytopathology*, 64(11), 1448-1450, 1974.

Provvidenti, R., Gonsalves, D. and Ranalll, P., “Inheritance of resistance to soybean mosaic virus in *Phaseolus vulgaris*”, *Journal of Heredity*, 73(4), 302-303, 1982.

Provvidenti, R., Gonsalves, D. and Taiwo, M. A., “Inheritance of resistance to blackeye cowpea mosaic and cowpea aphid-borne mosaic viruses in *Phaseolus vulgaris*”, *Journal of Heredity*, 74(1), 60-61, 1983.

Provvidenti, R., and Chirco, E. M., “Inheritance of resistance to peanut mottle virus in *Phaseolus vulgaris*”, *Journal of Heredity*, 78(6), 402-403, 1987.

Purcifull, D. E., Hiebert, E., Petersen, M. and Webb, S., “Virus detection–serology”, *Encyclopedia of Plant Pathology*, 2, 1100-1109, 2001.

Raccah, B., Huet, H. and Blanc, S., “Potyviruses. In Virus-insect-plant interactions”, *Academic Press*, pp. 181-206, 2001.

Raj, S. K., Khan, M. S., Snehi, S. K., Srivastava, S. and Singh, H. B., “*Candidatus* Phytoplasma asteris' isolate associated with a little leaf disease of pigeon pea in India”, *Plant Pathology*, 55(6), 2006.

Rao, G., Mall, S., Raj, S. and Snehi, S., “Phytoplasma diseases affecting various plant species in India”, *Acta Phytopathologica et Entomologica Hungarica*, 46(1), 59-99, 2011.

Rao, G. P., and Kumar, M., “World status of phytoplasma diseases associated with eggplant”, *Crop Protection*, 96, 22-29, 2017.

Rao, G. P., Thorat, V., Manimekalai, R., Tiwari, A. K. and Yadav, A., “A century progress of research on phytoplasma diseases in India”, *Phytopathogenic Mollicutes*, 7(1), 1-38, 2017.

Rao, G. P., Madhupriya, T. V., Manimekalai, R., Tiwari, A. K. and Yadav, A., “A century progress of research on phytoplasma diseases in India”, *Phytopathogenic Mollicutes*, 7(1), 1-38, 2017c.

Reyes-Moreno, C., Paredes-López, O. and Gonzalez, E., “Hard-to-cook phenomenon in common beans—A review”, *Critical Reviews in Food Science & Nutrition*, 33(3), 227-286, 1993.

Riechmann, J. L., Lain, S. and García, J. A., “Highlights and prospects of potyvirus molecular biology”, *Journal of General Virology*, 73(1), 1-16, 1992.

Roossinck, M. J., “Plant virus metagenomics: biodiversity and ecology”, *Annual review of genetics*, 46, 359-369, 2012.

Rubiales, D., Fondevilla, S., Chen, W., Gentzbittel, L., Higgins, T. J., Castillejo, M. A. and Rispail, N., “Achievements and challenges in legume breeding for pest and disease resistance”, *Critical Reviews in Plant Sciences*, 34(1-3), 195-236, 2015.

Santalla, M., Rodiño, A. and De Ron, A., “Allozyme evidence supporting southwestern Europe as a secondary center of genetic diversity for the common bean”, *Theoretical and applied genetics*, 104(6), 934-944, 2002.

Saraçoğlu, K., ve Erkan, S., “Fasulye tohumlarındaki viral etmenlerin saptanmasında tanı yöntemlerinin duyarlılıklarının incelenmesi”, *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 53(3), 309-315, 2016.

Schwartz, H. F., and Corrales, M. A. P., “Bean production problems in the tropics”, *CIAT*, 1989.

Schwartz, H. F., Steadman, J. R., Hall, R. and Forster, R. L., “Compendium of bean diseases (No. Ed. 2)”, *American Phytopathological Society*, 2005.

Seemüller, E., Marcone, C., Lauer, U., Ragozzino, A. and Göschl, M., “Current status of molecular classification of the phytoplasmas”, *Journal of plant pathology*, 3-26, 1998.

Sen Gupta, P. K., “Diseases of major pulse crops in India”, *PANS Pest Articles & News Summaries*, 20(4), 409-415, 1974.

Sengooba, T. N., Spence, N. J., Walkey, D. G. A., Allen, D. J. and Femi Lana, A., “The occurrence of bean common mosaic necrosis virus in wild and forage legumes in Uganda”, *Plant Pathology*, 46(1), 95-103, 1997.

Sertkaya, G., Martini, M., Ermacora, P., Musetti, R. and Osler, R., “Detection and characterization of phytoplasmas in diseased stone fruits and pear by PCR-RFLP analysis in Turkey”, *Phytoparasitica*, 33(4), 380-390, 2005.

Sertkaya, G., Osler, R., Musetti, R., Ermacora, P. and Martini, M., “Detection of phytoplasmas in *Rubus* spp. by microscopy and molecular techniques in Turkey”, *In X International Symposium on Small Fruit Virus Diseases*, 656, 181-186, 2004.

Sharma, A., Sharma, V., Sharma, P., Sharma, S. K. and Sharma, P. N., “Molecular characterization and genome organization of temperature insensitive necrosis inducing strain of BCMV infecting common bean in India”, *Australasian Plant Pathology*, 45(2), 219-227, 2016.

Sharma, P. N., Sharma, V., Sharma, A., Rajput, K. and Sharma, S. K., “Identification and molecular characterization of Bean yellow mosaic virus infecting French bean in Himachal Pradesh”, ***Virusdisease***, 26(4), 315-318, 2015.

Sharma, A., Sharma, S., Khan, I., Kaur, S. and Kaur, S. I., “Comparative study on detection efficacy of universal potyviruses primers”, ***Vegetos***, 34(4), 745-749, 2021.

Singh, S. P., and Schwartz, H. F., “Breeding common bean for resistance to diseases: a review”, ***Crop Science***, 50(6), 2199-2223, 2010.

Smart, C. D., Schneider, B., Blomquist, C. L., Guerra, L. J., Harrison, N. A., Ahrens, U. and Kirkpatrick, B. C., “Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region”, ***Applied and environmental microbiology***, 62(8), 2988-2993, 1996.

Sorel, M., Svanella-Dumas, L., Candresse, T., Acelin, G., Pitarch, A., Houvenaghel, M. C. and German-Retana, S., “Key mutations in the cylindrical inclusion involved in Lettuce mosaic virus adaptation to eIF4E-mediated resistance in lettuce”, ***Molecular Plant-Microbe Interactions***, 27(9), 1014-1024, 2014.

Spence, N. J., and Walkey, D. G. A., “Variation for pathogenicity among isolates of bean common mosaic virus in Africa and a reinterpretation of the genetic relationship between cultivars of *Phaseolus vulgaris* and pathotypes of BCMV”, ***Plant Pathology***, 44(3), 527-546, 1995.

Strausbaugh, C. A., Myers, J. R., Forster, R. L. and McClean, P. E., “bc-1 and bc-u—two loci controlling bean common mosaic virus resistance in common bean are linked”, ***Journal of the American Society for Horticultural Science***, 124(6), 644-648, 1999.

Strausbaugh, C. A., Miklas, P. N., Singh, S. P., Myers, J. R. and Forster, R. L., “Genetic characterization of differential reactions among host group 3 common bean cultivars to NL-3 K strain of Bean common mosaic necrosis virus”, ***Phytopathology***, 93(6), 683-690, 2003a.

Sutic, D. D., Ford, R. E. and Tomic, M. T., "Handbook of plant virus diseases", **CRC Press**, 1999.

Tamura, K., and Nei, M., "Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees", **Molecular biology and evolution**, 10(3), 512-526, 1993.

Thompson, J. D., Higgins, D. G. and Gibson, T. J., "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice", **Nucleic acids research**, 22(22), 4673-4680, 1994.

Thorat, V., Bhale, U., Sawant, V., More, V., Jadhav, P., Mane, S. S. and Yadav, A., "Alternative weed hosts harbors 16SrII group phytoplasmas associated with little leaf and witches' broom diseases of various crops in India", **Phytopathogenic Mollicutes**, 6(1), 50-55, 2016.

Trajkova, A., and Khristova, D., "Diagnostics of Bean common mosaic virus in common bean (*Phaseolus vulgaris* L.) seeds", **Plant Science (Bulgaria)**, 2008.

Udayashankar, A. C., Nayaka, S. C., Niranjana, S. R., Mortensen, C. N. and Prakash, H. S., "Immunocapture RT-PCR detection of Bean common mosaic virus and strain blackeye cowpea mosaic in common bean and black gram in India", **Archives Of Phytopathology And Plant Protection**, 45(13), 1509-1518, 2012.

Ulubaş Serçe, Ç., Gazel, M. ve Çağlayan, K., "Plum pox virus streynlerinin Türkiye'deki dağılımı (Distribution of Plum pox virus strains in Turkey)", **Türkiye IV. Bitki Koruma Kongresi Bildirileri**, Kahramanmaraş, 72, 2011.

Ulubaş Serçe, Ç., Gazel, M., Çağlayan, K., Baş, M. and Son, L., "Phytoplasma diseases of fruit trees in germplasm and commercial orchards in Turkey", **Journal of Plant Pathology**, 179-185, 2006.

Ulum, M., Çulal Kılıç, H. ve Yardımcı, N., “Burdur ilinde yetiştirilen fasulye bitkilerinde Bean Yellow Mosaic Virus (BYMV)'ünün araştırılması”, **Journal of Natural & Applied Sciences**, 24(1), 2020.

Valouzi, H., Golnaraghi, A., Hashemi, S. S., Abedini-Aminabad, L. and Yazdani-Khameneh, S., “Detection of potyviruses infecting Iranian common beans using broad-spectrum antibodies and universal primer pairs”, **Canadian Journal of Plant Pathology**, 41(4), 535-543, 2019.

Walkey, D. G. A., Innes, N. L. and Miller, A., “Resistance to bean yellow mosaic virus in *Phaseolus vulgaris*”, **The Journal of Agricultural Science**, 100(3), 643-650, 1983.

Wang, R. Y., and Ghabrial, S. A., “Effect of aphid behavior on efficiency of transmission of Soybean mosaic virus by the soybean-colonizing aphid, *Aphis glycines*”, **Plant Disease**, 86(11), 1260-1264, 2002.

Wortmann, C. S., “Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa (No. 297)”, **CIAT**, 1998.

Yeken, M. Z., Göksel, Ö. Z. E. R., Çelik, A. ve Çiftçi, V., “Türkiye’de ticari fasulye (*Phaseolus vulgaris* L.) çeşitlerinde bean common mosaic virus ve bean common mosaic necrosis virus etmenlerine dayanıklılıkla ilişkili genlerin karakterizasyonu”, **Türk Tarım ve Doğa Bilimleri Dergisi**, 5(4), 613-619, 2018.

Yılmaz, M. A., and Özaslan, D., “Fasulye ve börülce tohumlarında afıt kökenli mozayik virüsünün ELISA ile saptanması”, Çukurova Üniversitesi, Ziraat Fakültesi, **Bitki Koruma Bölümü**, 870, 873, 1987.

Zamora, L., Acosta, K. and Martínez, Y., “First report of ‘Candidatus Phytoplasma asteris’(16SrI group) affecting common bean in Cuba”, **New Disease Reports**, 25(4), 2044-0588, 2012.

Zheng, L., Rodoni, B. C., Gibbs, M. J. and Gibbs, A. J., “A novel pair of universal primers for the detection of potyviruses”, **Plant pathology**, 59(2), 211-220, 2010.

Zwolińska, A., Krawczyk, K. and Pospieszny, H., “Molecular characterization of stolbur phytoplasma associated with pea plants in Poland”, ***Journal of Phytopathology***, 160(7-8), 317-323, 2012.



APPENDICES

Appendix 1. Symptoms that occurred on common bean cultivars (Adabeyazı, Akbulut, Akman-98, Borsa, Canıpek, Cihan, Ergan, Güngör, Sururbey, and Zirve) after the first inoculation with BCMV

Cultivar Name	Replication	BCMV	
		Symptom	
		IL	EP
Güngör	GN1	Vc	Lc
	GN2	Vc	Lc
	GN3	-	Vc
	GN4	Vc	Vc
	GN5	-	Lc
	GN6	Vc, Lc	Sm, Vc
	GN7	Vc	Sm, Lc
	GN8	Vc	Lc, Vc
	Control	Sm	-
Akman-98	AB1	Vc	Sm
	AB2	-	Sm
	AB4	-	Vc
	AB5	-	Sm, Vc
	AB6	-	Vc
	AB7	-	Sm
	AB10	-	Vc
	AB15	-	Vc
	Control	Sm	-
Cihan	CIA3	-	Sm, Lc
	CIA5	-	Vc
	CIA7	-	Sm, Lc
	CIA9	Vc	Sm, Lc
	CIA10	Vc, Nl	Lc
	CIA12	-	Mm
	CIA13	Vc	Lc
	CIA14	-	Lc
	Control	Mm, Lc	-
Ergan	ERB1	Cl, Nl	Lc
	ERB2	Vc	Lc
	ERB3	Nl, Vc	Lc
	ERB5	Vc	Lc, Vc
	ERB6	-	Lc, Vc
	ERB8	Vc	Lc
	ERB12	Vc	Mm
	ERB13	Vc	Lc
	Control	Mm, Lc	-
Akbulut	AKB2	-	Lc
	AKB3	-	Sm, Cl, Lc
	AKB5	-	Lc
	AKB6	-	Sm
	AKB7	-	Sm
	AKB11	-	Sm
	AKB14	-	Nl
	AKB15	Vc	Lc
	Control	-	-
Sururbey	SB2	-	Sm
	SB3	-	Vc
	SB4	Vc	Vc
	SB5	Vc	Vc
	SB7	-	Sm

Appendix 1. Continued

Cultivar Name	Replication	BCMV	
		Symptom	
		IL	EP
Sururbey	SB6	-	Sm
	SB10	-	Cl
	SB13		Sm
	Control	Sm	-
Canipek	CB1	-	Vc
	CB2	-	Sm
	CB4	-	Sm
	CB7	-	Vc
	CB12	Vc	Vc
	CB13	-	Vc
	CB14	-	Cl, Sm
	CB15		Sm
	Control	Cl	-
Adabeyazı	ADB4	Vc	Vc
	ADB6	-	Lc
	ADB7	-	Lc
	ADB9	-	Vc
	ADB11	-	Lc
	ADB12	-	Lc
	ADB13	-	Lc, Vc
	ADB15	Vc	Lc
	Control	Vc	-
Zirve	ZB2	-	-
	ZB3	Vc	Vc
	ZB4		Vc
	ZB5	-	Nl, Vc
	ZB6	-	Vc
	ZB8	-	Lc, Vc
	ZB13	-	Lc, Vc
	ZB15		Lc, Vc
	Control	Vc	-
Borsa	BRB1	Vc	Lc
	BRB3	-	Lc
	BRB6	Nl	Lc
	BRB7	Vc	Lc
	BRB8	-	Lc
	BRB9	-	Lc
	BRB10	-	Vc, Cl
	BRB12	-	Lc
	Control	Cl	-

*IL: Inoculated leaves, EP: Entire plant, Cl: Chlorotic lesion, Lc: Leaf curling, Mm: Mild mosaic, Nl: Necrotic lesion, Sm: Systemic mosaic, Vc: Vein clearing

Appendix 2. Symptoms that occurred on common bean cultivars (Adabeyazı, Akbulut, Akman-98, Borsa, Canıpek, Cihan, Ergan, Güngör, Sururbey, and Zirve) after the second inoculation with BCMV

Cultivar Name	Replication	BCMV	
		Symptom	
		IL	EP
Güngör	GN1	Vc	Sm, Lc
	GN2	Vc	Sm, Lc
	GN3	Vc	Vc
	GN4	Vc	Vc
	GN5	-	Lc
	Control	Sm	-
Akman-98	AB1	Vc	Sm
	AB2	-	Sm
	AB3	-	Sm
	AB4	-	Sm, Vc
	Control	Sm	-
Cihan	CIA1	-	Sm, Lc
	CIA2	-	Mm, Vc
	CIA3	-	Lc
	CIA4	Vc	Sm, Lc
	Control	Sm, Lc	-
Ergan	ERB1	Cl, Nl	Sm, Lc
	ERB2	Vc	Mm, Lc
	ERB3	Nl, Vc	Lc
	ERB4	Vc	Lc, Vc
	Control	Sm, Lc	-
Akbulut	AKB1	-	Sm, Lc
	AKB2	-	Sm, Cl, Lc
	AKB3	-	Lc
	AKB4	-	Sm
	Control	-	-
Sururbey	SB1	-	Sm
	SB2	-	Sm
	SB3	-	Sm
	SB4	-	Sm
	Control	Sm	-
Canıpek	CB1	-	Sm
	CB2	-	-
	CB3	-	Sm
	CB4	-	Cl
	Control	Cl	-
Adabeyazı	ADB1	Vc	Lc, Vc
	ADB2	Vc	Sm, Vc
	ADB3	Vc	Lc, Vc
	ADB4	-	Vc
	Control	Vc	-
Zirve	ZB1	-	Vc
	ZB2	Vc	Sm, Vc
	ZB3	-	Vc
	ZB4	-	Vc
	Control	Vc	-
Borsa	BRB1	Vc	Sm, Vc
	BRB2	-	Vc
	BRB3	Vc	Cl, Sm
	BRB4	-	Sm
	Control	Cl	-

*IL: Inoculated leaves, EP: Entire plant, Cl: Chlorotic lesion, Lc: Leaf curling, Mm: Mild mosaic, Sm: Systemic mosaic, Vc: Vein clearing

Appendix 3. The first PTA-ELISA absorbance values of common bean cultivars (Adabeyazı, Akbulut, Akman-98, Borsa, Canıpek, Cihan, Ergan, Güngör, Sururbey, and Zirve) tested against BCMV by monoclonal antiserum

Cultivar Name	Replication	BCMV Isolate	Assessment
		*A405	
Güngör	GN1	0.178	Negative
	GN2	0.163	Negative
	GN3	0.159	Negative
	GN4	0.168	Negative
	GN5	0.173	Negative
	GN6	0.830	Positive
	GN7	0.439	Positive
	GN8	0.158	Negative
	Control	0.131	Negative
Akman-98	AB1	0.161	Negative
	AB2	0.154	Negative
	AB4	0.165	Negative
	AB5	0.166	Negative
	AB6	0.158	Negative
	AB7	0.175	Negative
	AB10	0.164	Negative
	AB15	0.157	Negative
	Control	0.118	Negative
Cihan	CIA3	0.133	Negative
	CIA5	0.148	Negative
	CIA7	0.138	Negative
	CIA9	0.142	Negative
	CIA10	0.136	Negative
	CIA12	0.139	Negative
	CIA13	0.147	Negative
	CIA14	0.145	Negative
	Control	0.121	Negative
Ergan	ERB1	0.158	Negative
	ERB2	0.170	Negative
	ERB3	0.166	Negative
	ERB5	0.164	Negative
	ERB6	0.171	Negative
	ERB8	0.158	Negative
	ERB12	0.165	Negative
	ERB13	0.161	Negative
	Control	0.117	Negative
Akbulut	AKB2	0.118	Negative
	AKB3	2.169	Positive
	AKB5	0.191	Negative
	AKB6	0.130	Negative
	AKB7	0.129	Negative
	AKB11	0.175	Negative
	AKB14	0.125	Negative
	AKB15	0.178	Negative
	Control	0.112	Negative
Sururbey	SB2	0.113	Negative
	SB3	0.128	Negative
	SB4	0.114	Negative
	SB5	0.124	Negative
	SB6	0.129	Negative
	SB7	0.123	Negative
	SB10	0.123	Negative

Appendix 3. Continued

Cultivar Name	Replication	BCMV Isolate	Assessment
		*A405	
Sururbey	SB13	0.118	Negative
	Control	0.209	Negative
Canipek	CB1	0.120	Negative
	CB2	0.128	Negative
	CB4	0.120	Negative
	CB7	0.133	Negative
	CB12	0.131	Negative
	CB13	0.122	Negative
	CB14	0.126	Negative
	CB15	0.123	Negative
	Control	0.125	Negative
Adabeyazı	ADB4	0.131	Negative
	ADB6	0.127	Negative
	ADB7	0.122	Negative
	ADB9	0.123	Negative
	ADB11	0.127	Negative
	ADB12	0.124	Negative
	ADB13	0.122	Negative
	ADB15	0.119	Negative
	Control	0.128	Negative
Zirve	ZB2	0.125	Negative
	ZB3	0.126	Negative
	ZB4	0.120	Negative
	ZB5	0.125	Negative
	ZB6	0.122	Negative
	ZB8	0.119	Negative
	ZB13	0.126	Negative
	ZB15	0.125	Negative
	Control	0.118	Negative
Borsa	BRB1	0.120	Negative
	BRB3	0.113	Negative
	BRB6	0.114	Negative
	BRB7	0.120	Negative
	BRB8	0.125	Negative
	BRB9	0.121	Negative
	BRB10	0.122	Negative
	BRB12	0.114	Negative
Stringless Green Refugee (HG1)	Control	0.106	Negative
	HG1-1	0.475	Negative
	HG1-2	0.468	Negative
	HG1-3	0.451	Negative
	HG1-4	0.444	Negative
	HG1-5	0.425	Negative
	HG1-6	0.450	Negative
	HG1-7	0.463	Negative
	HG1-8	0.442	Negative
	Control	0.460	Negative

*: Absorbance values were taken after 2 hrs of the addition of substrate.

Appendix 4. The PTA-ELISA absorbance values of common bean cultivars (Adabeyazı, Akbulut, Akman-98, Borsa, Canıpek, Cihan, Ergan, Güngör, Sururbey, and Zirve) from the second virus inoculation trial tested against BCMV by monoclonal antiserum

Cultivar Name	Replication	BCMV Isolate	Assessment
		*A405	
Güngör	GN1	2.987	Positive
	GN2	2.781	Positive
	GN3	0.968	Positive
	GN4	0.206	Negative
	Control	2.106	Positive
Akman-98	AB1	0.232	Negative
	AB2	0.218	Negative
	AB3	0.216	Negative
	AB4	0.214	Negative
	Control	0.212	Negative
Cihan	CIA1	0.198	Negative
	CIA2	0.211	Negative
	CIA3	0.190	Negative
	CIA4	0.212	Negative
	Control	0.196	Negative
Ergan	ERB1	0.209	Negative
	ERB2	0.208	Negative
	ERB3	0.209	Negative
	ERB4	0.197	Negative
	Control	0.201	Negative
Akbulut	AKB1	2.208	Positive
	AKB2	2.226	Positive
	AKB3	0.200	Negative
	AKB4	0.196	Negative
	Control	0.206	Negative
Sururbey	SB1	0.190	Negative
	SB2	0.194	Negative
	SB3	0.203	Negative
	SB4	0.196	Negative
	Control	0.200	Negative
Canıpek	CB1	0.211	Negative
	CB2	0.215	Negative
	CB3	0.213	Negative
	CB4	0.202	Negative
	Control	0.201	Negative
Adabeyazı	ADB1	0.198	Negative
	ADB2	0.196	Negative
	ADB3	0.203	Negative
	ADB4	0.206	Negative
	Control	0.205	Negative
Zirve	ZB1	0.205	Negative
	ZB2	0.214	Negative
	ZB3	0.209	Negative
	ZB4	0.211	Negative
	Control	0.207	Negative
Borsa	BRB1	0.206	Negative
	BRB2	0.192	Negative
	BRB3	0.210	Negative
	BRB4	0.204	Negative
	Control	0.213	Negative
Stringless Green Refugee (HG1)	HG1-1	0.209	Negative
	HG1-2	0.201	Negative
	HG1-3	0.210	Negative
	HG1-4	0.208	Negative
	Control	0.209	Negative

*: Absorbance values were taken after 2 hrs of the addition of substrate.

Appendix 5. The nucleotide sequence of the Isolate 186

> Isolate 186 nucleotide sequence:

ACTCAATGCAGAAGGAAAAGCACCATACATATCAGAGGTGGCTCTTAAGCA
TTTATACACTGAGGAGAAAAGTGACACCAGCAGAACTTGAACGGTATAATGT
GGCTTTAGTTGACAATGTTGAGCCTGAAGCCGATGAAGTGATATTGTGTTGC
TTTCAATCGGGCCAAGAAAAGCTTAATGCTGGGGAGGTTAAGAAGGAAAAA
GACAAGAAAGTGGAATCCACCTGGTGCTGAAACTGGGGAGAACAGCAA
TAGACAAATCATTCAAGACAAAGATGTGAACACAGGCACAGTTGGAACATT
TTCAGTTCCAAGACTTAAAAAGATAGCAGGGAAGCTTAATATCCCAAAGAT
TGGTGGAAAGATAGTCTTAAACTTGGATCATTTATTGGACTATAATCCGCCA
CAAGATGACATTTCAAATACCATTGCAACGCAGGAGCAGTTTGAAGCATGG
TACAATGGAGTGAAGCAGTCGTATGAAGTTGACGACTCACAAATGAGCATC
ATCCTGAATGGATTGATGGTGTGGTGCATTGAAAATGGAACCTTCAGGTGAC
CTGCAGGGTGAGTGGACCATGATGGATGGTGAGGAGCAGGTGACATATCCA
TTAAAACCCATTTTAGACAATGCAAAGCCAACATTTTCGCCAGATAATGTCAC
ATTTCTCACAAGTTGCAGAAGCTTACATTGAAAAGAGAAATGCGACTGAGA
GGTACATGCCTAGGTATGGAATTCAGAGGAACCTTAAGTACTATGGTTTGGC
TAGATATGCTTTTGATTTCTACAGGCTGACTTCAAAGACTCCTGTGCGTGCT
AGAGAAGCACATATGCAAATGAAGGCGGCAGCAATTAGAGGCAGGTCAAA
TCGATTATTTGGTCTTGATGGAAATGTTGGAACAGACGAGGAGAACACAGA
GAGACACACAGCAGAGATGTCAATCGTGATATGCATACCATGCTCGGTGTT
CGTATTTA

CURRICULUM VITAE

İrem KOÇ was born in . She received her high school degree from . She enrolled at Niğde Ömer Halisdemir University, Department of Plant Production and Technologies in September . She completed the second year of her undergraduate education at Wroclaw University of Environmental and Life Sciences, Poland under the Erasmus+ Program during 2017-2018. Also, she did her first internship in the laboratory of Molecular Plant Pathology at Udine University in Italy (2018). She did her second internship in the Department of Crop Development at Bayer Crop Science in Adana (2019). After getting her Bachelor's Degree, she registered Master's Degree at the Graduate School of Natural and Applied Sciences, Department of Plant Production and Technologies (English) at Niğde Ömer Halisdemir University under the supervision of Prof. Dr. Çiğdem ULUBAŞ SERÇE. She essentially focused on "Investigation of Potyvirus and Phytoplasma Diseases on Common Bean (*Phaseolus vulgaris* L.) Grown in Niğde Province" in her research works during her Master's Degree.

