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BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF BEAN COMMON MOSAIC NECROSIS VIRUS ISOLATES

Master's Thesis

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Supervisor

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ÖZET

FASULYE YAYGIN MOZAYİK NEKROZ VİRÜSÜ (BEAN COMMON MOSAIC NECROSIS VIRUS) İZOLATLARININ BİYOLOJİK VE MOLEKÜLER KARAKTERİZASYONU

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Bean common mosaic virus (BCMV) ve Bean common mosaic necrosis virus (BCMNV) baklagil bitkilerini enfekte eden önemli bitki virüsleridir. Her iki virüs türü *Potyviridae* familyası, *Potyvirus* cinsi üyesidir, ve tohumla ve yaprak biti türleri ile taşınma özelliğindedir. BCMNV, özellikle Afrika'da yaygın olup, *I* geni içeren fasulye bitkilerinde şiddetli tepe nekrozu sebebiyle önemli ürün kayıpları oluşturmaktadır. Dünyada şu ana kadar belirlenmiş olan BCMV ve BCMNV izolatları, sekiz patojenite grubuna (Patogrup; PG) ayrılmıştır. BCMNV ırkları PG III ve PG VI'da yer almıştır.

Bu çalışmanın ilk bölümü, BCMNV izolatlarının biyolojik olarak patogruplarının belirlenmesini ve kısmi Nuclear inclusion b (NIb) ve kılıf protein (CP) gen bölgelerinin moleküler karakterizasyonunu içermektedir. Öncelikle, BCMNV izolatlarının (NL-5-benzeri ve NWS) hassas fasulye çeşitlerine mekanik inokulasyon yöntemi ile bulaştırılarak, çoğaltımı sağlanmıştır. Daha sonra izolatların indikatör fasulye genotiplerinde oluşturdukları reaksiyonlara göre, patogrupları araştırılmıştır. Çalışmada, her iki izolatın PG VI' da yer aldığı, ancak NWS izolatının, NL-5-benzeri izolata göre daha virulent olduğu belirlenmiştir. Ayrıca, BCMNV izolatları ile enfekteli fasulye bitkilerinden dsRNA'lar izole edilmiş ve izolatların kısmi NIb ve CP gen bölgeleri, ters transkripsiyon-polimeraz zincir reaksiyonu (RT-PCR) ile çoğaltılmıştır. Takiben, sekans analizi ile PCR ürünlerinin nükleotid dizileri belirlenmiş ve BCMNV Türkiye izolatları ile diğer ülkelerde saptanan BCMNV izolatlarının genetik benzerlikleri BLASTn analizi ile incelenmiştir. NL-5-benzeri ve NWS izolatlarının, BCMNV'nin ABD orjinli NL-3 ve NL-5 ırklarına ait izolatlar ile sırasıyla %100 ve %99.04 benzerlik gösterdiği belirlenmiştir. Ayrıca, NWS ve NL-5-benzeri izolatların amino asit dizisi bakımından BCMNV NL-3-benzeri ırklar ile %100 benzerliğe sahip olduğu tespit edilmiştir.

Çalışmada, 20 ticari soya fasulyesi çeşidinin BCMNV NL-5-benzeri izolatına reaksiyonları araştırılmıştır. İnokule edilen soya çeşitlerinin DAS-ELISA ile test edilmesi sonucunda, Adasoy, Arısoy, Ataem, Atlas 3616, Atakişi, Blaze, Cinsoy, İlksoy, May-5451, Ohio, Samsoy ve SA-88 çeşitlerinin BCMNV'ye hassas olduğu belirlenmiştir. Çalışmada, Gapsoy-16, Mona, Nazlıcan, Prota Y, Safir, Soya Anam, Srebrina ve Umut 2002 çeşitleri ise dayanıklı bulunmuştur. BCMNV izolatlarının patogrup bilgileri ve dayanıklı olarak belirlenen soya çeşitleri, gelecekte virüs-dayanıklı yeni fasulye ve soya çeşitlerinin geliştirilmesi ile ilgili çalışmalarda kullanılabilir.

Anahtar Sözcükler: BCMNV, NIb, CP, Patogrup, DAS-ELISA, RT-PCR, Soya Fasulyesi

ABSTRACT

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF BEAN COMMON MOSAIC NECROSIS VIRUS ISOLATES

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Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) are major virus species infecting legumes. Both viruses are members of the *Potyvirus* genus of the *Potyviridae* family, and transmitted by aphids and seeds of the infected plants. BCMNV is especially prevalent in Africa, and causes significant yield losses due to severe top necrosis in the *I* gene-carrying common bean plants. Strains of BCMV and BCMNV are classified into eight pathogroups (PG). BCMNV strains identified so far have been assigned to PG III and PG VI.

The first part of this study involved the identification of pathogroups of BCMNV isolates biologically, and characterization of them molecularly in terms of the partial Nuclear Inclusion b (NIb) and coat protein (CP) gene-regions. First of all, BCMNV isolates, NL-5-like and NWS, were mechanically inoculated onto leaves of susceptible plants to propagate the virus. Then, according to the reactions of differential common bean hosts, the pathogroups of these isolates were investigated. Both NL-5-like and NWS were assigned to PG VI, but the NWS isolate was found to be more virulent than the NL-5-like isolate. Also, dsRNAs were isolated from bean plants infected with BCMNV isolates, and the partial NIb and CP regions of the isolates were amplified by reverse transcription-polymerase chain reaction (RT-PCR). Assessments were made after direct sequencing of PCR products, and the genetic identities of BCMNV isolates from Turkey and other parts of the world were analyzed by BLASTn. The results of comparisons showed that NL-5-like and NWS isolates had 100% and 99.04% identities with the isolates belonging to NL-3 and NL-5 strains (USA origin) of BCMNV, respectively. In addition, the amino acid sequences of NWS and NL-5-like isolates had 100% identity to those of NL-3-like strains of BCMNV.

In the second part of the study, twenty commercial soybean cultivars were screened for reactions to NL-5-like isolate of BCMNV. After mechanical inoculation and testing leaves of soybean cultivars by DAS-ELISA, Adasoy, Arisoy, Ataem, Atlas 3616, Atakişi, Blaze, Cinsoy, Ilksoy, May-5154, Samsoy, SA-88 and Ohio were found to be susceptible to BCMNV. However, Gapsoy-16, Mona, Nazlican, Prota Y, Safir, Soy Anam, Srebrina and Umut 2002 were determined to be resistant. The pathogroup information of BCMNV isolates and soybean cultivars determined to be resistant can be used to improve new virus-resistant bean and soybean cultivars in breeding studies in future.

Keywords: BCMNV, NIb, CP, Pathogroup, DAS-ELISA, RT-PCR, Soybean

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Abdul Razak AHMED

CONTENTS

SYMBOLS AND ABBREVIATIONS

BCMV	: Bean common mosaic virus
BCMNV	: Bean common mosaic necrosis virus
Bp	: Base pair
cDNA	: Complementary Deoxyribonucleic Acid
CP	: Coat Protein
cv.	: Cultivar
DAS-ELISA	: Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay
dsRNA	: Double-stranded Ribonucleic acid
HG	: Host Group
Kb	: Kilobase
ML	: Maximum Likelihood
NCBI	: National Centre for Biotechnology Information
nt	: Nucleotide
NWS	: New Strain
PG	: Pathogroup
Rpm	: Revolution per minute
RT-PCR	: Reverse Transcriptase-Polymerase chain reaction
TÜİK/TURKSTAT	: Türkiye İstatistik Kurumu / Turkish Statistical Institute
USDA-ARS	: United States Department of Agriculture-Agricultural Research Institute

FIGURE LEGENDS

TABLE LEGENDS

1. INTRODUCTION

Legumes are one of the most important food crops in the world. They make a significant contribution to the diet of mankind and animals. They are essential sources of carbohydrate, fibre, oil and essential food nutrients (calcium, magnesium, potassium, phosphorus and iron) coupled with various health benefits (Kamboj and Nanda, 2018). Close to 27% of total world crop cultivation is made up of legumes (Smýkal et al., 2020), and more than 2 billion of the world population consume legumes as part of their dietary requirements (Dogan, 2020). Between 2014 to 2019, soybean was the most consumed legume crop (346.2 million tons) followed by peanut with 62.2 million tons (Semba et al., 2021).

There are wide arrays of the plants in the leguminous group but as few as 20 of them, such as groundnut, soybean, cowpea, and common bean/ french bean, among others are being cultivated and consumed (Kumar and Pandey, 2020). The disparity in their nutrient compositions is one factor that accounts for the preference of some leguminous crops over others. For example, soybean has a protein composition of 35.142%, the common bean has 20.9-27.8% protein content, faba bean has 26.1-38% protein content, lentil and cowpea have 23-32% and 23.5% proteins, respectively (Kumar and Pandey, 2020). Concerning protein content, soybean is a good replacement for meat protein sources in comparison to common bean.

Ecologically, some countries have the ideal climate for the production of various leguminous crops whilst others are limited. In the year 2020, India ranked first in overall edible legume production in the world (Dogan, 2020). Additionally, America, some African and Asian countries are major producers of most legumes in the world. Also, India, Myanmar, the USA, Brazil, China, Mexico, Kenya, Rwanda are major dry bean producers (Sousa, 2019).

Common bean originated from Mesoamerica (currently Mexico) 4-6 million years ago (Nadeem et al., 2021). Different gene pools of common bean arose from Mexico, Central America and the Andean region of South America (Morales, 2008). However, gene pools from the Andean and Mesoamerica regions are considered the largest (Nadeem et al., 2021). Common bean is cultivated in subtropical and some tropic regions in the

world. Today, it is one of the staple foods in major African countries (South, East and West), South and Central America. Although soybean and groundnut are the most important legumes in terms of overall usage (human, industrial and animal), common bean are the most preferred food source for humans, offering it a higher market value in comparison to other legumes (Trott et al., 2016).

Common bean is mostly cultivated in the temperate and tropic regions with a rainfall requirement of 400 mm during cultivation season and a temperature requirement of 10-30^{o}C. Common bean may have determinate and indeterminate growth patterns with life spans from 2 months to 3 months (Katungi et al., 2009; Myers and Kmiecik, 2017).

The seeds of common bean crops are encapsulated in a pod of distinct shapes and sizes (Trott et al., 2016). They are self-pollinated, five-petaled flower (made up of 10 stamens) plants. Their flowers have mostly the shape of a butterfly which are usually pink or white (Figure 1.1). Common bean is consumed in either dry or fresh forms. Fresh bean pods are staple legumes in North America, Europe, the Middle East, Africa, Asia and Turkey (Myers and Kmiecik, 2017). Aside their dietary uses, common beans are known to be used in the treatment of several diseases. Results conducted by Laura et al. (2018) confirmed the uses of common bean in the treatment of jaundice in Ethiopia, rheumatism in Nigeria. Also, common bean has been reported to contain compounds that help in the fight against cancer. Katungi and Farrow Geagrofía (2009) related the importance of common bean in the treatment of coronary diseases due to their low-fat content and cholesterol free attributes.

Additionally, common bean helps to increase soil fertility through nitrogen-fixing abilities. As legumes, common bean establishes a mutualistic relationship with rhizobia (nitrogen-fixing bacteria), which offers a source of nitrogen (30-150 kg/ha) (Tongbram et al., 2021) through Symbiotic Nitrogen Fixing (SNF) activity. Although beans are less effective in their nitrogen fixing ability as compared to other legumes, they do help increase soil nitrogen content (Wilker et al., 2019).

Turkey, situated between southern Europe and Asia, has ideal climatic conditions for most crops. This rich ecologic advantage coupled with a vast area of arable land makes Turkey ideal and suitable for producing major legumes like common bean, soybean,

chickpea, cowpea, and others. In the year 2020, agricultural land of Turkey was reported to be close to 37,753,000 hectares (Tan and Yolcu, 2021). Also, the cultural significance of legumes such as common bean, lentil and chickpea in the diet of Turkish consumer leads to their massive production. Around the 17th century, common bean was introduced into Turkey. Despite of its foreign origin, common bean has been well received and spread in the Black Sea region (Bozoğlu and Sözen, 2007).

Common bean is the third most cultivated legume after chickpea and lentil. In 2020, 1 million 297 thousand tons of pulses were produced in Turkey. Turkey was the 4th highest global producer of chickpea per cultivation area, and ranks 19th in global dry bean production (Canlı and Çalkaya, 2022). In addition, in 2021, 305,000 tons of dry beans were produced in Turkey (TurkStat, 2022). Central Anatolia region has suitable climate for the production of dry bean. Out of these cultivated dry beans, 67.6% were from central Anatolia region. Niğde was responsible for 23.2% of the total production, 18.8% from Konya, 9.1% from Karaman and 11.3% from Nevşehir. The contribution of these four cities to total dry bean production of Turkey was massive (Table 1.1) (Kanat, 2022).

Table 1.1. Major dry bean producing provinces in Turkey in 2020/2021 season (Kanat, 2022)

Dry Bean Producing Provinces	**2020/2021 Production (Tons)**
Niğde	70760
Konya	57340
Nevşehir	34465
Karaman	27755

Turkey contributes not less than 3% to global fresh bean production (Çulal Kılıç et al., 2020). In 2020, a yield of 510.366 tons of fresh bean were produced (TurkStat, 2021). Black Sea, Mediterranean, Aegean, Central Anatolia regions are the main regions for fresh bean production. Samsun, Antalya, Mersin, İzmir, Tokat, Burdur, Muğla, Hatay and Konya are in decreasing order of production capacity of fresh bean.

Common bean production is threatened by biotic and abiotic factors. Temperature fluctuations, soil acidity and pathogens are notable factors that account for significant losses in common bean yield. Common bean is a suitable host for a range of plant viruses. Bean common mosaic virus (BCMV), Bean common mosaic necrosis virus (BCMNV)

and Cucumber mosaic virus (CMV) are amongst the most prevalent virus species discovered on common bean (Çulal Kiliç et al., 2020).

BCMV and BCMNV, belong to the *Potyvirus* genus in the *Potyviridae* family (Kyle and Provvidenti, 1993), have economic importance with not less than 35% of common bean yield losses (Çulal Kiliç et al., 2020). BCMV and BCMNV are highly widespread among common bean crops (Flores-Estévez et al., 2003). Both of them are transmitted through the seeds and pollen of infected plants, and through aphid species in a non-persistent manner (Silbernagel et al., 2001). The success rate of transmission of BCMNV through seeds can be as high as 50%, and BCMV has been reported to be stable in a seed for not less than 30 years (Chiquito-Almanza et al., 2017). This explains why seed transmission remains one of the commonest routes of BCMV and BCMNV transmission.

Potyvirus genome encodes a large polyprotein that is cleaved by viral proteases to produce ten functional proteins, known as genome-linked (VPg), P1, P3, CI (Cylindrical Inclusion), Nuclear Inclusion Protein a (NIa), Nuclear Inclusion Protein b (NIb), Coat Protein (CP), 6K1 and 6K2 proteins (Mishra et al., 2014).

Most BCMNV and BCMV strains cause almost similar symptoms (mosaic, mottling, and deformation on leaves) in susceptible common bean cultivars. However, BCMNV can be differentiated from BCMV by using the *I* gene carrying bean plants which normally show extreme resistance against most strains of BCMV. On the other hand, when the *I* gene-carrying plants are inoculated with BCMNV, vascular top necrosis occurs at temperatures below 30^{o}C. (Kelly, 1997).

The presences of BCMV and BCMNV have been reported in countries such as; Mexico (Flores-Estévez et al., 2003), Kenya (Wainaina et al., 2019), Tanzania (Mwaipopo et al., 2021), Turkey (Deligöz and Arli-Sokmen, 2008; Arli-Sokmen et al., 2016), USA (Feng et al., 2015). In Turkey, several studies been conducted on BCMV and BCMNV (Çulal Kiliç et al., 2020; Deligöz and Arli Sökmen, 2008), and other viruses infecting common bean. In the Aegean region of Turkey, Gümüş et al. (2001) sampled commercial common seeds and tested them for BCMV infection. The results of the study showed that approximately 24% of the seeds found to be positive for BCMV. In the Samsun Province located in the Black Sea region, Güzel and Arli-Sokmen (2003) investigated BCMV and

BCMNV infection in seed lots and leaves sampled from various sources. After testing the samples, it was found that 36% of the tested leaf samples were positive for BCMV, 10.8% for CMV, 2.8% for BCMNV and 2% for BYMV (Bean yellow mosaic virus). For the seed samples,18.9% were positive for BCMV, 17% for BCMNV and 17% for CMV. Similar studies by Arli-Sokmen et al. (2016) in Turkey discovered that out of the 367 seed and leaf samples collected from 15 common bean-producing provinces in Turkey, 67 samples were positive for BCMV while five were positive for BCMNV.

BCMNV have been reported to infect domesticated legumes, wild legumes and leguminous forages (Wainaina et al., 2019). Commonly, most researches performed on BCMV and BCMNV are related to common bean crops as compared to other susceptible leguminous crops. Soybean as a legume, is susceptible to BCMNV. In Kenya, five plants of a soybean cultivar inoculated with BCMNV showed a dominant symptom such as yellowing, but three out of the five inoculated soybean crops were positive for BCMNV in DAS-ELISA (Mangeni et al. 2020). BCMNV was reported to infect soybean crops in in soybean growing provinces in South Korea. Infected soybean crops showed symptoms such as chlorosis, mosaic, mottling, stunted growth and leaf yellowing (Jang et al., 2018).

BCMV and BCMNV strains are classified into eight pathogroups (PG) based on interactions of resistance genes in differential bean cultivars with virus pathogenicity genes (Drijfhout et al., 1978; Feng et al. 2015). These common bean cultivars are employed in the differentiation of BCMV and BCMNV pathogroups (PG) based on the symptoms that they generate. Host groups (HGs) of these differential common bean cultivars are numbered from 0 to 11. For instance, common bean cvs. Sutter Pink and Dubbele Witte, which contain the *ii* gene, are susceptible to all known strains of BCMV and BCMNV, whereas UI-36 Red Max (HG 2) and Redland Greenleaf C (HG 2) which involve the *bc-u* and *bc-1* genes, are susceptible to are susceptible to BCMV strains in pathogroup II-VIII, but resistant to strains in pathogroup I. Other differential hosts and their genes are given in Materials and Methods section.

Until 2015, seven pathogroups (I-VII) of BCMNV and BCMV have been identified. Then, Pathogroup eight (PG VIII) was added by Feng et al. (2015) when an isolate of BCMV was determined to overcome the resistance conferred by the *bc-2* and *bc-3* alleles.

The strains of BCMNV are classified into PG III (NL-8 strain), and PG VI (NL-3 and NL-5 strains), whereas the other pathogroups involve the strains of BCMV. Differential bean hosts placed in 8, 9 and 10 groups consist of *I*, *I+bc-1*, and *I+bc-1*^{2} gene combinations, respectively, are crucial for differentiation of BCMNV and BCMV strains (Drijfhout et al., 1978).

The main objective of this study is to determine the pathogroups of BCMNV isolates obtained from common bean leaf and seed samples, and characterize them molecularly in terms of the partial nuclear inclusion b (NIb) and coat protein (CP) regions. In addition, the study aims to investigate the reactions of twenty soybean cultivars to the NL-5-like isolate of BCMNV based on the phenotypic and serological evaluations.

2. LITERATURE REVIEW

2.1. Major Viruses in Common Bean

Environmental factors and pathogenic diseases reduce the productivity of legumes and other crops. Legumes are major host crops for viruses and other pathogens. Not less than 140 types of plant viruses infect leguminous crops (Edwardson and Christie, 2018). However, amongst the various leguminous crops, the common bean serves as a host for more than 30 different plant viruses (Morales and Bos, 1988). The majority of viruses that infect common beans mostly belong to the *Potyviridae*, *Bromoviridae* and *Geminiviridae* families (Table 2.1).

Table 2.1. Some Common Bean Viruses, Their Abbreviations and Families (Beatrice et al.,2017; Wamonje et al., 2020)

Virus	**Abbreviation**	**Family**
Bean common mosaic virus	BCMV	*Potyviridae*
Bean common mosaic necrosis virus	BCMNV	*Potyviridae*
Bean golden mosaic virus	BGMV	*Potyviridae*
Bean yellow mosaic virus	BYMV	*Potyviridae*
Cowpea aphid borne mosaic virus	CAbMV	*Potyviridae*
Cucumber mosaic virus	CMV	*Bromoviridae*
Calopogonium golden mosaic virus	CalCMV	*Geminiviridae*
Squash yellow mild mottle virus	SYMMoV	*Geminiviridae*
Bean golden yellow mosaic virus	BGYMV	*Geminiviridae*
Phaseolus vulgaris endornavirus 1	PvEV-1	*Endornaviridae*
Phaseolus vulgaris endornavirus 2	PvEV-2	*Endornaviridae*

Although these viruses cause considerable economic damages to common bean, CMV, BCMV and BCMNV are the most prevalent viruses, and they cause great amounts of losses to farmers as confirmed in a study by Wamonje et al. (2020).

2.2. Economic Losses Caused by BCMV and BCMNV

BCMV and BCMNV cause reduction in yield, deformation of bean pods and leaves, stunted growth on infected bean plants. Since the 1917's when BCMV was first reported in America up till today, countries all around the world have reported on the incidence, severity and economic impact of this virus (Strausbaugh et al., 2003a).

In Tanzania, Mwaipopo et al. (2018) attributed the reduction of common bean yield to as low as 1000 kg/ha to BCMV and BCMNV in combination with other pathogenic

diseases. Results from a study conducted by Pasev et al. (2014) in Bulgaria, confirmed high yield losses in local varieties less resistant to BCMV. The presence of BCMV and several losses in yield in India were reported by Sharma et al. (2011). Also, Omunyin et al. (1995) discovered the presence of BCMNV strains in most African countries with accompanying major losses recorded due to top necrosis.

BCMV causes not less than 50% losses in crop yield (Chiquito-Almanza et al., 2017; Deligöz and Arli Sökmen, 2008). *Potyvirus* mixed infections can result in the development of new strains through recombination event. These new strains tend to be more virulent than the previous *Potyvirus* strains (Worrall et al., 2015). Another study emphasized the devastating effects on common bean yield as a result of mixed infection between BCMV and BCMNV (Hema et al., 2014).

2.3. Hosts of BCMV and BCMNV

Although there are ranges of wild and domesticated crop families that serve as host for BCMV and BCMNV, six families are commonly reported. These are the Amaranthaceae, Chenopodiaceae, Leguminosae-Papilionoideae, Solanaceae and Tetragoniaceae. Groundnut, common bean, cowpea and soybean are among the crops that are regularly infected with BCMV and BCMNV. With regards to wild hosts, *Centrosema pubescens*, *Senna bicapsularis*, *Crotalaria* sp. and others are notable natural hosts (Lee et al., 2017; Worrall et al., 2015).

2.4. Soybean as a Host of BCMV and BCMNV

Soybean (*Glycine max*) are known as a host of BCMV and BCMNV. In Kenya, Mangeni et al. (2020) confirmed the infection of soybean plants after inoculating several 3-leaf stage soybean plants with BCMNV. The five replicates of a variety of soybean "Nyala" were inoculated with BCMNV. After three weeks, all the plants inoculated showed yellowing symptoms. However, only three plants were found to be positive after DAS-ELISA method. This indicated the susceptibility of soybean varieties to BCMNV.

According to the findings of a study conducted by Golnaraghi et al. (2002) in Iran, BCMV was confirmed to infect soybean crops. In South Korea, Jang et al. (2018) reported that soybeans were infected with the NL-5 strain of BCMNV. Out of 31 samples tested for BCMNV, a relatively low percentage (9.6%) was identified to be infected with

BCMNV. However, they pointed out that care must be taken in order to prevent the spread of this virus.

2.5. Transmission of BCMV and BCMNV

Almost all strains of BCMV and BCMNV are known to be transmitted by seed, aphids and in some cases through pollen, contaminated farm equipment and sap (Silbernagel et al., 2001). The tendency of transmission through seed is reduced when infection occurs after flowering. However, when infection occurs before flowering, transmission rate can be as high as 83% (Francisco José Morales and Bos, 1988) whereas the transmission rate of BCMV was 22% in Tapari bean plants (Hema et al., 2014).

BCMV and BCMNV, like most potyviruses, are transmitted by aphid species (Table 2.2) in a non-persistent and non-circulative manner within various monocotyledonous and dicotyledonous hosts. Virulent BCMV strains can be preserved in common bean seeds for up to 30 years. The predominance of BCMV and BCMNV may be due to the persistence of virulent strains in the seed. Seed route transmission was proposed to be the main source of the disastrous outbreaks of BCMV and BCMNV in America and Europe. In high quality commercial seeds, the rate of seeds infected with BCMV or BCMNV can be as high as 1% (Worrall et al., 2015).

Table 2.2. Some Potyviruses and Their Common Aphid Vectors (Gadhave et al., 2020; Nigam et al., 2019; Strausbaugh et al., 2003a).

Potyvirus Species	**Aphid vectors**
Bean common mosaic necrosis virus	*Aphis fabae, Acyrthosiphon pisum, Aphis craccivora, Myzus persicae, Macrosiphum solanifolii, Macrosiphum pisi, Macrosiphum ambrosiae, Aphis rumicis, Aphis gossypii, Aphis medicaginis, Hyalopterus atriplicis, Rhopalosiphum pseudobrassicae*
Bean common mosaic virus	*M. solanifolii, M. pisi, M. ambrosiae, M. persicae, A. rumicis, A. gossypii, A. medicaginis, H. atriplicis, R. pseudobrassicae, Metopholophium dirhodum, Rhapolasiphum padi, Schizaphis graminum, Sitobion avenae, M. persicae, Aphis pisum, A. craccivora, A. fabae*
Beet mosaic virus	*M. persicae, R. padi, Acyrthosiphon kondoi, Diuraphis noxia, A. fabae, A. pisum, Macrosiphum euphorbiae, M. persicae, R. padi, Cavariella aegopodi*
Clover yellow vein virus	*Aphis glycines, M. persicae, A. pisum, Aulacorthum solani, M. euphorbiae*

2.6. BCMV and BCMNV Differentiation

BCMV and BCMNV have undergone several modifications in terms of their nomenclature. Before 1934, BCMV was given names such as Bean virus 1, Bean mosaic virus and Phaseolus virus 1. Symptoms generated by BCMV strains on various common bean cultivars and the coat protein profiles were used as identification into the various serotypes. Previously, all BCMNV and BCMV strains were classified as Serotype A and serotype B, respectively, based on the serology and restriction analysis of the capsid protein. After 1992, they were reassigned to the names BCMNV (serotype A) and BCMV (serotype B) (Vetten et al., 1992; McKern et al., 1992; Mink et al., 1994), which are still in use till today. Taxonomic renaming reclassified the strains such as NL-3, NL5, and NL-8 as BCMNV strains (Strausbaugh et al., 2003a), while NL-1(US-1), NL-2, NL-4 (US-6), NL-6 (US-4), NL-7, US-2 and US-5 were designated as the strains of BCMV (Serotype B) (Drijfhout, 1978).

BCMV and BCMNV elicit similar symptoms (mosaic, chlorosis, stunted growth and leaf mottling) in susceptible common bean cultivars (Worrall et al., 2015). However, common bean cultivars carrying the dominant *I* gene response differently to BCMV and BCMNV infections. If the *I* gene-bearing bean plant is infected with a BCMV-necrotic strain, the plant may express systemic top necrosis above temperatures of 30^{o}C [Temperature-dependent Necrosis (TDN)], whilst it shows top necrosis at all temperatures [Temperature-independent Necrosis (TIN)] when infected with BCMNV (Feng et al., 2017; Gilbertson et al., 2001). Top necrosis or Whole Plant Necrosis (WPN) or black root are synonymic names given to this symptom. Top necrosis is characterized by the progression of vascular necrosis due to hypersensitive reaction, and it may finally lead to the death of plant.

The molecular weight of BCMV coat protein is 34.5-25 kDa, whilst that of BCMNV is 33 kDa. Also, the particle length of BCMV is 847-886 nm, however, BCMNV particle has a length of 810-818 nm (Vetten et al., 1992). Also, because of their serological dissimilarity, Feng et al. 2017 proposed the use of monoclonal or polyclonal antisera to differentiate between the various strains of BCMV and BCMNV.

2.7. The Pathogenicity of BCMV and BCMNV

BCMNV and BCMV are positive-stranded RNA viruses. Resistance to these viruses are governed by a single dominant gene, *I* and six recessive genes (*bc-1, bc1*^{2}, *bc-2,bc-2*^{2}, *bc-3* and *bc-u*), which are found at four loci (Drijfhout, 1978). Aside *bc-1, bc-1*^{2}, *bc-2* and bc-2^2 which are strain specific genes, *bc-u* is an effector gene that helps in the expression of the other genes. In the absence of *I* gene, *bc-u* is a necessary gene for the functionality of the others, namely, when it is present, *bc-1*, *bc-1*^{2} and *bc-3* confer resistance to their hosts against BCMV and BCMNV strains. Amongst these genes, *bc-u* and *bc-1* have been proposed to involve some form of relation. The *bc-3* gene in combination with *bc-u* or the dominant *I* gene, is proposed to confer resistance against all known strains of BCMV and BCMNV with the exception of the NL-3K strain (Hart and Griffiths, 2013).

The *I* gene provides resistance in common bean plants against most of BCMV strains at temperatures lower than 30^{o}C. However, in the last decade, strains that induce top necrosis in common bean cv. Jubila carrying *I+bc-1* (Feng et al. 2014, Arli-Sokmen et al. 2016) and cvs. Amanda and Isabella having *I+bc-1*^{2} (Arli-Sokmen et al. 2016) at lower temperatures were identified. On the other hand, the *I* and *bc-1* genes cause necrotic local lesion and vein necrosis on inoculated leaf of common bean when infected with the NL-8 strain of BCMNV, whilst they lead to top necrosis leading to the death of the plant after inoculation with the NL-3 and NL-5 strains of BCMNV at all temperatures (Strausbaugh et al., 2003b). Also, *I*, *bc-1*^{2} and *bc-3* have been reported to confer resistance against some other potyviruses (Larsen et al., 2008).

The *I* gene appears in two forms. These are the protected *I* gene and the unprotected *I* gene. A protected *I* gene occurs when strain-specific recessive genes are integrated with the *I* gene in order to protect the *I* gene from necrosis-causing isolates of BCMV in which they infer resistance. Unprotected *I* gene occurs when isolate-specific genes are not integrated with the *I* gene. It is also worth knowing that in the situation of protected *I* gene, viruses are hardly transmitted through seed in comparison to the unprotected *I* gene. Crop varieties that contain the unprotected *I* gene experience major crop losses if cultivated in close proximity to susceptible varieties infected with BCMNV in the presence of aphids. The recessive genes *bc-1*, *bc-1*^{2} and *bc-3* protect the *I* gene in the absence of *bc-u*, but *bc-*

2^{2} requires the help of *bc-u* to perform the same function (Larsen et al., 2008; Miklas et al., 2002; Soler-Garzón et al., 2021). The protected *I* gene provides immunity in common bean crops against several BCMV strains under 30^{o}C.

Virus pathogenicity tests assist the grouping of BCMV and BCMNV strains. Eight pathogenic groups (PG) have been established on the basis of interrelationship between the plant resistance genes and viral pathogenicity genes. These are PG I, PG II, PG III, PG IV, PG V, PG VI, PG VII and PG VIII, respectively. Based on the reaction of the BCMV and BCMNV strains on the selected differential bean cultivars, BCMV and BCMNV strains were first classified into seven pathogenic groups (I-VII) (Drijfhout, 1978). Previously, the *bc-3* gene was known to provide resistance against all BCMV and BCMNV strains. However, a study by Feng et al. (2015) identified a new isolate (1755a) infecting a common bean cultivar having the *bc-3* gene, and this isolate was assigned to PG VIII (Table 2.3). Feng et al. (2015) observed mutations in the amino acid sequence of this new strain as compared to other strains that could not overcome the *bc-3* gene. Also, upon subjecting it to the various differential cultivars such as IVT 7214 (*I, bc-u, bc-2, bc3*), Dubbele Witte and Stringless Green Refugee (*i, bc-u*) and Sanilac (*i, bc-u, bc-2*), various symptoms indicating their susceptibility was observed. This shows that mutation and recombination can lead to the development of more virulent strains of BCMV and BCMNV. Strains in PG I-PG II contain only one pathogenicity gene, whereas those in PG III-PG IV contain two pathogenicity genes, and those in PG V- PG VIII have three pathogenicity genes as shown in the Table 2.3 (Drijfhout, 1978; Feng et al., 2017; Silbernagel et al., 2001).

Before the discovery of other members of Pathogroup VI, RU-1 was the only strain assigned to the group. Larsen et al. (2005) dismissed the theory that the RU-1 strain was first identified in the experimental fields in Washington or Idaho but rather, it was determined in seed accessions belonging to USDA that were imported from Russia in 1985. Feng et al. (2017) stated that BCMNV isolates identified so far were classified into PG III, and PG VI along with RU-1 strain of BCMV.

BCMNV has more limited spread than BCMV in the world. In most African countries, the NL-3 and NL-8 strains of BCMNV were discovered to be dominant (Vetten

& Allen, 1991). Almost no harvest was recorded for most varieties that contain the *I* gene due to top necrosis (Omunyin et al., 1995). Mangeni et al. (2020) attributed the high incidence and severity of BCMNV and BCMV in Kenya to the uncontrolled use of noncertified seeds by farmers. Also, the duration of rain was found to positively correlate with the incidence and severity of BCMV and BCMNV. Short rain positively influenced the incidence and severity of BCMNV and BCMV, whilst long rain season decreased their incidence and severity.

In Tanzania, a review by Beatrice et al. (2017) reported that the incidences of BCMNV and BCMV were approximately 37% and 12.4%, respectively, in common bean seed lots (from eastern and western Tanzania) collected from local farmers and market, whereas zero incidences were recorded for seed lots collected from wild legumes. It was also confirmed that the Tanzanian strain of BCMNV (TN 1) has 98.4% nucleotide identity to the other BCMNV isolates. On the other hand, the P1 protein of the NL-3K strain of BCMNV has a 98% identity to that of RU1 (BCMV) (Larsen et al., 2005). This result shows that NL-3K strain is a recombinant strain between BCMNV NL-3 and BCMV RU-1. There is a limited number of complete genome sequences of BCMNV isolates available in the GenBank database, and the biological diversity of BCMNV isolates is not as wide as that of BCMV isolates.

A study conducted by Feng et al. (2017) used three isolates of BCMNV (1755b, TN1a and NL-8 CA) and tested them biologically using 12 differential cultivars of common bean. At the end of the study, the isolates 1755b and TN1a were placed in PG VI, whilst NL-8 CA was placed in PG III. It was observed that NL8-CA triggered necrosis on inoculated leaves of differential host group (HG) 9, which have the resistance genes *I* and *bc-1*, but less systemic mosaic infection was observed in the host groups (HGs) 2 and 3. It was found that TN1 and 1755b strains caused top necrosis in Top Crop (HG 9) between a week to two after inoculation. It was also revealed that *bc-1* gene plays a role in the suppression of the systemic spread of BCMNV strain within the common bean plants.

Table 2.3. Pathogenicity genes of BCMV and BCMNV strains and their pathogenicity groups

Pathogenicity Genes	**Pathogenicity groups**	**Virus Strains**
P0	PG I	BCMV NL-1, BCMV PR-1, BCMV US-1
P1	PG II	BCMV NL-7
P1, $P1^2$	PG III	BCMNV NL-8 BCMNV NL8-CA
P1, P2	PG IV	BCMV US-5 BCMV NL-6
P1, $P1^2$, P2	PG V	BCMV US-2 BCMV NL-2
P1, $P1^2$ P2	PG VI	BCMNV NL-3 BCMNV NL-5 BCMV RU-1 BCMNV TN1 BCMNV 1755b BCMNV TN1a
P1, $P1^2$, $P2^2$	PG VII	BCMV NL-4 BCMV TR-180 BCMV US-10 BCMV RU1-OR BCMV RU1-M BCMV US-6
P0, P2, P3	PG VIII	BCMV 1755a

Virus of the same strain, but of different isolates trigger dissimilar symptoms in their host plants. NL-3K strain causes extreme symptoms in host groups with the *I* and *bc-3* genes in comparison with other strain (NL-3D). This occurrence of different symptoms was attributed to the recombination leading to genomic transformation, hence, the differences in their pathogenicity (Larsen et al., 2005).

In California, Guzman et al. (1997) after the discovery of NL-3 strain of BCMNV, it was found out that the strain caused top necrosis in BTS T-39 (HG 8) and Top Crop (HG 9). They discovered general mosaic symptoms in the systemic leaves of Sutter Pink (HG 0).

In the study done by Güzel and Arlı-Sökmen (2003), the presence of BCMNV infection was first reported in common bean leaf and seed samples in Turkey. Out of 499 plant samples, 2.8% were found to be positive by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) for BCMNV, 36% for BCMV.

In the previous study, 53 seed samples were obtained from seed markets and local farmers. After planting the seeds, 18.9% of them were positive for BCMV and 17% for BCMNV (Güzel and Arlı-Sökmen, 2003).

In Turkey, Çulal Kılıç et al. (2020) recorded 15.27% incidence of BCMNV and 26.18% of BCMV in leaf samples, and 2.32% incidence of BCMV and 6.97% incidence of BCMNV in seed samples. Mixed infection cases were also recorded. Percentages of mixed infection in leaf and seed samples were approximately 25% and 56%, respectively. This confirms findings that seed transmission is the most common route of infection.

Seed and leaf samples were collected from various dry and snap bean growing areas in Samsun province and tested for BCMV and BCMNV. After DAS-ELISA testing, 34.4% were determined to be positive for BCMV, whilst 7.6% were for BCMNV. Some of the samples were subjected to pathogrouping. Based on the symptoms, three isolates (BTür2, ÇAhu-2, ÇAhu-3) were identified to be similar to the NL-3 strain of BCMNV (Deligöz and Arli Sökmen, 2008).

Sengooba et al. (1997) conducted a study using different BCMNV isolates on a range of differential host cultivars. The isolates 197 and 473 were obtained from *Senna hirsuta*, the isolate 218 from *Crotalaria* sp., the isolate 463 from *Crotalaria incana*, the isolate 496 from *Senna bicapsularis* and the isolate 741 from *Centrosema pubescens*. On their natural hosts, the isolates numbered 218, 463 and 741 triggered mild mosaic symptoms, the isolate 496 caused chlorotic spots, the isolate 197 caused mosaic symptoms, and the isolate 473 triggered clear mosaic symptoms. However, after inoculation onto differential cultivars, the isolates 197, 218, 463, 473, 496 and 741 caused susceptible reactions (mosaic) in HG 1-HG 5. However, there was resistant reaction in HG 6 and HG 10, but necrotic symptoms at temperatures below 30^{o}C (Temperature Insensitive Necrosis; TIN) in HG 8, HG 9a and HG 9b.

2.8. The *Potyviridae* Family

The one of most important families of plant RNA viruses, the *Potyviridae*, has gotten a lot of attention. The *Potyviridae* family currently has twelve genera (*Arepavirus*, *Bevemovirus*, *Brambyvirus*, *Bymovirus*, *Celavirus*, *Ipomovirus*, *Macluravirus*, *Poacevirus*, *Potyvirus*, *Roymovirus*, *Rymovirus*, *Tritimovirus*) and 237 species (Valli et

al., 2018). These virus family are distinguished by their genomic composition, form, RNA sequences, and vector transmission. Their virions have nonenveloped and flexible filamentous shape, with diameters ranging from 11 to 15 nanometers and a helical pitch of 3.4 nanometers coupled with a 680-900 nm length (Gibbs et al., 2020). Viruses from the *Potyviridae* family are contagious. Their genomes consist of positive-sense single stranded RNA in a range of 8.2–11.5 kb, the genome is usually monopartite with the exception of *Bymovirus*. Furthermore, their genomes encode a large polyprotein that splits itself into different sets of proteins with different roles (Rabenstein and Kühn-institut, 2012). Unlike the others with monopartite genomes, Bymoviruses have bipartite RNA genomes, which are individually translated. When the viruses belonging to the *Potyviridae* family enter plant cells, their viral RNA is uncoated and translated into polyprotein, which is then processed by proteinases. The genome structure and protein sequences of most viruses in *Potyviridae* have some similarities with Comoviruses and Nepoviruses (Gibbs and Ohshima, 2010).

2.9. The *Potyvirus* Genus

In 1959, the Potyvirus group was started to be used. After being named by Kenneth Smith as Potato virus Y (PVY) in the 1920's, viruses possessing common features to PVY were given the name "Potyvirus". In relation to improvements in the methods of identification and classification of viruses, the number of identified potyviruses have increased**.** *Potyvirus*, the largest genus of the *Potyviridae* family, contains around 195 members according to the latest report of International Committee on Taxonomy of Viruses (ICTV) (ICTV, 2021). Until 1991, the NIb-CP region of 57 potyviruses have been sequenced, whereas in recent times, over 26,000 *Potyvirus* sequences have been identified**.** The first *Potyvirus* is estimated to be originated from a grass host approximately 15,000 to 30,0000 years back (Gibbs et al., 2020)**.** Infected planting materials and seeds are primary inoculum source of potyviruses. Potyviruses are considered to have a high risk to plant production due to their negative effect on yield. As such, they are one of the most studied among the plant virus genera.

2.10. The *Potyvirus* Components

Like most viruses in the *Potyviridae* family, potyviruses have flexible, filamentous particles, which are 680-900 in length and 11-15nm in diameter (Fig 2.1). They have positive sense single stranded RNA genome which comprises of nearly 10,000 nucleotides (Gibbs and Ohshima, 2010). Potyviruses are made up 2000 copies of coat protein which encapsulates the RNA genome. Molecules of the coat protein are assembled and used in the construction of the virions. The genome has a 5'viral genome-linked protein (VPg) (Mishra et al., 2014) and a 3' poly-A tail.

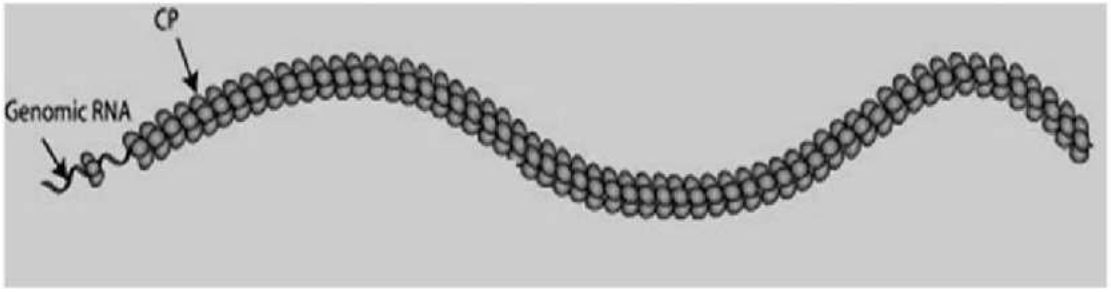

Figure 2.1. Structure of *Potyvirus* genomic particle (Mishra et al., 2014)

2.11. Potyvirus Genome Structure

The genomes of Potyviruses consist of an extended Open Reading Frame (ORF) that is made up of a 350 kDa polyprotein. This polyprotein, with the help of proteolytic enzymes "proteases", is cleaved into several smaller proteins. It is worth noting that the number of cleaved proteins can vary. Worrall et al. (2015) indicated that ribosomal frameshifting increases the number of viral proteins. The *Potyvirus* proteins are viral protein genome-linked (VPg), P1, P3, CI (Cylindrical Inclusion), Nuclear Inclusion Protein a (NIa), Nuclear Inclusion Protein b (NIb), Coat Protein (CP), 6K1 and 6K2 proteins as shown in Fig. 2.2. (Mishra et al., 2014).

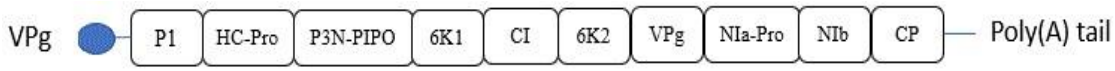

Figure 2.2. A structure of the polyprotein in the *Potyvirus* genus

Initially, it was believed that polyproteins were the source of all proteins of Potyviruses. But another research from explained the theory through the discovery of P3N-PIPO (pretty interesting *Potyviridae* ORF) which was established to be responsible

for movements within cells of potyviruses. In BCMNV and BCMV, P3N-PIPO is believed to arise from polymerase slippage (Chung et al., 2008).

Inside the *Potyvirus* genome, there are stable (HC-Pro, NIb) and variable (P1, P3 and CP) components. These protein components have distinct functions for virus infection. *Potyvirus* particles, however, involve only VPg and the coat protein (Shukla et al., 1991).

P1, a serine proteolytic enzyme, slices at the boundary of P1/Helper Component Protease (HC-Pro) region. Even though the function of P1 protein remains to be fully understood, it is suggested that it play a couple of roles in coalescing of protein (Martínez and Daròs, 2014). Furthermore, studies from Mishra et al. (2014) postulated that the P1 protein may be involved in cell-to-cell movement. Also, P1 protein is known to have an essential role in virus replication and pathogenicity.

The HC-Pro region of *Potyvirus* genome is proposed to contain proteinase, involved in aphid transmission of the virus and aids in cell-to-cell movement of the virus. In addition, the HC-Pro is also responsible for transmission of the virus not belonging to the *Potyvirus* genus. For example, HC-Pro is useful in the semi-persistent transmission of Wheat streak mosaic virus (Tritimovirus) which has eriophyid mites as their vectors (Valli et al., 2018). Other function of HC-Pro region includes the interference in RNA silencing mechanism by suppressing antiviral defense of the host plants and symptom initiation.

The viral protein genome-linked (VPg) located on the exterior of the P1 component is identical to the CAP structure (N7- methylated guanosine linked to the first nucleotide of the RNA) seen on most cellular mRNAs. Eukaryotic Initiation Factors (eIFs) such as eIF4E plays a role in the initiation of the translation and the production of viral proteins (Tavert-Roudet et al., 2017). Hart and Griffiths (2013) proposed that further protein-protein research on eIF4E protein and Potyvirus VPg interaction may play a significant role in understanding the resistance scale of Potyviruses.

The N-terminal VPg and the C-terminal protease make up the NIa component. The NIa- VPg acts as a primer for the start of RNA synthesis, that aids genome replication indirectly, whereas the NIa-Pro contains a significant proteinase. The RNA-dependent RNA polymerase (RdRp) found in the NIb protein is responsible for nucleic acid binding

and genome replication. Splitting of seven proteins, out of the numerous polyproteins, are performed by NIa-Pro. For some potyviruses the NIa and NIb interacts with each other, while there is interaction of NIa and VPg for others. The proteins of NIa and VPg trigger NIb-related RNA polymerase activity.

The proteins 6K1 and 6K2 have their established functions. Whilst 6K1 is responsible for formation of tubules for replication (Nigam et al., 2019), 6K2 as reported by Worall et al. (2015), is responsible for viral replication. In addition, 6K1 is proposed to participate important in RNA replication and other processes (Hull, 2013; Mishra et al., 2014; Worrall et al., 2015).

2.12. The *Potyvirus* Coat Protein (CP)

In contrast to other gene products, the coat protein has an amino acid content that is unique to the *Potyvirus* genus, namely, an amino acid sequence of it exhibits no significant homogeneity amongst various plant virus groups. It is also the virion's main gene product, accounting for 95% of the potyvirus particles (Shukla and Ward, 1989).

Basically, molecules of coat protein are used in the synthesis of virions in addition to the protection of the virions. The amino acid sequence and molecular weight of the CP varies according to the type and strain of *Potyvirus*.

Aside the protection of the RNA component of the virus, the CP of most potyviruses in conjunction with other components of the genomes perform a series of function. The interrelationship between HC-Pro and CP are useful in the transmission of certain potyviruses. Worrall et al., (2015) confirmed that the transmission of Soybean mosaic virus by aphids are propelled by the interrelationship between HC-Pro and CP. However, variations in the amino acid sequence of the CP directly affects the transmissibility of the *Potyvirus*. Atreya et al. (1990) discovered aphid transmissible strains of Tobacco vein mottling virus (TVMV) to be no longer transmittable by aphids after the mutation of CP amino acid sequence at location of 2747. This confirms the hypothesis that a variation in the amino acid sequence of *Potyvirus* immensely may decrease the probability of the *Potyvirus* to be transmitted by aphids.

Like all potyviruses, Asp-Ala-Gly (DAG) motif in CP amino acid terminal must be present because they are critical in the transmissibility of the virus by aphids (Atreya et

al., 1990), although there could be exception [Asp-Ala-Ala-Ala (DAAA) motif] as in the case of Peanut mottle virus which is also highly transmittable by aphids (Flasinski and Cassidy, 1998). Worrall et al. (2015) confirmed the preserved CP amino acid sequence in the NL-1, NL-4 and NL-7 strains of BCMV.

Coat protein has a crucial role in most stages of the virus infection cycle. Coat Protein is responsible for activities such as RNA expansion, transmission of virus by aphids, cell to cell movement and among others. It is evident that the aid of HC-Pro protein must be implored by CP in order to perform certain functions to the maximum. In PVY, HC-Pro binds to the virions and helps the uptake of the virus by aphids (Blanc et al., 1997). In addition, Flasinski and Cassidy (1998) discovered that when aphids feed in a synthetic diet including filtered virus and Pepper mottle virus (PeMoV), HC-Pro restored aphid transmissibility of the PStV-DAAA mutant. Molecular analysis of the CP and HC-Pro of BCMV and BCMNV demonstrated that the interaction between these components is a necessity for insect transmission of these viruses (Worrall et al., 2015). Symptoms and other variables can be used as a source of distinction between potyviruses. Information on the protein structure is needed to reclassify potyviruses and their strains. Johnsongrass mosaic virus (JGMV) was distinguished from Sugarcane mosaic virus (SCMV). Bean yellow mosaic virus was found to be a distinct *potyvirus*, and Pepper mottle virus (PeMV) was associated with PVY (Shukla and Ward, 1989).

2.13. The *Potyvirus* Nuclear Inclusion b (NIb) Protein

Nuclear Inclusion b (NIb) protein, one of the components of the potyvirus genome, is RNA-dependent RNA polymerase responsible for viral replication. Aside this role, NIb has been reported to help in various virus-host interactions. NIb uses many host proteins in the synthesis of virus replication complexes (VRC's), which are useful in the formation of functional VRC's for virus multiplication, and have a role in suppressing Non-Expresser of Pathogenesis Related Genes 1 (NPR-1)-mediated immunity response (Shen et al., 2020).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1 Virus Sources

In this study, the seeds infected with NL-5-like isolate of BCMNV were originated from the previous study by Arli-Sokmen et al. (2016) and maintained at Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayis University at a temperature of 4^{o}C. Also, the seeds of a commercial common bean cultivar were supplied by the Department of Field Crops of Ondokuz Mayis University and used as a source of the NWS isolate of BCMNV identified in this study.

3.1.2. Soybean Seed Source

The seeds of registered commercial soybean cultivars were obtained from the Black Sea Agricultural Research Institute in Samsun, Turkey. The soybean cultivars subjected to virus inoculation were Adasoy, Arisoy, Ataem, Atakişi, Atlas 366, Blaze, Cinsoy, Gapsoy-16, Ilksoy, May 5451, Mona, Nazlican, Ohio, Prota Y, Sa-88, Safir, Samsoy, Soy Anam, Srebrina and Umut 2002.

3.1.3. Differential Common Bean Cultivars

The seeds of common bean genotypes belonging to 12 differential host groups (HGs: HG 0- HG 11), which are used in the differentiation of BCMV and BCMNV pathogroups were supplied by USDA-ARS (Prosser, USA) (Table 3.1).

3.1.4. Antisera

Polyclonal antisera specific to BCMNV, BCMV, and CMV were supplied from Bioreba (Switzerland).

3.1.5. Buffers used in DAS-ELISA

I. Phosphate Buffer Saline (PBS) (pH 7.4)

0.13 M NaCl (8 gr), 0.014 M KH_2PO_4 (0.2 gr), 0.002 M KCl (0.2 gr), 0.08 M $Na_2HPO_4.12H_2O$ (2.9 gr).

The components were dissolved in 1 liter of distilled water. 0.1 M of NaOH or HCL was added to adjust the pH (7.4), and PBS solution was stored at a temperature of 4^{o}C.

Table 3.1. Differential host groups, bean cultivars and their resistance genes

Host Group (HG)	Bean Cultivar	Resistance Genes
0	Dubbele Witte, Sutter Pink	*ii*
1	Stringless Green Refugee	*i, bc-u*
2	Redland Greenleaf C, UI 36 Red Max	*i, bc-u*, bc-*1*
3	Redland Greenleaf B	*i, bc-u*, bc-1^2
4	Pinto UI-111	*i, bc-u, bc-2*
5	Pinto UI-114	*i, bc-u, bc-1, bc-2*
6	Othello	*i, bc-u, bc-*1^2*, bc-*2^2
7	IVT 7214	*i, bc-u, bc-2, bc-3*
8	Widusa, Black Turtle Soup-1	*I*
9a	Jubila	*I, bc-1*
9b	Improved Tendergreen 40031	*I, bc-1*
10	Amanda, Isabella	*I, bc-*1^2
11	IVT 7233	*I, bc-u, bc-*1^2*, bc-*2^2

II. Coating Buffer

0.015 M Na_2CO_3 (1.59 gr), 0.035 M $NaHCO_3$ (2.93 gr).

The components above were dissolved in 1 L of distilled water. The pH (9.6) was then adjusted with 0.1 M of NaOH or HCL and stored at a temperature of 4^{o}C.

III. Washing Buffer

Washing buffer was prepared by adding 0.5 ml Tween-20 to 1 L PBS buffer.

IV. Extraction buffer

It was prepared by mixing 1 L of washing buffer solution with 20 g (2%) Polyvinylpyrrolidone 40,000 (PVP-40) and 1g of low-fat milk powder.

V. Conjugate Buffer

Extraction buffer was also used as conjugate buffer solution.

VI. Substrate buffer (pH 9.8)

It was prepared by mixing 9.7 ml of diethanolamine and 80ml of distilled water. After adjusting the pH to 9.8 with HCl, the volume was completed to 100 ml with distilled water.

3.1.6. ELISA Microplate

In this study, a 96-welled flat bottom microplate (Thermo Scientific, Denmark) made of polystyrene material was used.

3.1.7. Buffers used in dsRNA Extraction

I. Lysis Buffer

200 mM (2.422 g) Tris, 500 mM (2.922 g) NaCl, 10 mM (0.203 g) $MgCl_2$, 3 g SDS, 10% (10 ml) Ethanol and 1% (1 ml) 2-mercaptoethanol.

The above chemicals were dissolved in 80 ml of distilled water before adding ethanol and 2- Mercaptoethanol and sterilized in an autoclave for 15 min at 121^{o}C. Then, the buffer was kept at a temperature of +4^{o}C until used.

II.1×STE Buffer

10 mM (0.121 g) Tris, 10mM (0.827 ml) HCl,100 mM (0.584 g) NaCl,1 mM (0.037 g) EDTA

The chemicals were dissolved in 100 ml of distilled water and the pH adjusted to 8.0. Then, the buffer was autoclaved.

III. 16% Ethanol Containing STE Buffer

8 ml of absolute ethanol was added to 42 ml of prepared 1XSTE buffer.

3.1.8. Buffer used in Agarose Gel Electrophoresis

I. 1×TBE Buffer

TBE Buffer contained 89 mM (10.8 g) Tris base, 89 mM (5.5 g) Boric acid, 2 mM (0.337 g) EDTA**.** The chemicals were dissolved in 1 L of distilled water and autoclaved**.**

3.2. Methods

3.2.1. Planting of BCMNV-infected Seeds

The seeds of susceptible common bean cultivars (Sutter Pink or Dubbele Witte) infected with BCMNV isolates were planted in small plastic pots filled with soil-pasture mixture. After three-four weeks, the leaves showing typical BCMNV symptoms were

collected and used as the main sources of virus inoculum. Also, the inoculums of BCMNV isolates were maintained on susceptible bean cvs. Sutter Pink and Dubbele Witte throughout the study.

3.2.2. Inoculum Preparation and Mechanical Virus Inoculation

Inoculum was obtained from Dubbele Witte and Sutter Pink cultivars showing clear signs of BCMNV infection. The leaves are collected and grinded in a sterile mortar and pestle [1 g of infected leaf: 10 ml of inoculation buffer (pH 7.5)]. Inoculation buffer contained 1% K_2HPO_4 and 0.1% Na_2SO_3 (Sengooba et al., 1997).

When the primary leaves of common bean plants reached to at least a size of ¾ whole leaf, a 400-mesh carborandum powder was sprinkled on them. Then, the sap of grinded infected leaves was applied on the primary leaves of bean plants by wearing plastic gloves. Two plants of each bean cultivar were inoculated with the virus whilst one was maintained as control. Carborandum powder and distilled water was applied on the primary leaf of the control plant. After inoculation, the inoculated leaves were washed under running tap water. The plants were then placed in a climate room at temperatures of 20^{o}C (dark) and 25^{o}C (light) for 14 hours of photoperiod.

Also, in this study, the reactions of some commercial soybean cultivars against BCMNV were investigated. For this aim, the primary leaves of soybean plants at about 10-day old were mechanically inoculated with NL-5-like isolate (PG VI) of BCMNV. Five replications of each 20 soybean cultivars (stated in section 3.2.1) were used in phenotypic and serological screening of them after sap-inoculation, which was applied as mentioned above.

3.2.3. Differentiation of BCMNV Isolates and Identification of Their Pathogroups

For identification of the pathogroups of BCMNV isolates, the seeds of differential bean cultivars (Table 3.1), which are known to possess different combination of genes, were used (Drijfhout et al., 1978). For confirmation of the pathogroup of a BCMNV isolate (NL-5-like isolate obtained in the previous study) and identification of the pathogroup of a new BCMNV isolate obtained in this study, two BCMNV isolates were firstly propagated and maintained in common bean cvs. Dubbele Witte and Sutter Pink.

Secondly, each BCMNV isolate was used in mechanical inoculation of differential bean cultivars (Table 3.1). Symptoms observed on differential hosts after inoculation were weekly recorded. Cultivars showing systemic infection from 7-28 days post inoculation were designated as phenotypically susceptible (S), while those that did not show systemic symptoms were classified as resistant (R). Also, at the fourth week of inoculation, the upper non-inoculated trifoliate leaves of plants that showed no obvious virus symptoms or mild symptom were tested by DAS-ELISA to confirm the absence or presence of systemic BCMNV infection. Those determined to be positive in ELISA were considered to be susceptible (S), while those found to be negative were considered as resistant (R). Also, the cultivars responded to BCMNV infection by giving systemic necrosis at lower temperatures (below 30^{o}C) were established as Temperature-independent Necrosis (TIN). Evaluations were made based on available pathogen-host interactions (Table 3.2.) (Drijfhout, 1978; Silbernagel et al., 2001; Feng et al., 2014).

3.2.4. Testing Plants by DAS-ELISA

Common bean and soybean cultivars inoculated with BCMNV isolates were tested using DAS-ELISA. In the ELISA test, polyclonal virus-specific antisera were used as recommended by Clark and Adams (1977). The dilution of the antisera was made according to the recommendation of the antiserum manufacturer. A total amount of 100 µl of antiserum was loaded into each well of the ELISA microplate and placed in an incubator at a temperature of 30^{o}C for 4 hours. The plates were then coated with samples prepared in 1:10 of the extraction buffer. After incubation at 4^{o}C overnight, the wells were washed for 5 times and the diluted conjugate (100 µl) was loaded into the plate wells. After incubation of 5 hours at 30^{o}C, the substrate (p- nitrophenyl phosphate) (1mg substrate: 1ml substrate buffer) was added into the wells. After each step, plate wells were washed at least three times with washing buffer (PBS+ Tween 20). The results (absorbance values) of ELISA were obtained by a microplate reader (Tecan Spectra II, Austria) at 405nm wavelength, 60-120 min after the addition of the substrate. Samples that had absorbance values at least more than twice of the mean absorbance values of the negative controls (healthy plant leaves) were evaluated as positive (Strausbaugh et al., 2003a).

Table 3.2. Pathogen-host interactions for known BCMNV strains (Drijfhout, 1978; Silbernagel et al., 2001; Feng et al., 2014)

HG/Bean Cultivars (Genes involved)	**NL-3**	**NL-5**	**NL-8**	**TN-1**	**1755b**	**NL-8 CA**
0. Dubbele Witte (*i*)	S	S	S	S	S	S
1. Stringless Green Refugee (*i*, *bc-u)*	S	S	S	S	S	S
2. Redland Greenleaf C (*i*, *bc-u*, *bc-1*)	S	S	R	S	S	R
3. Redland Greenleaf B (*i*, *bc-u*, *bc-1*^{2})	S	S	R	S	S	R
4. Sanilac (*i*, *bc-u*, *bc-2*)	S	S	S	S	S	S
5. Pinto UI 114 (*i*, *bc-u*, *bc-1*, *bc-2*)	S	S	R	S	-	-
6. Monroe (*i*, *bc-u*, *bc-1*^{2}, *bc-2*^{2})	R	R	R	R	S	S
7. IVT 7214 (*i*, *bc-u*, *bc-2*, *bc-3*)	R	R	R	R	S	S
8. Widusa, BTS-1 (*I*)	S*	S*	S*	R	-	-
9. I.T.40031, Top Crop (*I*, *bc-1*)	S*	S*	R*	R	S	S
10. Amanda (*I*, *bc-1*^{2})	S**	S*	R*	R	S	S
11. IVT 7233 (*I*, *bc-u*, *bc-1*^{2}, *bc-2*^{2})	R*	R*	R*	R	S	S

R: Resistant, S: Susceptible, S*: Susceptible, systemic and vascular necrosis occur at 25^{o}C and 30^{o}C (temperature-independent necrosis: TIN), S**: Susceptible, systemic and vascular necrosis are at 30^{o}C or above (temperature-dependent necrosis; TDN). Systemic necrosis increases with increasing temperature R*: Resistant, no systemic necrosis, local lesion occurs as pinpoint necrosis. -: Not available, BTS-1: Black Turtle Soup-1

3.2.5. Isolation of Nucleic Acids from BCMNV-infected and uninfected Common Bean Plants

Double strand RNA (dsRNA) isolation method performed by Khabbazi et al. (2017) was modified in this study. The leaves of the BCMNV-infected common bean plants were collected for dsRNA isolation. A mass of 1.5 g (three replicates of 0.5 g) of infected leaf was used for each BCMV and BCMNV isolates in the isolation process.

Isolation Procedure:

1. A total of 1.5 g of common bean leaf infected with BCMV or NL-5-like isolate of BCMNV or a new BCMNV isolate identified in this study was individually mixed with 4ml of lysis buffer (Section 3.1.7) and homogenized using a sterile mortar and pestle,
2. The homogenized liquid mixture was loaded into a 2 ml sterile microcentrifuge tube and divided into four microcentrifuge tubes of 2 ml (1ml per each),
3. The tube was placed on a vortex (Isolab) for 10 seconds, mixed vigorously and incubated in a water bath at 37^{o}C for 10 minutes.
4. Chloroform was then added to mixture at a ratio of 1:1 followed by centrifugation at 11.300 rpm for 20 min at 4^{o}C,
5. The upper layer was transferred into a new tube and chloroform added in a ratio of 1:1. Then, a 7-minute centrifugation is performed at 11.300 rpm (this step could be repeated severally to remove leftover leaf tissue particles),
6. The upper layer was transferred to a new tube and 0.2ml of absolute ethanol was added per 1ml of supernatant. The mixture was inverted several times and a 5-minute centrifugation was undertaken at 11.300 rpm at 4^{o}C,
7. After centrifugation, 15 mg of cellulose fibers (catalogue No: C6288, Sigma Aldrich. ST. Louis, MO, USA) was measured and added to each tube. The tubes were mixed vigorously and maintained at room temperature for 15 minutes. The original procedure involves Whatman Cellulose CF-11 instead of cellulose fibers to absorb dsRNAs under 16% ethanol concentration and incubation time of 5 min. However, CF-11 cellulose powder is no longer available worldwide. Therefore, Sigma's cellulose fiber was tried to be used to specifically bind dsRNA fraction to the column in this study. After adding cellulose fibers and incubation, centrifugation was performed at 4^{o}C at 11.300 rpm for 7 minutes,
8. Upper liquid phase was separated, and 1 ml of washing buffer (1XSTE buffer containing 16% absolute ethanol, Section 1.3.7) was added to the pellet and mixed vigorously.
9. Centrifugation was performed at 11.300 rpm at 4^{o}C for 5 minutes. The upper layer was discarded and the pellet maintained, and the washing step was repeated once more.

10. To rinse the pellet, 150µl 1×STE buffer (Section 1.3.7) was added and incubated at a room temperature for 15 minutes, and then, the upper liquid phase was transferred into a new microcentrifuge tube,

11. The divided samples (as stated in step 2) of the same virus strain were collected in one tube, then absolute ethanol was added as twice volume of the final solution and maintained at -20^{o}C for 60 minutes,

12. For the precipitation of the dsRNA, the centrifugation process of the tubes was done for 20 minutes at a temperature of 4^{o}C. The precipitated dsRNA was dried at a room temperature for 15 minutes in inverted position on a paper towel.

13. The pellet was dissolved in 30 µl ddH_2O, nucleic acid quantification was made in a spectrophotometer (Implen NanoPhotometer®) at 260 nm, and the dsRNA samples were kept at a temperature of -20^{o}C until used.

3.2.6. Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

A single step RT-PCR was performed to amplify the partial NIb and CP regions of BCMNV isolates. Degenerate primers were used for the amplification of the partial BCMNV genome (Table 3.3). These primers were obtained from the previous studies conducted by Melgarejo et al. (2007) on the amplification of the partial NIb and CP regions of the strains of BCMV and BCMNV.

Table 3.3. Degenerate Primers used for amplification of the partial NIb and Capsid Protein (CP) Regions of Bean common mosaic necrosis virus and Bean common mosaic virus

			The Size of Expected PCR Product (bp)		
Virus	**Code**	**Primer Sequence (5'….3')**	**NIb**	**CP**	**Total**
BCMV	DgF	GARRAGCHCCRTAYATAGCAGA	162	495	657
	DgR	GCTTTGCATTTYCAACCATTGG			
BCMNV	DgF	GARRAGCHCCRTAYATAGCAGA	162	417	579
	DgR	GCTTTGCATTTYCAACCATTGG			

For single step RT-PCR, the following mixture was undertaken (Table 3.4). Before mixing components of RT-PCR, dsRNAs were denatured at 80^{o}C for five min and then immediately chilled in ice for 10 min.

Table 3.4. Reaction mixture of Single-step reverse transcription-polymerase chain reaction

Reaction Components	Volume (µl)	Final Concentration
RNase-free H_2O	3.375	
5× QIAGEN OneStep RT-PCR buffer*	2	
dNTP Mix (10 mM)	0.4	400 µM of each dNTP
Primer DgF (10 µM)	0.6	0.6 µM
Primer DgR (10 µM)	0.6	0.6 µM
QIAGEN OneStep RT-PCR Enzyme Mix	0.4	-
dsRNA	2.5	
Total	**10**	

* 5× QIAGEN OneStep RT-PCR buffer contains a total of 12.5mM $MgCl_2$.

The amplification was performed in MJ Mini Thermocycler (Bio-Rad) using the following reaction conditions.

Table 3.5. Single-step RT-PCR conditions used for BCMNV Identification

Steps	Temperature (ºC)	Duration	Number of Cycles
RT –Step	50	30 min	1
Initial denaturation	95	15 min	1
Denaturation	94	1 min	
Annealing	51	1 min	35
Extension	72	1 min 30sec	
Final Extension	72	10 min	1

3.2.7. Agarose Gel Electrophoresis

After the RT-PCR stage, the PCR products obtained were subjected to analysis by agarose gel electrophoresis. A total of 8 µl of the product was mixed with 2 µl of loading dye (6×TriTrack DNA Loading Dye, Promega). Then, the samples were loaded in 1% agarose gel containing 0.5 µg/ml ethidium bromide in a TBE buffer and run for an hour. Gel Doc 2000 (Biorad, USA) gel imaging system was used in the viewing of the DNA fragments after electrophoresis. The PCR products formed were photographed and used in further analysis of the nucleotide sequencing

3.2.8. Nucleotide Sequence Analysis of BCMNV isolates

PCR products that gave sufficient result in terms of quantity and quality were further analyzed by sequencing. The sequence data belonging to the partial NIb and CP

regions of BCMNV isolates were obtained by direct sequencing of the purified PCR products with the Sanger Method via a commercial company (BM Laboratories, Ankara). The raw data of nucleotide sequencing of PCR products were edited by Bio-edit Version 7.2, and multiple alignments of sequencing were performed by the CLUSTALW Method (Thompson et al. 1994) in Mega-11 Software. Also, the sequence data of each BCMNV isolates was subjected to BLASTn analysis available at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3.2.9. Phylogenetic Analysis

Phylogenetic analysis of the partial NIb and coat protein regions of BCMNV isolates (NWS and NL-5-like) was conducted in MEGA11 (Tamura et al., 2021). First of all, the sequences of BCMNV isolates and N-Wilga strain of Potato virus Y (PVY) isolate (Table 3.6) were retrieved from the National Centre for Biotechnology Information (NCBI) database. The N-Wilga strain of PVY was used as outgroup.

The evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei, 1992). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and are in the units of the number of base differences per site. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm (Nei and Kumar, 2000) at a search level of 1. The Neighbor-joining algorithm (Saitou and Nei, 1987) was used to generate the initial tree. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 519 positions in the final dataset.

Table 3.6. BCMNV and PVY isolates used in phylogenetic analysis

Accession Number	Isolate Name	Strain Name	Origin	References
MH169566	K1	-	Kenya	Musembi Mutuku et al., 2018
LC493096	-	BG12	Kenya	Unpublished
LC433691	NB2-11	TN-1	Kenya	Unpublished
KY659306	TN1a	-	USA	Feng et al., 2017
KY659305	1755b	-	USA	Unpublished
KY659304	NL-8 CA	-	USA-California	Feng et al., 2017
KX302007	TM70	-	Australia	Unpublished
HQ229994	-	NL8	USA	Larsen et al., 2011
HQ229993	-	NL5	USA	Larsen et al., 2011
AY138897	Washington	-	USA	Unpublished
AY864314	Kimberly	NL-3K	USA	Larsen et al., 2005
MN987555	MP-1	-	Zambia	Unpublished
MF078483	HXH-1	-	Tanzania	Unpublished
MK069983	CN3	-	Mexico	Unpublished
MG640399	INIFAP CN61	-	Mexico	Unpublished
NC_004047	Michigan	NL3(Necrotic)	USA	Fang et al., 1995
HG792063	PV 0413	-	UK	Unpublished
AY282577	Michigan	NL-3	USA	Unpublished
MN987556	EPb	-	Zambia	Unpublished
MH567340	BeCa8	-	Kenya	Unpublished
MF405187	HXH2- Pooled RNA	-	Tanzania	Unpublished
MH169564	N2	-	Kenya	Musembi Mutuku et al., 2018
MH169568	K3	-	Kenya	Musembi Mutuku et al., 2018
MF179113	SRF97	-	Australia	Unpublished
OK094708	BK4	-	India	Unpublished
MT350289	-	PVY N-Wilga	Slovenia	Unpublished

-: Not available; CB: Common bean; WCB: Wild Common bean; NB: *Nicotiana benthamiana*

4. RESULTS

4.1. Acquisition, Propagation and Maintenance of BCMNV Isolates

First of all, a BCMNV isolate named as NL-5-like, that had been obtained from snap bean samples collected from Antalya Province and maintained in bean seeds at 4^{o}C since the previous study (Arli-Sokmen et al., 2016), was tried to be re-acquired from a group of infected seeds. The bean seedlings obtained from the NL-5-like-infected seeds induced mosaic, leaf curling symptoms, which were typical phenotypes of BCMV or BCMNV on susceptible bean plants (Fig. 4.1). Therefore, symptomatic leaves of seed-grown plants were subsequently tested by DAS-ELISA using BCMV and BCMNV-specific polyclonal antisera to confirm that the plants had a single infection of BCMNV.

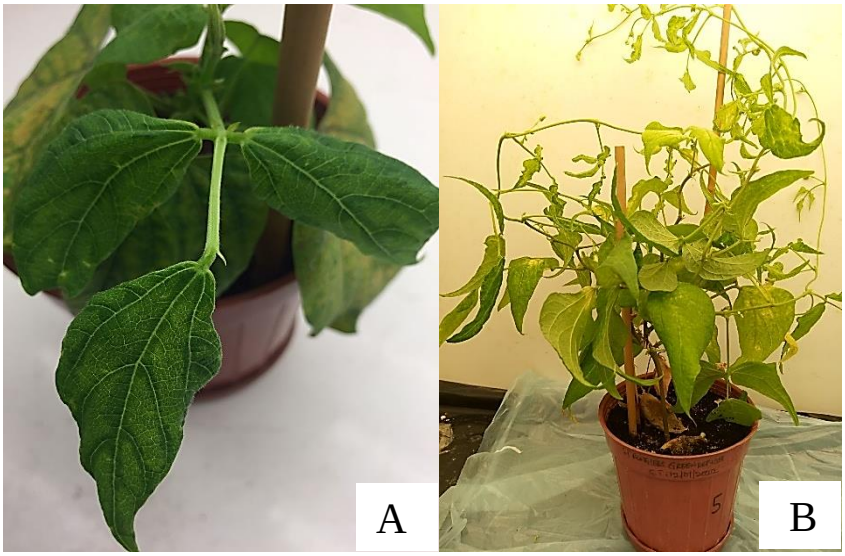

Figure 4.1. Typical mosaic and leaf curling symptoms of NL-5-like isolate on Sutter Pink (A) and Stringless Green Refugee (B)

Secondly, a commercial seed lot provided by the Department of Field Crops at Ondokuz Mayis University was subjected to a growing-on test during the thesis work. One of the young bean seedlings induced a distinct type of progressive necrotic symptom on primary leaf (Fig. 4.2A) and then severe mosaic, vein clearing and distortion on the upper trifoliate leaves (4.2B). Symptomatic leaves of this plant were tested by DAS-ELISA using BCMV, BCMNV and CMV antisera. The sample reacted positively against only BCMNV polyclonal antiserum in ELISA, and the mean absorbance value ($A_{405\ nm}$)

of the sample reached to 0.721 which was about 3 times higher than that of negative (healthy) controls (0.250). Thus, this new BCMNV isolate was named as NWS.

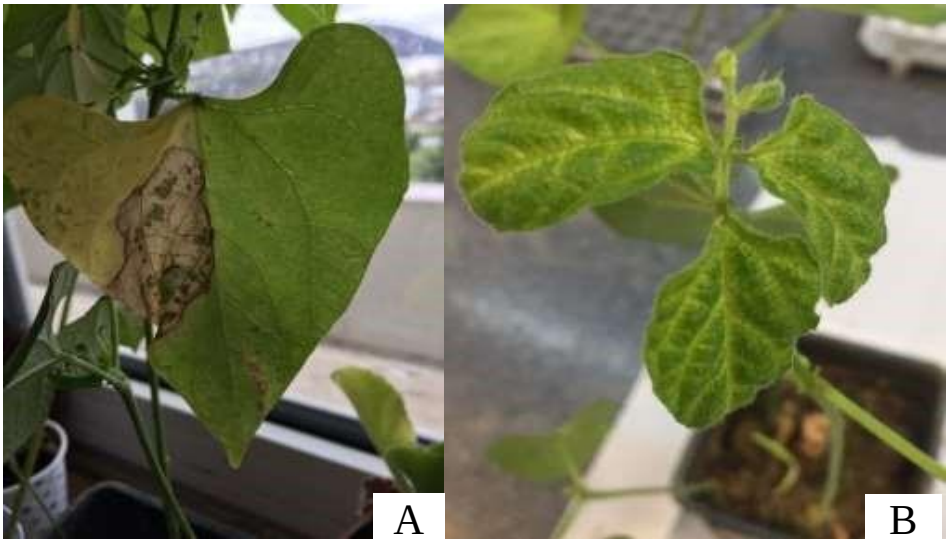

Figure 4.2. Common bean plant infected with a new isolate (NWS) of BCMNV showing a large necrotic lesion on primary leaf (A) and mosaic, vein clearing and distortion on trifoliate leaves (B)

4.2. Biological Characterization of BCMNV Isolates

A set of differential bean cultivars representative of the host groups (HGs) was used to evaluate the pathogroups of BCMNV isolates. First of all, the bean seedlings infected with NWS or NL-5-like isolate, were used as inoculum source to mechanically inoculate susceptible bean cultivars for virus propagation. The NWS isolate caused shortened nodes when inoculated on Stringless Green Refugee, which contains the recessive *i* and *bc-u* genes. This symptom was followed by rosetting, leaf mottling, mosaic, curling coupled with dwarfism on the plants (Fig. 4.3). BCMNV isolates were separately inoculated on 12 differential host cultivars for their pathogroup (PG) characterizations. According to the results of phenotypic scoring and DAS-ELISA, a differential bean cultivar that showed no symptom or induced symptom only on inoculated leaf and had a negative result for BCMNV in DAS-ELISA was described as resistant (R), while bean cultivar that had systemic symptom and/or positive result in DAS-ELISA was declared as susceptible (S). The reactions of the differential host plants against BCMNV isolates were given in Table 4.1.

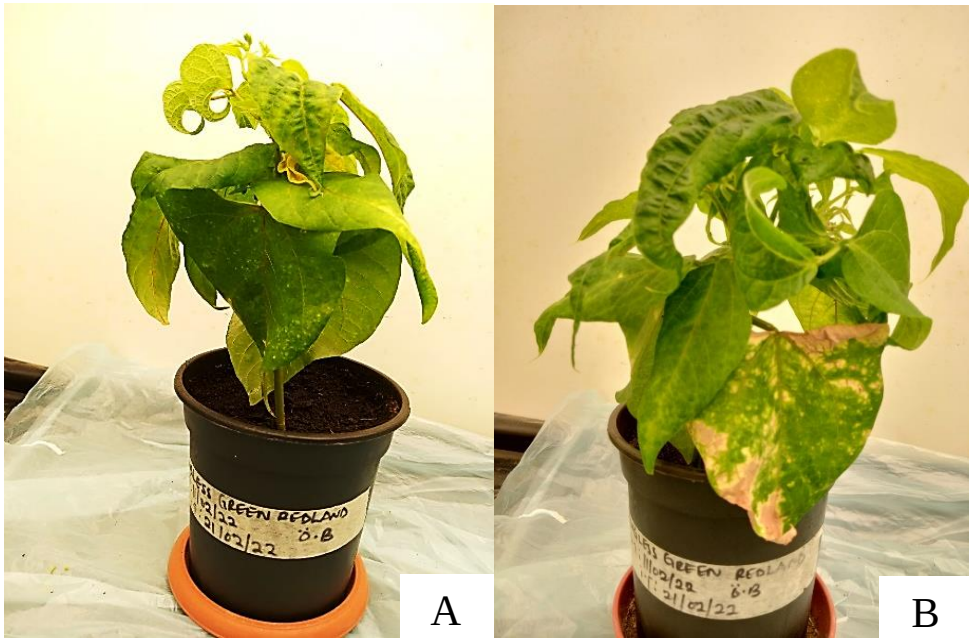

Figure 4.3. Mosaic, leaf mottling, curling and rosette-like symptoms observed on bean cv. Stringless Green Refugee after inoculation with the NWS isolate of BCMNV (A and B)

4.2.1. Reactions of Differential Bean Cultivars to the NL-5-like isolate of BCMNV

The NL-5-like isolate of BCMNV elicited mosaic symptoms in susceptible cvs. Sutter Pink (HG 0), Stringless Green Refuge (HG 1), Redland Greenleaf C (HG 2), Redland Greenleaf B (HG 3), Sanilac (HG 4), Pinto UI 114-8 (HG 5), Black Turtle Soup (BTS)-1 (HG 8), Jubila (HG 9a), Improved Tendergreen (HG 9b) and Isabella (HG 10).

Table 4.1. The Reactions of Differential Bean Hosts against BCMNV Isolates

Host Groups (HG)	**NWS**	**NL-5-like**	**NL-3$^+$**	**NL-5$^+$**
0. Dubbele Witte/ Sutter Pink	S	S	S	S
1. Stringless Green Refugee	S	S	S	S
2. UI-36 Red Max/ Redlands Greenleaf C	S	S	S	S
3. Redlands Greenleaf B	S	S	S	S
4. Pinto UI-111	S	S	S	S
5. Pinto UI-114-8	S	S	S	S
6. Othello	R	R	R	R
7. IVT-7214	R	R	R	R
8. Widusa/Black Turtle Soup	S*	S*	S*	S*
9a. Jubila	-	S*	-	-
9b. Improved Tendergreen 40031	S*	S*	S*	S*
10. Amanda/Isabella	S*	S*	S**	S*
11. IVT 7233	R	R	R*	R*

+: Information on the reaction of differential hosts to NL-3 and NL-5 was obtained from Drijfhout (1978). R: Resistant, S: Susceptible, S*: Susceptible, systemic necrosis at a temperature less than 30^{o}C, S**: Susceptible, systemic necrosis at a temperature higher than 30^{o}C, R*: Resistant, no systemic necrosis, pinpoint necrotic lesion, -: No information available.

On cv. Sutter Pink (HG 0), general chlorosis, chlorotic lesions, mosaic, leaf mottling and reduced in leaf size were observed. The reactions of cv. Sutter Pink and cv. Stringless Green Refugee (HG 1) were different. Stringless Green Refugee (HG 1) plants had elongated stem coupled with a tiny and few leaves, and a shoe-string symptom developed. Also, chlorosis and wrinkled leaves were dominant among the symptoms determined (Fig. 4.4). On Sutter Pink (HG 0), leaf distortion and mosaic were prominent symptoms.

UI-36 Red Max (HG 2) had no visible systemic symptoms on the upper non-inoculated leaves, except for yellowing of the inoculated leaves (Fig. 4.5). In Redland Greenleaf B (HG 3), chlorosis occurred on the inoculated leaf, while systemic chlorotic lesions appeared after a month of inoculation (Fig. 4.5). On Pinto UI-111 (HG 4), necrotic spots were present on the upper non-inoculated leaves, whilst the inoculated leaves had necrotic spots and chlorotic symptoms (Fig. 4.5).

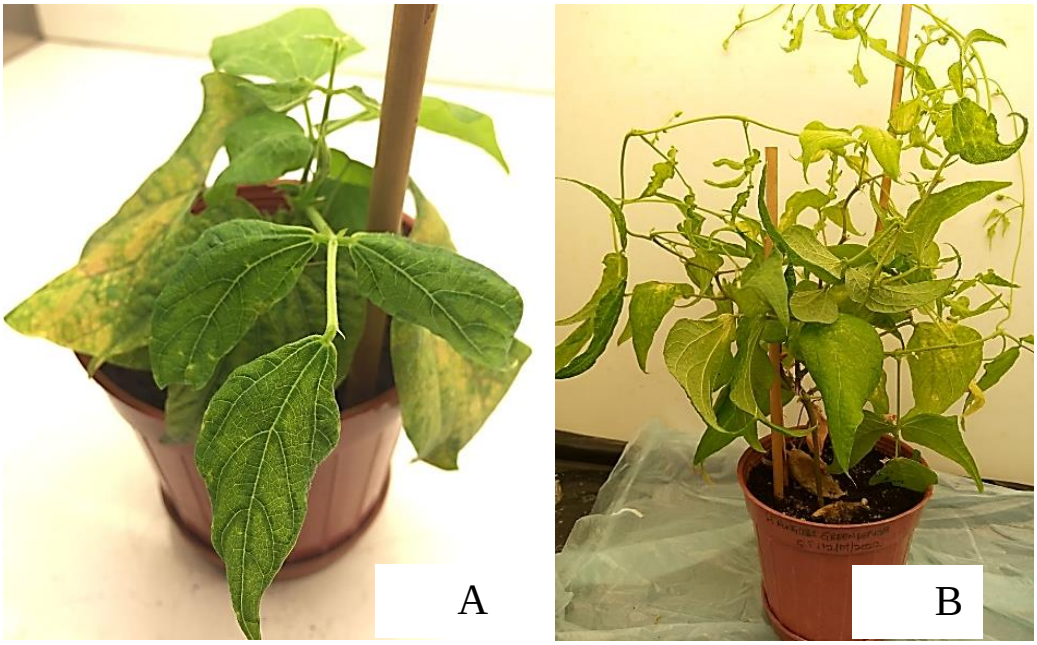

Figure 4.4. Mosaic and leaf distortion in cv. Sutter Pink (A), curling and shoe-string of leaves in cv. Stringless Green Refugee (B) 4 weeks after inoculation with NL-5-like isolate of BCMNV

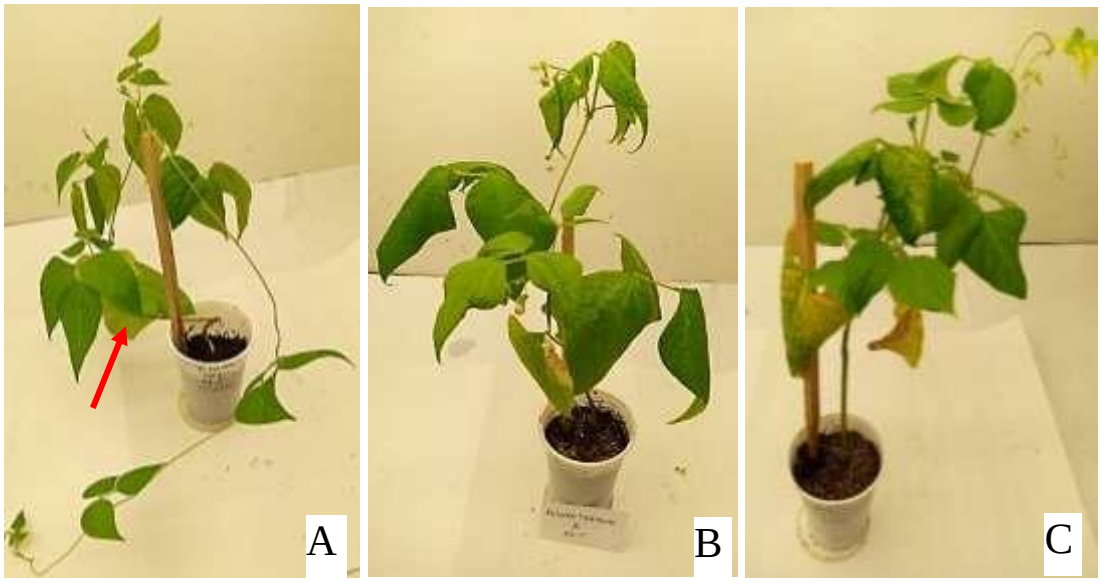

Figure 4.5. UI 36-Red Max had yellowing only on inoculated leaf (A), Redland Greenleaf B showed chlorosis (B) four weeks after inoculation, and Pinto UI-111 had induced necrotic spots on inoculated and non-inoculated upper leaves (C) three weeks after inoculation with NL-5.

Bean cv. Pinto 114-8 (HG 5) only showed yellowing and necrotic spots on the inoculated leaves, but no symptoms were observed on the upper leaves (Fig. 4.6).

Figure 4.6. Chlorosis and necrotic spot symptoms in cv. Pinto UI 114 (HG 5) a month after inoculation

Pin-point necrosis was observed on the inoculated leaves of cv. Black Turtle Soup-1 (HG 8) three days after inoculation, followed by vein and top necrosis on upper leaves seven days after inoculation (Fig. 4.7). Top necrosis also occurred in cvs. Jubila (HG 9a) and Improved Tendergreen (HG 9b) at the first week of inoculation at temperatures of 20-25°C on (Fig. 4.8). The partial necrosis on the upper leaves and necrosis on the bean pods of cv. Jubila were observed a month after inoculation with the NL-5-like isolate (Fig. 4.9).

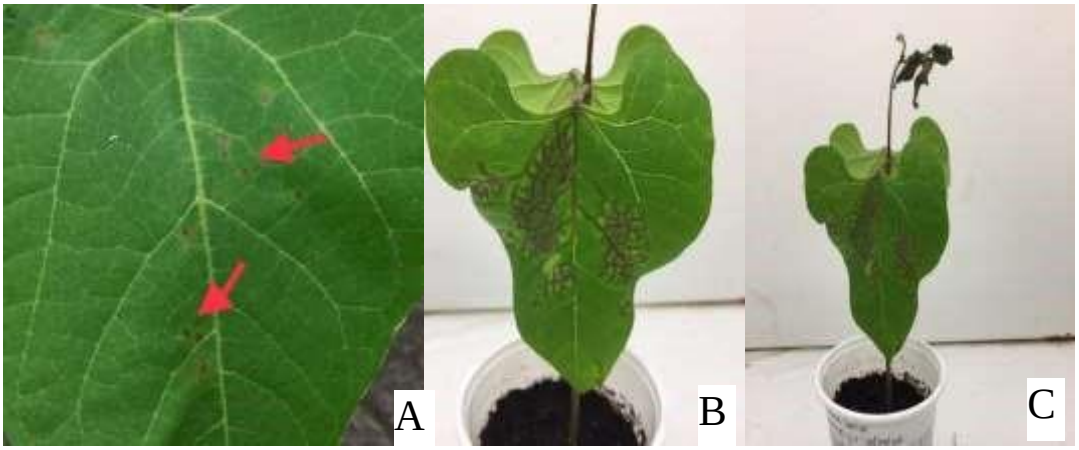

Figure 4.7. Pin-point necrotic lesions (A), vein necrosis on inoculated leaves of Black Turtle Soup-1 (B) and top necrosis (C) at 20-25ºC a week after inoculation with NL-5-like

Three days after inoculation, pin-point necrotic lesions were occurred on inoculated leaf of cv. Isabella (HG 10) (Fig. 4.10). Partial and progressive necrosis of the upper non-inoculated leaves appeared on Isabella 5 weeks after inoculation with NL-5-like isolate (Fig. 4.10).

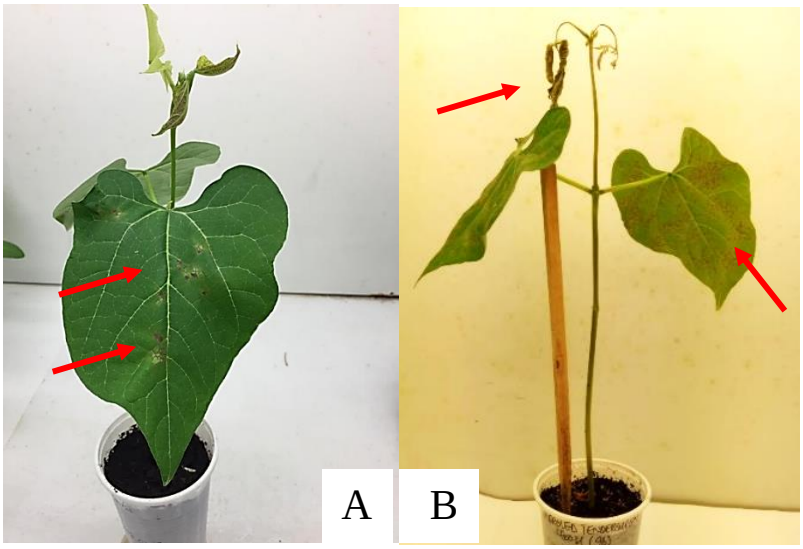

Figure 4.8. Necrotic lesions and vein necrosis on inoculated leaves, top necrosis on Jubila (HG 9a) (A) and Improved Tendergreen (HG 9b) (B) a week after inoculation with NL-5-like at 20-25ºC

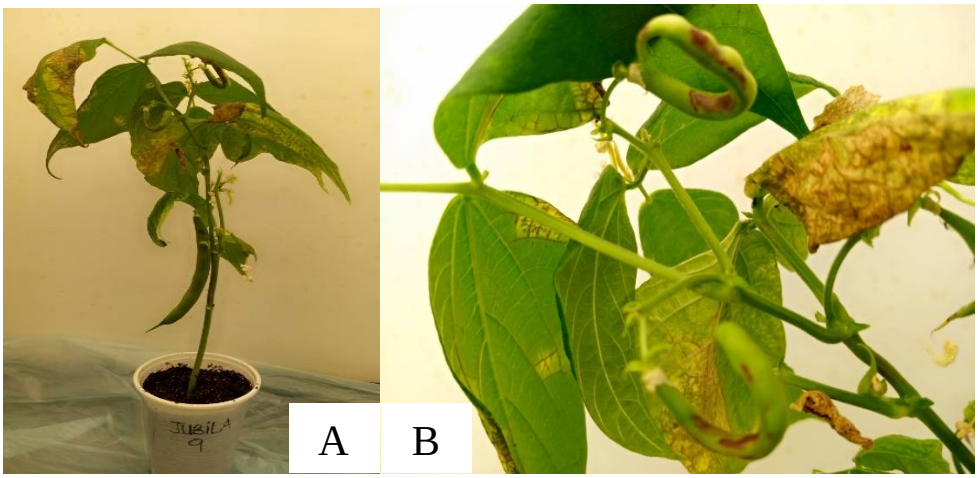

Figure 4.9. Systemic necrosis (A), necrotic spots on pods (B) of Jubila (HG 9a) infected with the NL-5-like isolate of BCMNV

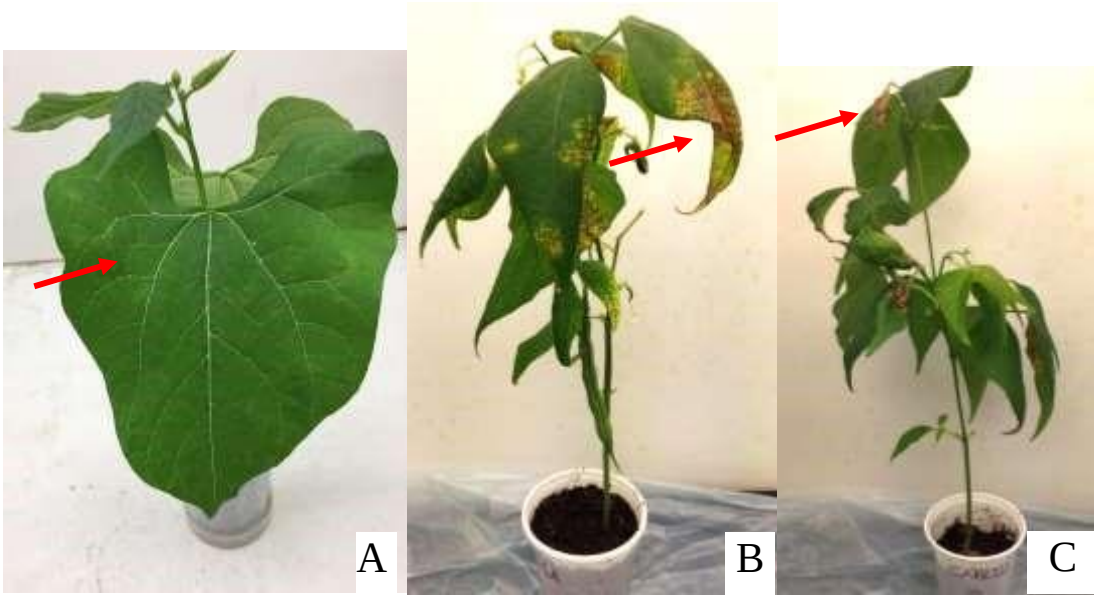

Figure 4.10. Pin-point necrotic lesions on inoculated leaf (A) at three days post- inoculation, progressive systemic necrosis (B) of Isabella (HG 10) (C) at five days post-inoculation with the NL-5-like isolate at 20-25^{o}C

The phenotypic evaluations in terms of interactions of the pathogenicity genes of BCMNV with the resistance genes in common bean cultivars and the assessment through ELISA (Appendix 1) suggested that the NL-5-like isolate belongs to Pathogroup VI.

4.2.2. Reactions of Differential Bean Cultivars to the NWS isolate of BCMNV

UI-36 Red Max (HG 2) had yellowing symptoms on virus-inoculated leaves, whilst some non-inoculated leaves had necrotic spots, chlorotic lesions (Fig 4.11). Three weeks after inoculation, chlorotic lesions, mosaic and leaf mottling were observed on inoculated leaves, whilst systemic mild chlorosis and leaf mottling coupled with a reduced leaf size were observed on bean cv. UI-111 (HG 4) (Fig. 4.12)

Bean cv. Pinto UI 114-8 (HG 5) showed necrotic spots on the inoculated leaves, while the upper non-inoculated leaves had symptoms of mild chlorosis (Fig 4.13).

In Othello (HG 6), vein necrosis was observed on the inoculated leaves (Fig 4.11), and chlorosis and chlorotic lesions were observed on the upper non-inoculated leaves three weeks after inoculation.

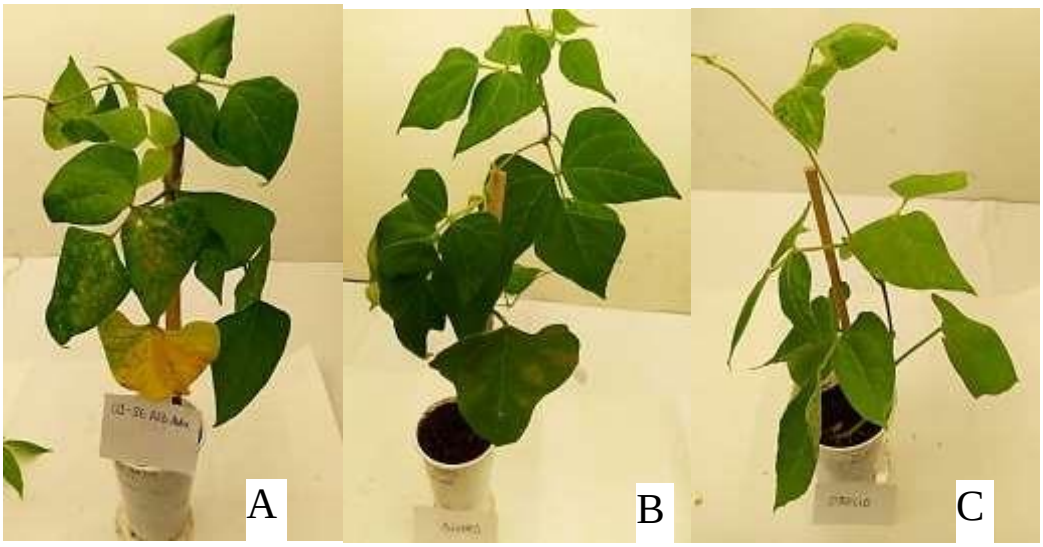

Figure 4.11. Systemic mosaic, necrotic spots on UI-36 Red Max (HG 2) (A), necrotic lesions on inoculated leaf (B) and systemic chlorotic lesions (C) in Othello (HG 6) three weeks after inoculating with the BCMNV NWS isolate

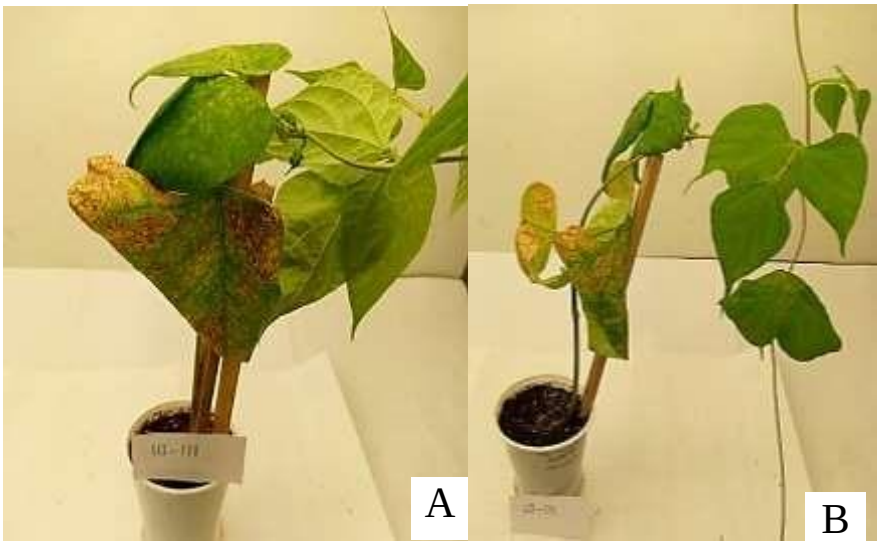

Figure 4.12. Systemic necrotic and chlorotic symptoms on UI-111 (HG 4) three weeks after inoculation with the BCMNV NWS isolate (A and B)

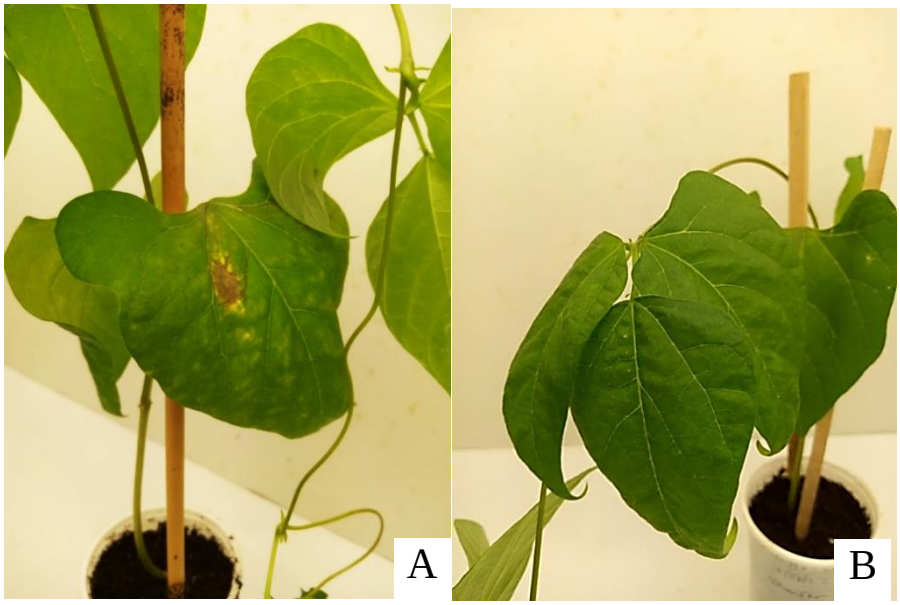

Figure 4.13. Necrotic symptoms on inoculated leaf (A) and leaf distortion on non-inoculated leaves (B) on Pinto UI 114 (HG 5) after inoculation with the BCMNV NWS (A and B)

Bean cvs. Widusa (HG 8) and Improved Tendergreen (HG 9b), which have the dominant *I* gene, and a combination of the dominant *I* gene and *bc-1* gene, respectively, showed vein necrosis on the inoculated leaves and top necrosis seven days after inoculation with the NWS isolate of BCMNV (Fig 4.14).

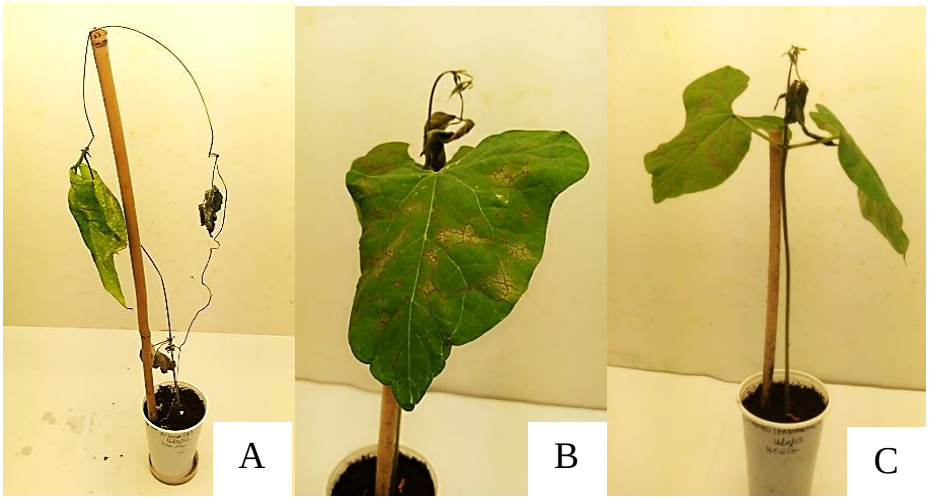

Figure 4.14. Whole plant necrosis on Widusa (A), vein necrosis on inoculated leaf (B) and top necrosis on Improved Tendergreen (C) (HG 9a) a week after inoculating with BCMNV NWS isolate at 20-25ºC

Amanda (HG 10), which has the *I* and *bc-1*^{2} genes, had a few pinpoint necrotic spots on the inoculated leaves after four days (Fig. 4.15A). However, vein necrosis was observed on the inoculated leaves, and top necrosis were discovered within two weeks post inoculation (Fig. 4.15B). Also, another Amanda plant experienced the partial necrosis on the upper leaves (Fig 4.15C) after three weeks of inoculation. On the fourth week post inoculation, necrosis on the upper leaves was more evident (Fig. 4.15D).

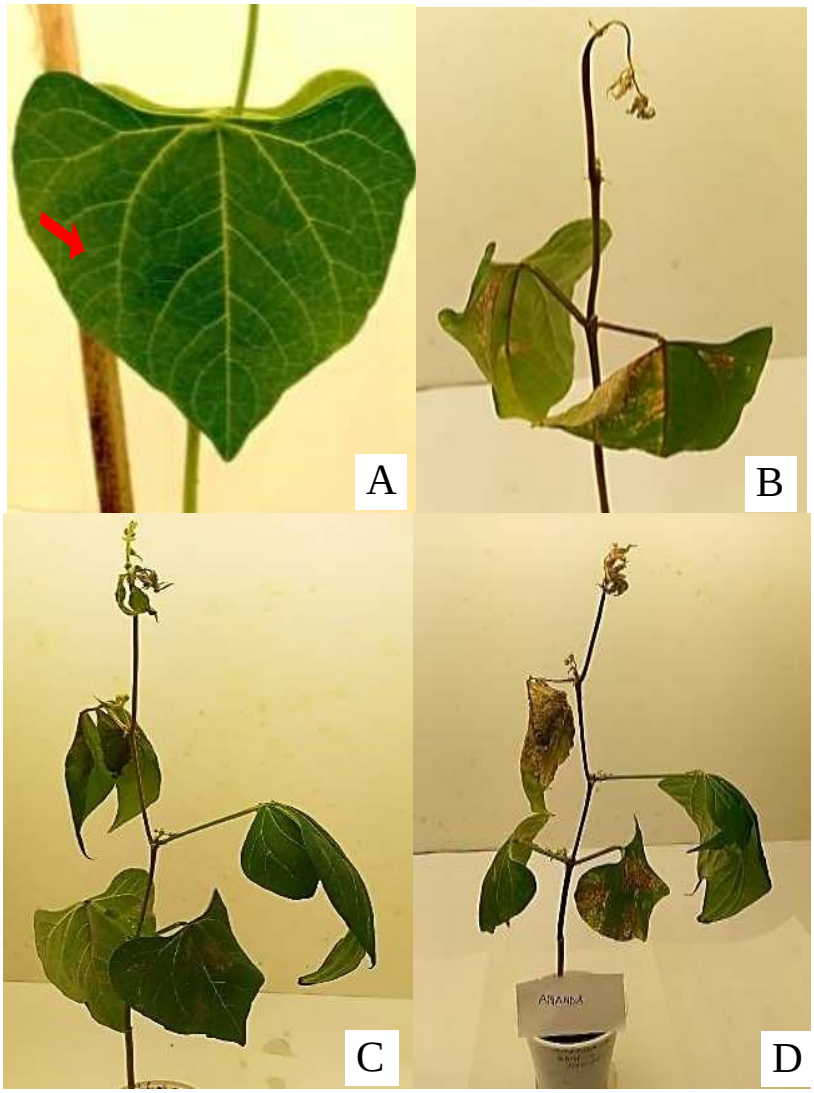

Figure 4.15. Pin-point necrotic lesions on inoculated leaves of Amanda at four days post-inoculation (A), vein necrosis and top necrosis on Amanda two weeks after inoculation (B) and progressive systemic necrosis after three (C) and four weeks (D) after inoculation with NWS at 20-25ºC

IVT 7233 (HG 11) had sporadic cases of leaf mottling on its leaves (Fig 4.16). This symptom was observed three weeks after inoculation with the NWS isolate of BCMNV. However, after DAS-ELISA applied for BCMNV, negative results were obtained. According to the results of differential hosts and DAS-ELISA (Appendix-1), NWS was identified to be in Pathogroup VI, as the NL-5-like isolate of BCMNV.

Figure 4.16. Mottle-like symptom on IVT 7233 at three weeks post-inoculation with the NWS isolate of BCMNV

4.3. Reactions of Soybean Cultivars to the NL-5-like Isolate of BCMNV

A total of 20 soybean cultivars were screened for their reactions to BCMNV. Symptoms were weekly recorded from 7 to 35 days after virus inoculation. A week after inoculation, no symptoms were observed on both inoculated and non-inoculated leaves of the soybeans. However, symptoms like chlorotic and/or necrotic lesions began to be visible on the inoculated leaves of some cultivars after two weeks (Fig. 4.17-4.18). Symptoms were more prevalent at three weeks after inoculation.

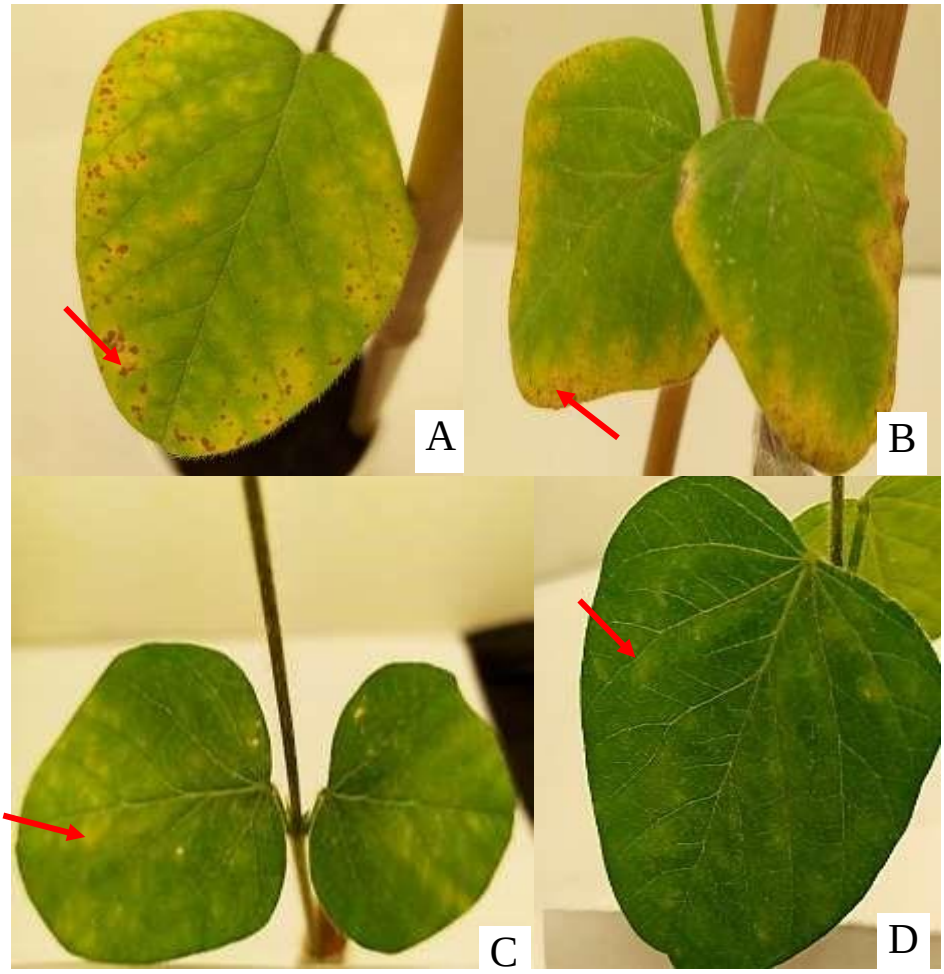

Figure 4.17. Necrotic lesions on inoculated primary leaves of SA-88 (A) and Ilksoy (B), chlorotic lesions on inoculated leaves of Samsoy (C) and May 5451(D) two weeks after inoculation

The systemic symptoms on non-inoculated upper leaves were quite different from those detected on the inoculated leaves. More than 50% of the 20 cultivars of soybean tested had prevalent symptoms of BCMNV such as mosaic, chlorosis, reduced leaf size and leaf mottling. Nevertheless, these symptoms were more pronounced in Adasoy, Arisoy, Ataem, Atlas 3616, Blaze, Cinsoy, Gapsoy-16, May 5451, Nazlican, Ohio, Prota-Y, SA-88 and Samsoy (Fig. 4.19-4.22) at the fifth week following inoculation.

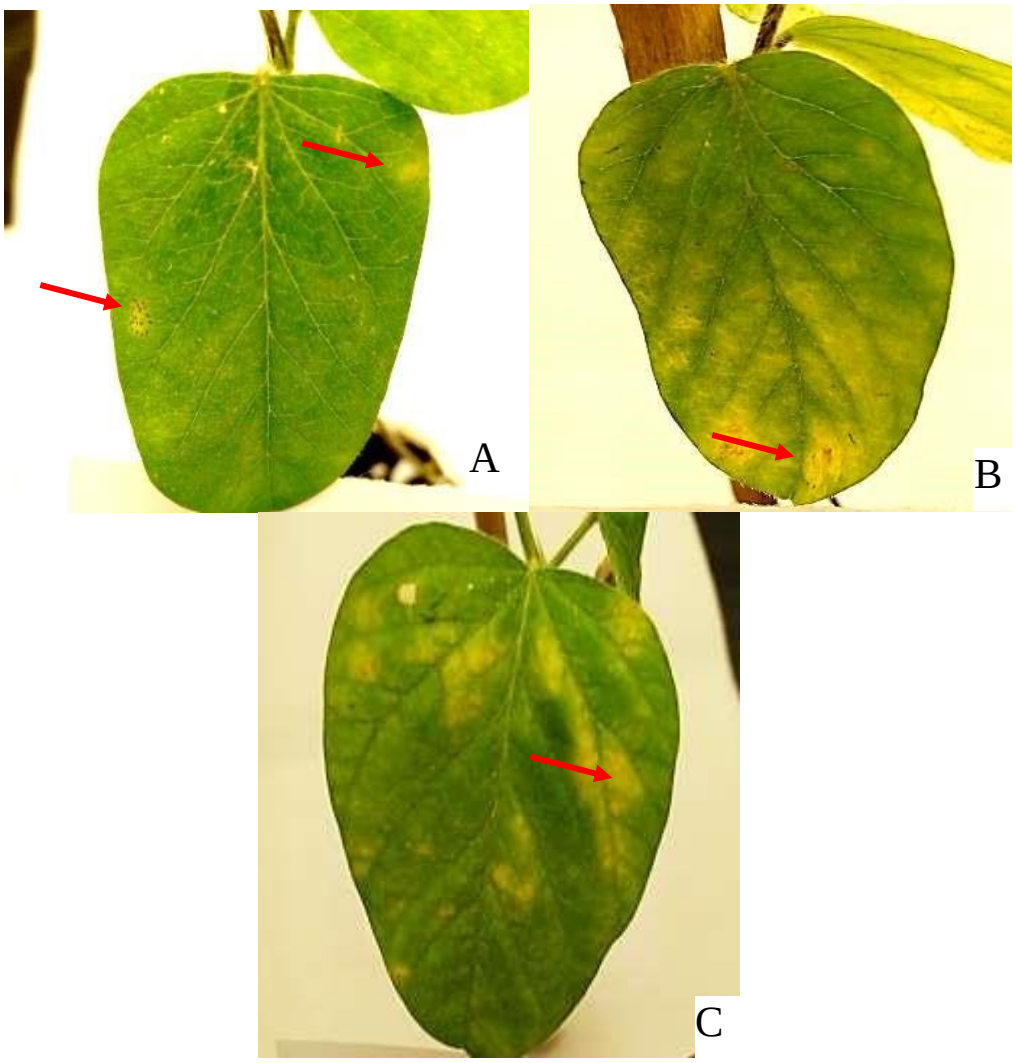

Figure 4.18. Pin-point necrotic lesion on inoculated primary leaves of Soy Anam (A, B) and chlorotic patches on primary leaf of Ohio (C) two weeks after inoculation

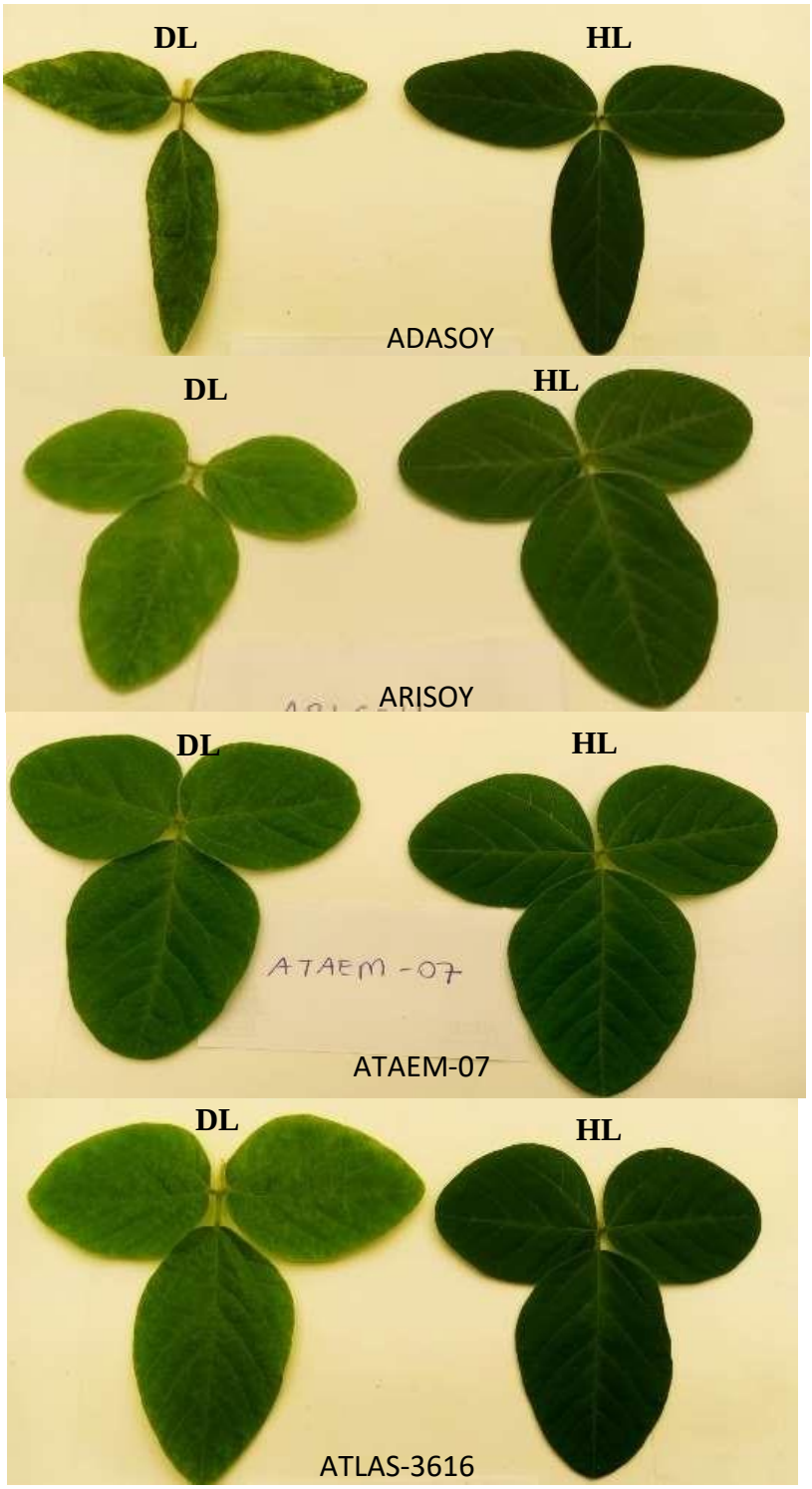

Figure 4.19. Soybean cvs. Adasoy, Arisoy and Atlas 3616 showing clear mosaic, and Ataem07 with leaf crinkling symptoms at five weeks post-inoculation. **DL**: Diseased Leaf; **HL**: Healthy Leaf

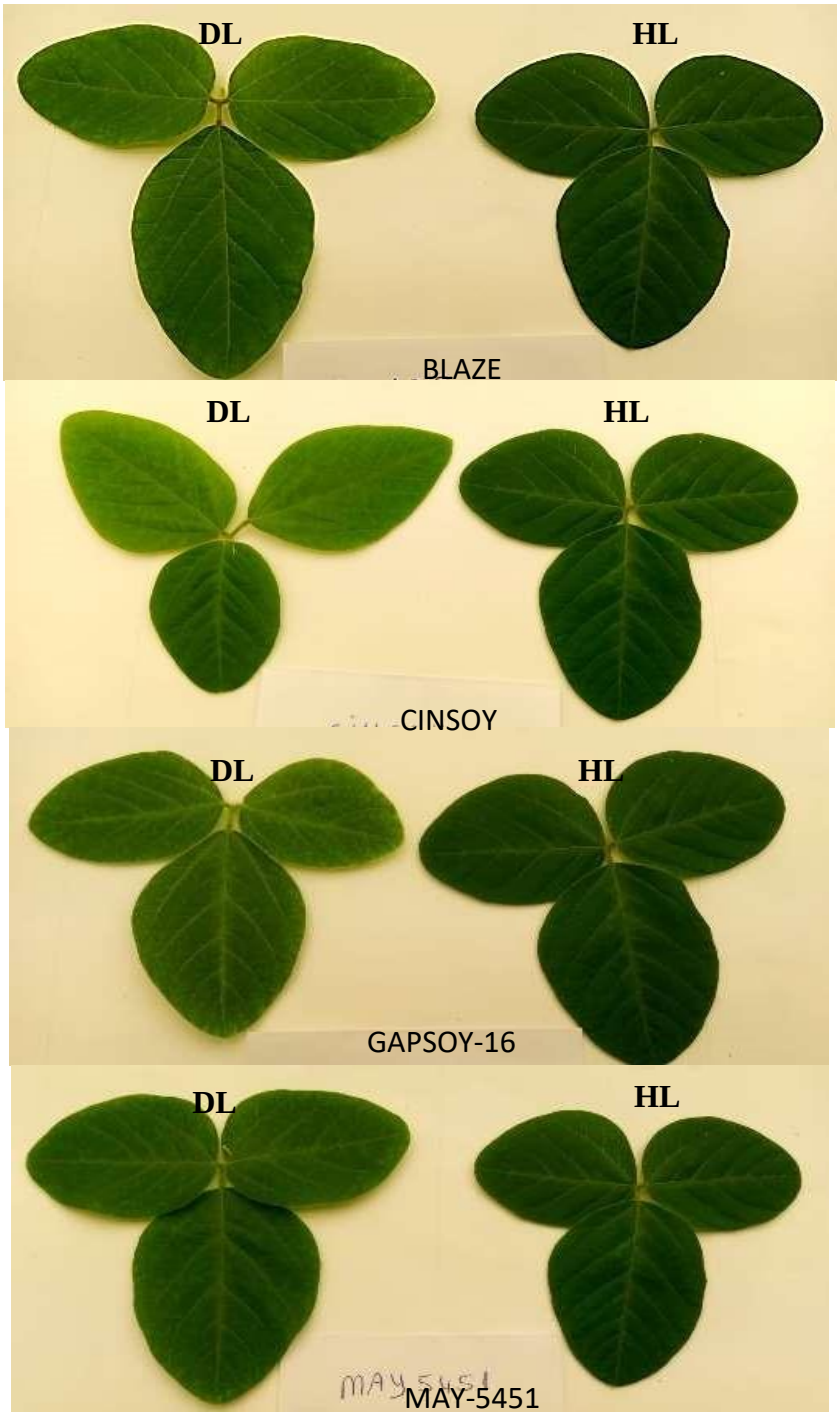

Figure 4.20. Soybean cvs. Blaze, Cinsoy, Gapsoy-16 and May-5451 showing mild mosaic and leaf crinkling symptoms at five weeks post-inoculation **DL**: Diseased Leaf; **HL**: Healthy Leaf

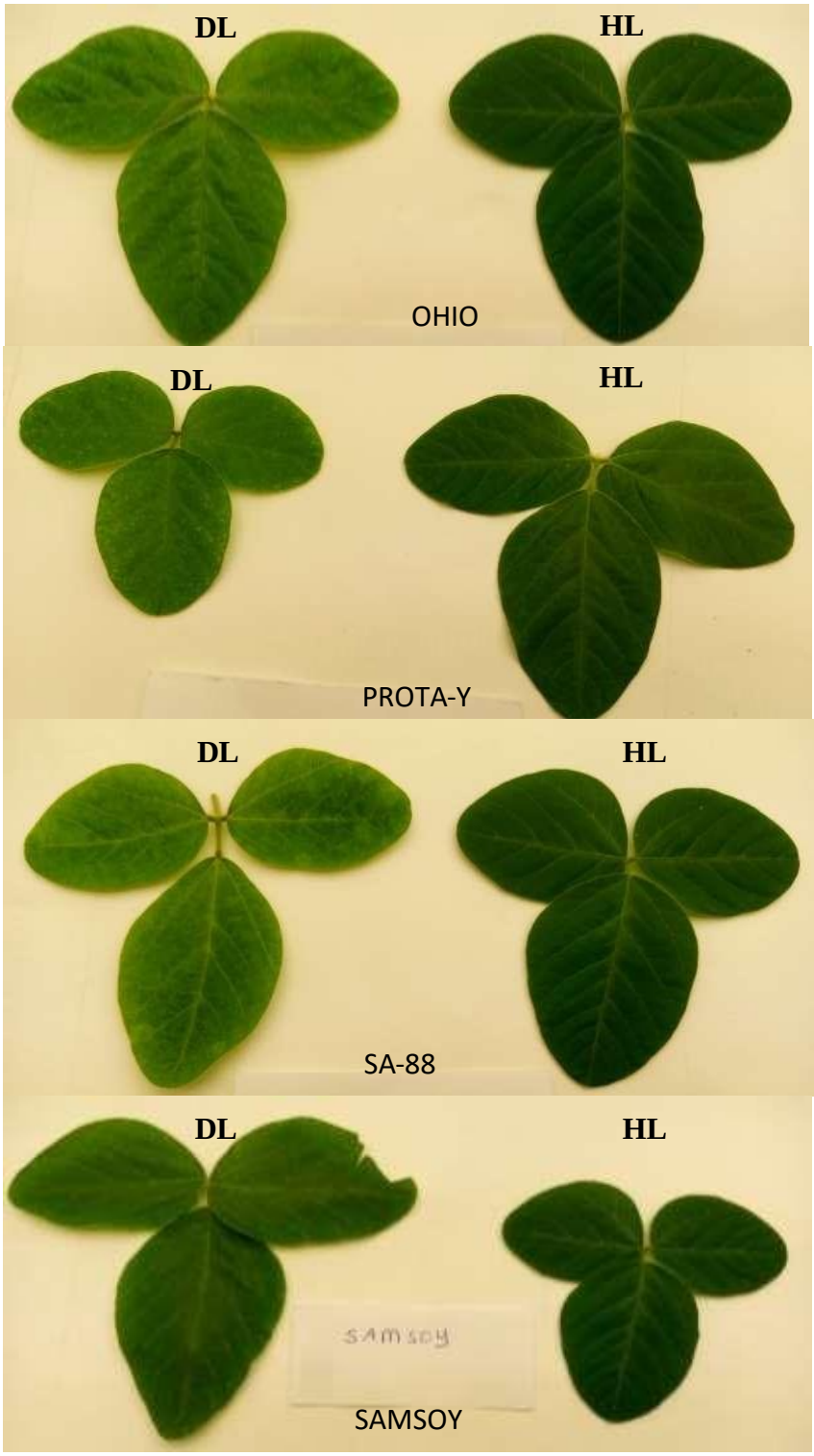

Figure 4.21. Soybean cvs. Ohio, Prota-Y, Samsoy indicating leaf crinkling symptoms and SA-88 with severe mosaic symptoms at five weeks post-inoculation **DL**: Diseased Leaf; **HL**: Healthy leaf

Figure 4.22. Soybean cv. Nazlican inducing chlorotic spots and light green leaf colour at five weeks post-inoculation

Also, DAS-ELISA was performed twice to detect the presence of BCMNV infection in soybean cultivars at three- and five-weeks post-inoculation. Although soybean cvs. Prota-Y and Nazlican expressed virus-like symptoms (chlorotic spots) (Fig. 4.21-4.22), and also Gapsoy-16 (Fig. 4.20) and Soy Anam exhibited crinkles only on the upper trifoliate leaves of some replicates, these cultivars were found to be negative at the third and the fifth week's ELISA (Table 4.2). According to phenotypic evaluations and ELISA-based screening (Appendix-2), out of 20 soybean cultivars, 12 (Adasoy, Arisoy, Ataem, Atakişi, Atlas-3616, Blaze, Cinsoy, Ilksoy, May-5451, Ohio, SA-88 and Samsoy) were found to be susceptible to BCMNV. On the other hand, the virus was not detected in eight cultivars (Gapsoy-16, Mona, Nazlican, Prota-Y, Safir, Soy Anam, Srebrina and Umut-2002) (Appendix-2). The detailed results of biological and serological tests belonging to each replication of soybean cultivars; namely, symptoms on non-inoculated upper leaves and DAS-ELISA results of each replication have been summarized in Table 4.2.

Table 4.2. Systemic symptoms and the results of ELISA of Soybean Cultivars

Cultivars	Systemic Symptoms					Results of DAS-ELISA				
	1	2	3	4	5	1	2	3	4	5
Adasoy	-	+	+	+	+	**P**	NT	NT	NT	**P**
Arisoy	+	+	+	-	+	**P**	N	NT	**P**	N
Ataem	-	+	+	-	-	NT	N	N	NT	**P**
Atakişi	-	+	+	+	-	**P**	NT	N	N	NT
Atlas 3616	+	+	+	-	+	NT	N	**P**	N	N
Blaze	+	+	+	+	+	NT	NT	**P**	NT	N
Cinsoy	+	+	+	+	+	N	**P**	NT	NT	NT
Gapsoy 16	-	+	+	-	-	N	N	N	NT	N
Ilksoy	+	+	+	+	+	NT	**P**	N	NT	NT
May 5451	+	+	+	+	+	NT	N	NT	**P**	N
Mona	-	-	-	-	-	N	N	N	N	N
Nazlican	+	-	+	+	+	N	N	N	N	N
Ohio	+	+	+	+	+	**P**	NT	NT	NT	**P**
Prota	+	+	+	+	+	N	N	N	NT	N
SA-88	+	+	+	+	+	**P**	NT	**P**	NT	NT
Safir	-	-	-	-	-	NT	N	NT	N	NT
Samsoy	+	+	+	+	+	**P**	N	NT	N	N
Soy Anam	+	-	+	+	+	N	NT	N	N	N
Srebrina	-	-	-	-	-	NT	N	NT	N	N
Umut 2002	-	-	-	-	-	N	N	N	N	N

+: Present -: Absent **P**: Positive **N**: Negative **NT**: Not tested. Grey areas show positivity.

As seen in Table 4.2, the positivity in ELISA along with systemic symptom appearance helped to evaluate soybean cultivars as susceptible (S) or resistant (R). Also, numbers 1-5 represent the replicates of soybean cultivars. At the third week of testing, only four cultivars (Adasoy, Blaze, SA-88 and Ohio) were found to be positive, whereas seven other cultivars (Arisoy, Atlas 3616, Ataem, Atakişi, Cinsoy, Ilksoy, May 5451) were positive for BCMNV at the fifth weeks's evaluation. Soybean cv. Samsoy was identified to be infected six weeks after inoculation.

4.4. Amplification of The Partial NIb and Capsid Protein Regions of BCMNV Isolates

dsRNA extractions were carried out using the common bean plants infected with the NL-5-like and NWS isolates as described in Method 3.2.6. In order to amplify the partial NIb and coat protein regions of BCMNV from the isolated dsRNAs, a single step RT-PCR method was used. In the first attempt to amplify the partial NIb and coat protein

regions, a 2 µl of the undiluted dsRNAs of NL-5-like isolate was used in 10µl RT-PCR reaction, and an expected size (579 bp) of PCR product was obtained (Fig 4.23). Then, undiluted dsRNAs extracted from the common bean plant infected with NWS isolate, and from the healthy bean plant were used in the same volume of PCR reaction. However, no PCR product was obtained with dsRNA samples extracted from the NWS-infected plants, both dsRNA samples of infected and healthy plants had primer-dimers (Fig 4.24). When the dilution of the extracted dsRNAs belonging to the NL-5-like isolate (dilution, 1:1) and the NWS isolate (dilution, 1:5), depending on their RNA concentrations (Table 4.3) for the same volume of RT-PCR was tried, it gave satisfactory and clear products at the expected size (Fig 4.25), with a highly reduced amount of primer-dimer formation.

Table 4.3. The results of spectrophotometric measurement of dsRNA concentrations

BCMNV/BCMV Isolate	**Concentration (µg/ml)**
NWS	56.1
NL-5-like	22
BCMV	294

Moreover, the dilution of dsRNAs of NL-5-like (1:1), NWS (1:5) and a BCMV isolate (1:10), which was used as a comparison sample, generated the reasonable amount of PCR products above 500 bp, as expected with the degenerate primers. The DNA fragment of BCMV isolate was slightly higher than those of the other two (NWS and NL-5-like) isolates (Fig 4.26). The amount of primer-dimer was barely observed in the gel as a result of the dilution of RNA templates.

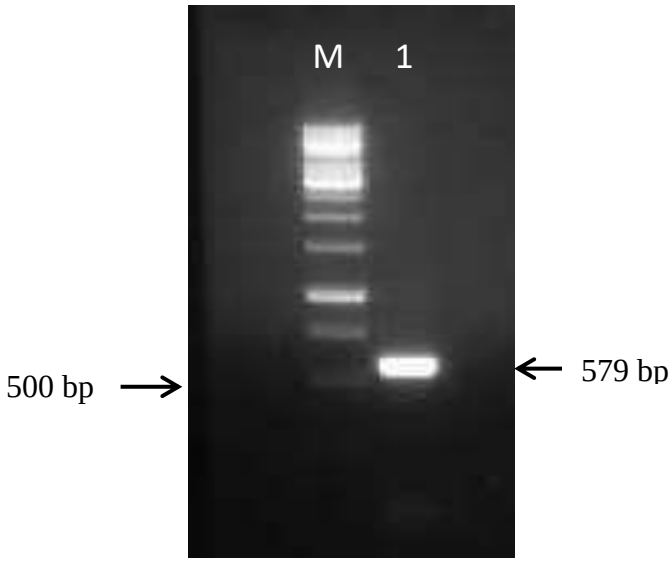

Figure 4.23. dsRNA amplification results of the partial NIb + Capsid Protein regions of the BCMNV-NL-5-like isolate. M: 1 Kb Ladder (Promega), 1: NL-5-like isolate

Figure 4.24. dsRNA amplification results of the partial NIb + Capsid Protein regions of the BCMNV-NWS isolate. M: 1 Kb Ladder (Promega), 1: NWS isolate, 2: Healthy bean plant

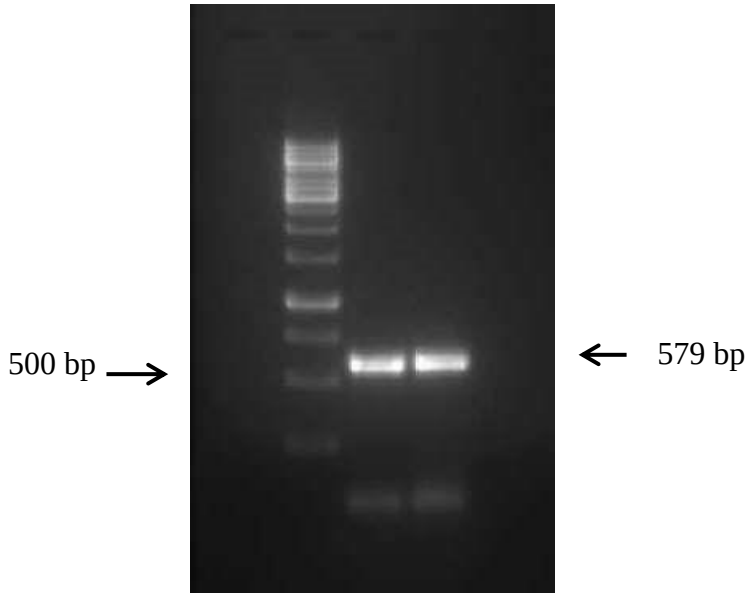

Figure 4.25. The diluted dsRNA amplification products of the partial NIb + Capsid Protein regions of BCMNV isolates. M: 1 Kb Ladder (Promega), 2: NL-5-like isolate (1:1 dilution) 2: NWS isolate (1:5 dilution)

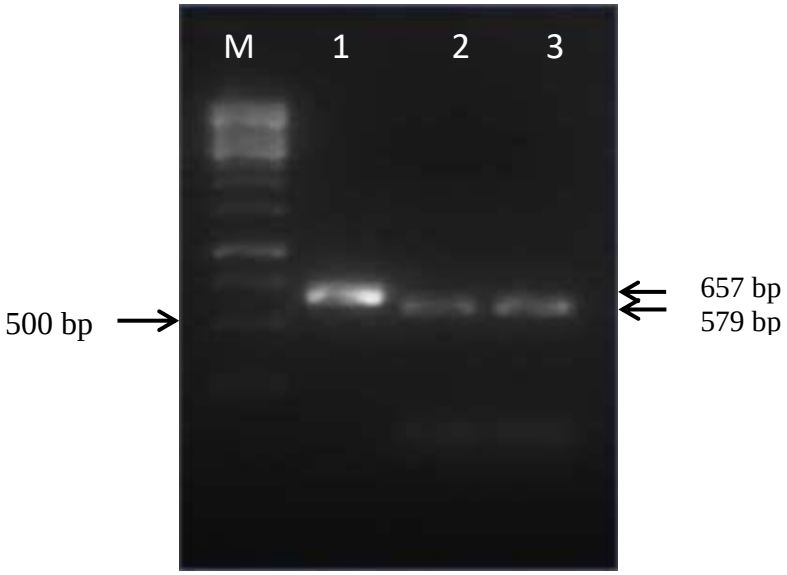

Figure 4.26. Diluted dsRNA amplicons of the partial NIb + Capsid Protein regions of BCMNV isolates, and BCMV isolate. M: 1 Kb Ladder (Promega), 1: BCMV isolate (1:10 dilution), 2: NWS (1:5 dilution), 3: NL-5-like (1:1 dilution)

4.5. BLAST Analysis of BCMNV Isolates

4.5.1. BLAST analysis of nucleotide sequences of BCMNV Isolates

A nucleotide sequence of 519 bp obtained after editing (Appendices-3 and -4) was used in this study, and the exact positions of the sequences on BCMNV polyprotein gene were determined by using the BLASTn analysis. The sequences of NWS and NL-5-like isolates covered the nucleotide position of 8458-8976, that was based on the reference isolate (GenBank Accession number-HQ229993). On the other hand, a BCMV isolate recovered by coincidence from the bean seed sample in this study and used in RT-PCR studies as comparison was also sequenced, although BCMV was not in the scope of the thesis's work. A nucleotide sequence of 658 bp (Appendix-5) was obtained after editing the sequences of the BCMV isolate, and corresponded the positions of 8760-9417 nt on the polyprotein region of BCMV NL-1 strain (Accession number: KM023744) (data not shown).

In order to analyze the genetic identities amongst the BCMNV isolates studied, and the other BCMNV isolates from the world, BLASTn algorithm was employed. It was observed that the NWS and NL-5-like isolates of BCMNV had 100% identity to each other (data not shown). Also, the partial NIb and CP regions of NWS and NL-5-like isolates were compared with other strains of BCMNV by the use of BLASTn analysis (Table 4.4). The results obtained by the NWS isolate were similar to those obtained by the NL-5-like isolate of BCMNV. The isolates NWS and NL-5-like had 100% nucleotide identity to NL-3 (AY282577, U19287) and NL-3K (AY864314) strains of BCMNV, which were from Michigan and Washington in the USA, respectively.

4.5.2. BLAST analysis of amino acid sequences of BCMNV Isolates

A deduced amino acid sequence for residues 172 corresponding to the partial NIb and coat protein regions was used in this study, and the exact positions of the amino acid sequences on BCMNV polyprotein were determined by using BLASTp analysis. The positions of the amino acid sequences of NWS and NL-5-like isolates covered the positions of 2764-2935 of the reference BCMNV isolate (GenBank Accession number-ADR80231).

BLASTp analysis of the amino acid sequences of NWS and NL-5 like isolates of BCMNV revealed a 100% identity to four strains of BCMNV (Table 4.5). The amino acid sequence analysis of NWS or NL-5 like isolates showed a higher amino acid identity to the NL-3 strain of BCMNV belonging to the USA. However, the least amino acid identity rates were obtained with the K1, N1, HXH-1 and 1A-F isolates of BCMNV, which were of Kenya, Kenya, Tanzania and Zambia, respectively. As such, the NWS and NL-5 like isolates bears a much higher similarity with BCMNV strains of the USA origin, and less similarity to strains of African origin.

Table 4.4. BLASTn analysis of the partial NIb and CP regions of BCMNV Isolates*

Isolate of This Study	NCBI Accession Number	Isolate Name	Strain Name	Country	Query Cover (%)	Identity (%)
	AY138897	Washington	-	USA	100.00	100.00
	AY282577	Michigan	NL-3	USA	100.00	100.00
	U19287	Michigan	NL-3 (Necrotic)	USA	100.00	100.00
	AY864314	Kimberly	NL-3 K	USA	100.00	99.42
NWS	HG792063	PV 0413	-	UK	100.00	99.23
	HQ229993	-	NL5	USA	100.00	99.04
	MH169563	N1	-	Kenya	100.00	98.46
	LC493096	-	BG12	Kenya	100.00	98.07
	MH169564	N2	-	Kenya	100.00	98.07
	MF179108	SRF122	-	Australia	100.00	98.07
	AY138897	Washington	-	USA	100.00	100.00
	AY282577	Michigan	NL-3	USA	100.00	100.00
	U19287	Michigan	NL-3 (Necrotic)	USA	100.00	100.00
	AY864314	Kimberly	NL-3 K	USA	100.00	99.42
NL-5	HG792063	PV 0413	-	UK	100.00	99.23
	HQ229993	-	NL5	USA	100.00	99.04
	MH169563	N1	-	Kenya	100.00	98.46
	LC493096	-	BG12	Kenya	100.00	98.07
	MH169564	N2	-	Kenya	100.00	98.07
	MF179108	SRF122	-	Australia	100.00	98.07

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

Table 4.5. BLASTp analysis of the partial NIb and coat protein amino acid sequences of NWS and NL-5- like isolates*

NCBI Accession Number	Isolate Name	Strain Name	Country	Query Cover (%)	Identity (%)
AAA62509	Netherlands	NL-3	USA	100.00	100.00
NP_660175	Michigan	NL-3 (Necrotic)	USA	100.00	100.00
AAN27999	Washington	-	USA	100.00	100.00
AAP38183	Michigan	NL-3	USA	100.00	100.00
CDK12644	PV 0413	-	UK	100.00	99.42
AAW50598	Kimberly	NL-3 K	USA	100.00	99.42
AYV99858	K1	-	Kenya	100.00	98.84
AYV99855	N1	-	Kenya	100.00	98.84
AWX66760	HXH-1	-	Tanzania	100.00	98.84
QNT09928	1A-F	-	Zambia	100.00	98.84

-: Not available, *: The first ten BCMNV isolates with the highest amino acid identities were given.

4.6. Phylogenetic Analysis of BCMNV Isolates

The phylogenetic tree consisting of BCMNV strains and PVY strain as outgroup generated two major clads. Clade I involved a cluster of BCMNV strains which are of East African origins (HXH-1, K1, EPb, HXH-2, BG12, TN-1, MP-1 and HXH-2). Their genomic similarities can be attributed to their geographical proximity. Also, the NWS and NL-5-like isolates of BCMNV determined to be closely related to each other by the BLAST analysis were found to be in the same sub-clade along with the isolates of BCMNV NL-3 strain (Fig 4.27). In Clade II, although most of BCMNV strains were of America origin, the couple of other strains from different origins were observed. It seems that the origin of plant virus plays a huge role in clade formations and clustering.

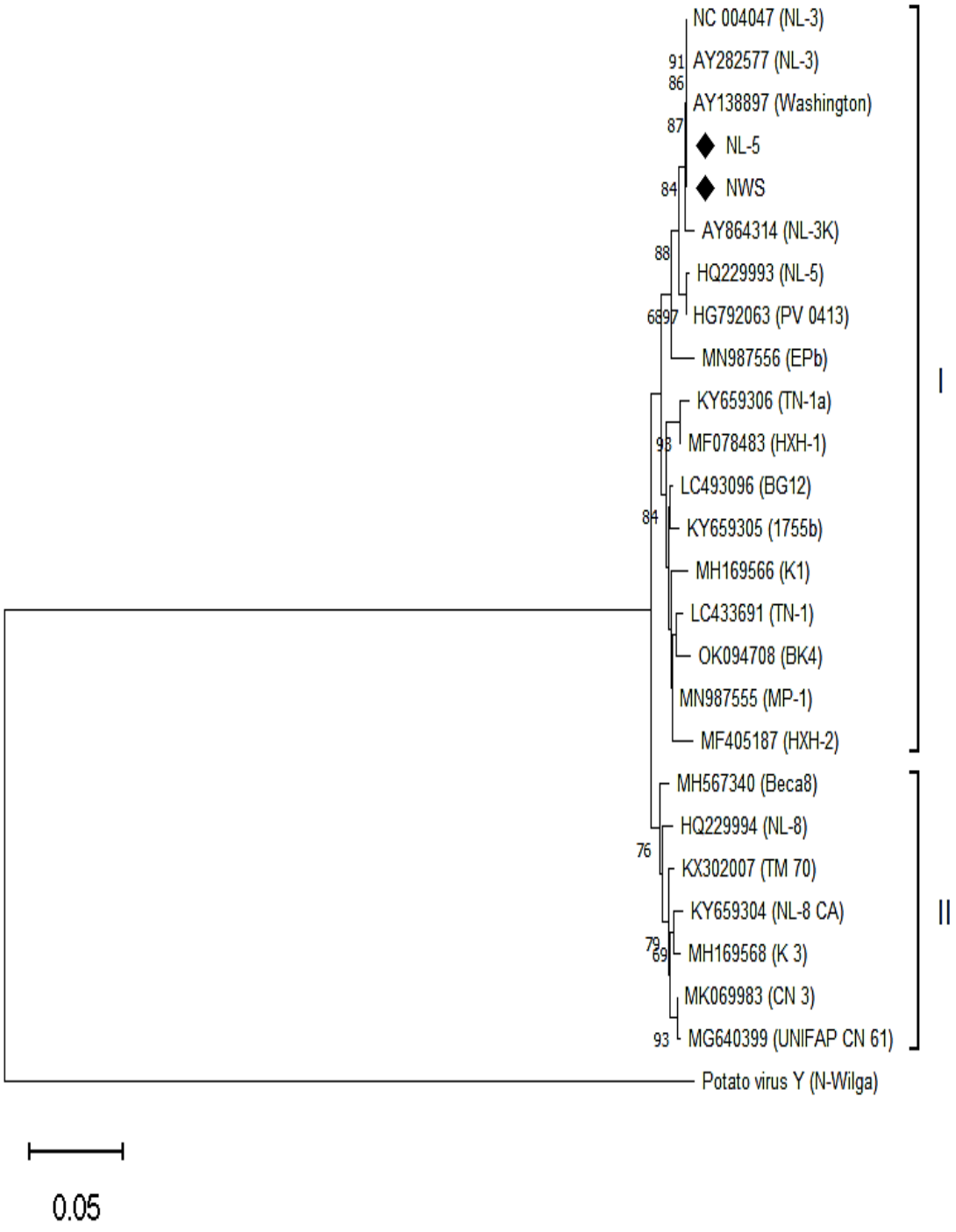

Figure 4.27. Phylogenetic analysis of the partial NIb and coat protein region of Bean common mosaic necrosis virus isolates (NWS and NL-5-like) conducted in MEGA11. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown below the branches (Felsenstein, 1985). Numerical values less than 60% were not shown. There were a total of 519 positions in the final dataset. The N-Wilga strain of Potato virus Y was used as outgroup.

5. DISCUSSION

In the first part of this study, the pathogenicity groups of two different BCMNV isolates (NL-5-like and NWS) were investigated by using 12 differential common bean cultivars, which carry various combination of recessive and dominant genes. The responses of bean cultivars were summarized in Table 4.1. Also, BCMNV isolates were molecularly detected and characterized for the first time in Turkey. In addition to these, the reactions of 20 commercial soybean cultivars to the NL-5 isolate of BCMNV were determined. This is the first study of screening soybean cultivars for their responses to BCMNV in Turkey.

After inoculating 12 differential common bean cultivars with the NWS isolate of BCMNV, three bean cultivars were responded as resistant. These cultivars were: Othello (HG 6) with the *bc-u, bc-1*^{2} and *bc-2*^{2} resistant genes (Fig 4.11C), IVT-7214 (HG 7) which has the *bc-u*, *bc-2* and *bc-3* resistant genes, and IVT-7233 (HG 11) with the resistant genes *I, bc-u*, *bc-1*^{2} and *bc-2*^{2}. IVT 7233 (Fig. 4.16) had a few chlorotic patches on its some upper leaves, but when these leaves were tested, they were determined to be negative for BCMNV. Chlorotic patches in IVT 7233 and mosaic-like symptom in some replicates of cv. Othello (4.11.C) was thought to be a type of physiological disorder related to abiotic stress. Top necrosis (Fig 4.14C) was observed in cv. Improved Tendergreen (*I* and *bc-1*) after a week, and in cvs. Widusa (*I*) (Fig 4.14A) and Amanda (*I* and *bc-1*^{2}) (Fig 4.15D) after 2 weeks. These results indicate that the NWS isolate of BCMNV has highly virulent character. Studies by Feng et al. (2014) showed that the TN1 isolate of BCMNV, which belongs to the Pathogroup VI, generated necrosis only on inoculated leaves of Jubila (HG 9a) (*I* and *bc*-1), but no necrosis was elicited on non-inoculated upper leaves. Similarly, no systemic symptoms were observed in Amanda (*I* and *bc-1*^{2}) (HG 10). As such, the reaction of TN1 is dissimilar to the NWS of BCMNV in terms of symptoms generated in Jubila and Amanda.

Strausbaugh et al. (2003) discovered NL-3K, NL-5 and NL-3D strains causing top necrosis in Black Turtle Soup (HG 8) (*I*). Top crop (HG 9b), which has the same resistance genes as that of Improved Tendergreen (HG 9b), exhibited top necrosis after inoculation with the NL-3K strain of BCMNV. Notably, vein necrosis observed in Amanda due to

NL3K strain was similar to vein necrosis triggered by the new isolate (NWS) of BCMNV in the inoculated primary leaf of Amanda (Fig. 4.15B) in this study. The difference was highlighted that the duration necessary for top necrosis to occur was shorter in the current study than in that of Strausbaugh et al. (2003). Top Crop (HG 9b) responded as top necrosis after the third week in the previous study, however, Improved Tendergreen (HG 9b) showed it just within a week after inoculation in the present study. The reactions of HG 8 (Widusa), HG 9b (Improved Tendergreen) and HG 10 (Amanda) to the NWS isolate of BCMNV had consistency with the findings reported by Arli Sokmen et al. (2016) in which the isolate in VIb (NL-5-like) triggered top necrosis in cvs. Widusa (HG 8), Improved Tendergreen (HG 9b) and Amanda (HG 10) at temperatures below 30^{o}C (Authors, *personal communication*), indicating that the NWS is a member of PG VI as similar to the NL-5 strain, but seems to be more virulent than NL-5-like strain of the previous study.

Feng et al. (2017) investigated three BCMNV isolates (NL-8 CA, TN1a and 1755b) as well as TN1 (PG VI) as control. They distinguished the isolates by using 12 bean differentials and whole-genome sequencing followed by BLASTn analysis. They reported that TN1a and 1755b were assigned to PG VI, while NL-8 CA was assigned to PG III. The isolates TN1a, 1755b and TN-1 (control) were found to cause rapid whole plant necrosis (WPN) in Top Crop (HG 9b) 7 to 14 days post inoculation, however, systemic necrosis without WPN occurred in Jubila (HG 9a) 3 to 5 weeks post inoculation. Similarly, the NWS isolate, which was assigned to PG VI in the current study, also resulted in rapid WPN and top necrosis in Widusa (HG 8, *I*) and in Jubila (HG 9a, *I+bc-1*) within 7 days after inoculation, and in Amanda (HG 10, *I+bc-1+ bc-1*^{2}) two to three weeks after challenged with NWS. On the other hand, in plants of HG 2 (*ii+bc-u+bc-1*) and HG 3 (*ii+bc-u+bc-1*^{2}), NL-8-CA systemically infected a small proportion (less than 3% and 13%, respectively) of upper non-inoculated leaves. Sequence differences between the isolates in PG III and PG VI were located mostly in P1/HC-Pro and HC-Pro/P3 cistrons (Feng et al., 2017). Phenotypic differences between BCMNV PG III and PG VI isolates in HG 2, HG 3 and HG 9 suggested that *bc-1* may have a role in systemic movement of BCMNV (Feng et al., 2017).

According to the phenotypic appearance of the plants after inoculated with the NL-5-like isolate and the results of ELISA to determine the presence of systemic infection in the differential host plants, the pathogroup of this isolate was confirmed and determined to be PG VI as suggested by Arli-Sokmen et al. (2016). Black Turtle Soup-1 (HG 8), Improved Tendergreen (HG 9b), Jubila (HG 9a) and Isabella (HG 10) resulted in differential necrotic symptoms. Top necrosis occurred in cvs. Black Turtle Soup-1 (Fig 4.7B and C) and Improved Tendergreen (Fig 4.8B) after one week. Jubila (Fig 4.9A) also expressed progressive necrosis on upper leaves (Fig 4.9A) and necrosis on seed pods (Fig 4.9 B), while Isabella had slowly progressive necrosis on the non-inoculated leaves (Fig 4.9 B and C), without top necrosis and plant death below 30^{o}C, which differed from the NWS isolate (4.15 B, C).

Top necrosis is due to hypersensitive reaction which certainly leads to the death of plants. Bean cultivars containing the dominant *I* resistance gene experience top necrosis at regular temperatures (Temperature insensitive necrosis; TIN) when infected with the strains of BCMNV. As such, BCMNV and BCMV strain differentiation can be made by elevating the temperature to 30^{o}C (Gilbertson et al., 2001; Kelly, 1997). Top necrosis response in Black Turtle Soup-1 (HG 8) and Improved Tendergreen (HG 9b) serves as a defense mechanism to prevent the spread of the virus within the vascular system of the plant. Thus, the death of the plant eliminates the possibility of transmission of the virus to other generations through the seeds. Symptoms of susceptible cultivars ranged from leaf mottling, reduced leaf size, mosaic, chlorosis, chlorotic lesions against the NL-5-like isolate. These cultivars not possessing the dominant *I* gene, but having other genes were: Sutter pink (*i*) (Fig. 4.1A), UI-36 Red Max (*bc-u, bc-1*) (Fig.4.11), Redland Greenleaf B (*bc-u, bc-1*^{2}), Pinto UI-111 (*bc-u, bc-2*) (Fig. 4.12) and Pinto UI 114 (*bc-u, bc-1, bc-2*) (Fig. 4.13).

After confirmation of the pathogroup of the NL-5-like isolate, twenty soybean cultivars were tested against it. Necrotic spots and chlorotic lesions (Fig. 4.17 and Fig. 4.18) were observed on the inoculated primary leaves, whereas leaf mottling, leaf crinkling, mosaic, reduced leaf size, yellowing and chlorotic spots were induced on the non-inoculated leaves (Fig. 4.19-4.22). Other cultivars had shortened plant stem coupled with less leaves. These symptoms had similarity with those in the previous studies with

BCMNV isolates in several leguminous crops. Sengooba et al. (1997) reported the occurrence of chlorotic spots, mild mosaic, clear mosaic, general mosaic on leguminous crops such as *Centrosema pubescens*, *Vigna vexillata*, *Senna hirsuta*, *Sena sophera* and other wild and forage legumes. Feng et al. (2014) emphasized that leaf deformation and mosaic symptoms that accompany BCMNV infection impeded the plant growth in susceptible cultivars. Jang et al. (2018) in South Korea, reported similar symptoms such as chlorosis, mosaic, stunted growth on soybean plants inoculated with BCMNV. Also, after inoculating a soybean variety with BCMNV, Mangeni et al. (2020) stated that the dominant symptom was yellowing at the time of phenotypic evaluations. During the studies of this thesis, the most prominent symptoms observed on soybean cultivars were mosaic and leaf crinkling (Fig. 4.19-4.21). Emphasis should be made on the fact that the perceived chlorotic lesions observed on the leaves of some soybean cultivars such as Nazlican and Prota-Y developed into rust-like symptoms later. Therefore, a care must be taken during phenotypic evaluations as some disparate chlorotic lesions may be incipient symptoms of other plant diseases. Cultivars found to be positive for BCMNV at the first DAS-ELISA (three weeks post inoculation) were Adasoy, Blaze, SA-88 and Ohio. Seven additional soybean cultivars were identified to be systemically infected with BCMNV after the second DAS-ELISA test (five weeks post inoculation). These cultivars were Arisoy, Ataem, Atlas, Atakişi, Cinsoy, Ilksoy and May 5451 (Table 4.2). Additionally, soybean cv. Samsoy was positive at the third DAS-ELISA at six weeks post-inoculation. Most of cultivars were detected to be BCMNV-infected four weeks after inoculation. This may be due to the fact that the replication and spread of virus within the plant reached the peak level about a month later. However, Gapsoy-16, Mona, Nazlican, Prota Y, Safir, Soy Anam, Srebrina and Umut 2002 were negative for BCMNV, even after that period.

RT-PCR was used to amplify the partial NIb and coat protein regions of BCMNV using degenerate primers which were specific for BCMV and BCMNV. The RT-PCR with dsRNAs extracted from the leaves infected with BCMNV isolates (NL-5-like and NWS) resulted in the size of products of 579 bp (Fig. 4.26). However, the dilution of isolated dsRNA had a positive correlation with the quality of the PCR-product formation. The primer-dimer formation was significantly reduced after the dilution of dsRNAs to 1:1 (BCMNV-NL-5-like) or 1:5 (BCMNV-NWS) (Fig 4.25). However, the dilution of the

RNA samples of the of NWS (1:5) and NL-5-like (1:1) produced the precise sizes of PCR fragments (Fig 4.26). The results obtained agreed with the findings of the study by Melgarejo et al. (2007) in which they explained that the products for the recommended primers were expected to be around 500 bp. Thus, the degenerate primers amplified 162 nucleotides (nt) of the NIb and 417 nt of the coat protein of BCMNV (Table 3.3).

The NWS and NL-5-like isolates of BCMNV had 100% nucleotide identity for the genome region analyzed (Table 4.4, Appendices 3 and 4). It can be inferred that these two isolates of BCMNV may have similar sequences in the complete NIb and CP regions. Phylogenetic tree analysis revealed that NWS and NL-5-like isolates were in close genetic relationship with the isolates belonging to NL-3 strain of BCMNV (Fig 4.27). Furthermore, the clades formed by phylogenetic tree analysis in the present study agreed with the results of Wainaina et al. (2019), whose phylogenetic tree also included two main clades on the basis of BCMNV origin, similar to the results of this thesis. As far as our knowledge, this is the first study on molecular identification and characterization of BCMNV isolates in Turkey.

6. CONCLUSION AND RECOMMENDATIONS

The first aim of this study was to biologically and molecularly characterize two isolates of BCMNV. The second objective of the study was to evaluate 20 soybean cultivars by inoculating them with the NL-5-like isolate (PG VI) of BCMNV.

The NL-5-like isolate elicited symptoms similar to that of the new isolate (NWS) of BCMNV. It induced top necrosis in Improved Tendergreen (HG 9b) and Black Turtle Soup (HG 8). However, the partial and slow progressive necrosis induced in Jubila (HG 9a) and Isabella (HG 10) discriminated the isolate NL-5-like from the NWS, which produced a rapid and an accelerated spreading of necrosis in the *I* gene-carrying cultivars in HG Group 9a and HG 10. The complete coat protein or other genome components of BCMNV isolates, especially the ones responsible for divergent pathogenic properties should be investigated in order to increase the data on gene functions in future research.

Soybean cultivars against the NL-5-like isolate induced symptoms similar to the previously described symptoms of BCMNV. Twelve cultivars were found to be susceptible to BCMNV, while eight were negative. Although some cultivars had symptoms such as chlorotic spots and leaf deformation, they were identified to be negative in DAS-ELISA. These symptoms do not necessarily reflect the presence of BCMNV infection. As such, further serological tests such as DAS-ELISA tests should be used to confirm the presence of BCMNV infections on plants. More soybean genotypes as well as groundnut and cowpea genotypes are needed to be evaluated against BCMNV and BCMV in the future, and the use of resistant ones in breeding studies are recommended.

Additionally, the identification of the pathogroups of the studied isolates of BCMNV provides useful information to legume breeders in Turkey and around the world. These isolates may use help to seek virus-resistant legume genotypes and increase crop yield.

REFERENCES

Arli-Sokmen, M., Deligoz, I., and Kutluk-Yilmaz, N. D. (2016). 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. https://doi.org/10.1007/s10658-016-0886-x

Atreya, C. D., Raccah, B., and Pirone, T. P. (1990). A point mutation in the coat protein abolishes aphid transmissibility of a *potyvirus*. *Virology*, 178(1), 161–165. https://doi.org/10.1016/0042-6822(90)90389-9

Beatrice, M., Susan, N.-M., Paul, N., Fred, T., Magdalena, W., Papias, B., Elisiana, K., Michael, K., and Deusdedith, M. (2017). 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), 1486–1500. https://doi.org/10.5897/ajar2017.12236

Blanc, S., López-Moya, J. J., Wang, R., García-Lampasona, S., Thornbury, D. W., and Pirone, T. P. (1997). A specific interaction between coat protein and helper component correlates with aphid transmission of a *potyvirus*. *Virology*, 231(1), 141–147. https://doi.org/10.1006/viro.1997.8521

Bozoğlu, H., and Sözen, Ö. (2007). Some agronomic properties of the local population of common bean (Phaseolus vulgaris L.) of Artvin Province. *Turkish Journal of Agriculture and Forestry*, 31(5), 327–334. https://doi.org/10.3906/tar-0609-17

Canlı, Z. and, Çalkaya, M. (2022). *Turkey has a say in the production of dried pulses in the world*. https://www.aa.com.tr/tr/ekonomi/turkiye-kuru-bakliyat-uretiminde-dunyada-soz-sahibi/2498688 (Retrieved on February,10)

Chiquito-Almanza, E., Acosta-Gallegos, J. A., García-Álvarez, N. C., Garrido-Ramírez, E. R., Montero-Tavera, V., Guevara-Olvera, L., and Anaya-López, J. L. (2017). Simultaneous detection of both RNA and DNA viruses infecting dry bean and occurrence of mixed infections by BGYMV, BCMV and BCMNV in the Central West Region of Mexico. *Viruses*, 9(4). https://doi.org/10.3390/v9040063

Chung, B. Y. W., Miller, W. A., Atkins, J. F., and Firth, A. E. (2008). An overlapping essential gene in the Potyviridae. Proceedings of the National Academy of Sciences of the United States of America, 105(15), 5897–5902. https://doi.org/10.1073/PNAS.0800468105

Clark, M. F., and Adams, A. N. (1977). Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, 34(3). https://doi.org/10.1099/0022-1317-34-3-475

Çulal Kiliç, H., Kök, H., and Yardimci, N. (2020). Göller Bölgesi Fasulye Üretim Alanlarında Fasulye Adi Mozayik ve Fasulye Adi Mozayik Nekroz Virüs'ü Enfeksiyonları. *European Journal of Science and Technology*, 386–392. https://doi.org/10.31590/ejosat.705686

Deligöz, İ., and Arli Sökmen, M. (2008). Differentiation of Bean Common Mosaic Virus (BCMV) and Bean Common Mosaic Necrosis Virus (BCMNV) Strains Infecting Common Bean in Samsun Province. *The Journal of Turkish Phytopathology*,37,1-3.

Deligoz, İ., Arlı-Sökmen, M., and Tekeoglu, M. (2021). 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. https://doi.org/10.24326/asphc.2021.6.2

Dogan, H. G. (2020). Projection of Dry Beans Cultivation Area for Turkey: Case of Center Anatolian Region. *Journal of Global Innovations in Agricultural and Social Sciences*, 195–201. https://doi.org/10.22194/JGIASS/8.922

Drijfhout, E. (1978). Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. *Verslagen van Landbouwkundige Onderzoekingen*, 872.

Drijfhout, E., Silbernagel, M.J. and Burke, D.W. 1978. Differentiation of strains of Bean common mosaic virus. *Netherland Journal of Plant Pathology*, 84: 13-26.

Edwardson, J. R., and Christie, R. G. (2018). CRC Handbook of Viruses Infecting Legumes. In CRC Handbook of Viruses Infecting Legumes. CRC Press. https://doi.org/10.1201/9781351071192

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Feng, X., Guzmán, P., Myers, J. R., and Karasev, A. V. (2017). Resistance to Bean common mosaic necrosis virus conferred by the bc-1 gene affects systemic spread of the virus in common bean. *Phytopathology*, 107(7), 893–900. https://doi.org/10.1094/PHYTO-01-17-0013-R

Feng, X., Myers, J. R., and Karasev, A. V. (2015). Bean common mosaic virus isolate exhibits a novel pathogenicity profile in common bean, overcoming the bc-3 resistance allele coding for the mutated eIF4E translation initiation factor. *Phytopathology*, 105(11), 1487–1495. https://doi.org/10.1094/PHYTO-04-150108-R

Feng, X., Poplawsky, A. R., and Karasev, A. V. (2014). A recombinant of Bean common mosaic virus induces temperature-insensitive necrosis in an I gene bearing line of common bean. Phytopathology, 104(11), 1251–1257. https://doi.org/10.1094/PHYTO-02-14-0048-R

Flasinski, S., and Cassidy, B. G. (1998). Potyvirus aphid transmission requires helper component and homologous coat protein for maximal efficiency. *Archives of Virology*, Volume 143 Issue 11, 2159–2172. https://doi.org/10.1007/s007050050449

Flores-Estévez, N., Acosta-Gallegos, J. A., and Silva-Rosales, L. (2003). Bean common mosaic virus and bean common mosaic necrosis virus in Mexico. *Plant Disease*, 87(1), 21–25. https://doi.org/10.1094/PDIS.2003.87.1.21

Gadhave, K. R., Gautam, S., Rasmussen, D. A., and Srinivasan, R. (2020). Aphid transmission of potyvirus: The largest plant-infecting RNA virus genus. *Viruses*,12(7). https://doi.org/10.3390/v12070773

Gibbs, A. J., Hajizadeh, M., Ohshima, K., and Jones, R. A. C. (2020). The potyviruses: An evolutionary synthesis is emerging. *Viruses*,12(2). https://doi.org/10.3390/v12020132

Gibbs, A., and Ohshima, K. (2010). Potyviruses and the digital revolution. Annual Review of Phytopathology, 48, 205–223. https://doi.org/10.1146/annurev-phyto073009-114404

Gilbertson, R. L., Guzman, P., Rojas, M., Crnov, R., & Mkandawire, A. (2001). Bean/ Cowpea Collaborative Research Support Program-East Africa Proceedings: Bean Seed Workshop Arusha, Tanzania January 12-14 Detection of Bean Infecting Viruses In California With An Emphasis On The Crsp-Facilitated Work. 4.

Golnaraghi, A. R., Shahraeen, N., Pourrahim, R., Farzadfar, S., & Ghasemi, A. (2002). First report of the natural occurrence of eight viruses affecting soybeans in Iran. *Plant Pathology*, 51(6). https://doi.org/10.1046/j.1365-3059.2002.00764.x

Gümüş, M., Erkan, S., Yorganci, Ü and Duman, İ. (2001). Bazı sebzelerin tohumlarında bulunan viral etmenlerin saptanması üzerine araştırmalar. *Ege Üniversite Fakülte Dergisi*,50 (3) 190–197.

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

Guzman, P., Rojas, M. R., Davis, R. M., Kimble, K., Stewart, R., Sundstrom, F. J., & Gilbertson, R. L. (1997). First report of bean common mosaic necrosis potyvirus (BCMNV) infecting common bean in California. *Plant Disease*, 81(7). https://doi.org/10.1094/PDIS.1997.81.7.831B

Hart, J. P., and Griffiths, P. D. (2013). 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. https://doi.org/10.1007/s00122-013-2176-8

Hema, M., Sreenivasulu, P., Patil, B. L., Kumar, P. L., & Reddy, D. V. R. (2014). Tropical Food Legumes: Virus Diseases of Economic Importance and Their Control. Advances in Virus Research, 90, 431–505. https://doi.org/10.1016/B9780-12-801246-8.00009-3

Hull, R. (2013). Plant virology. Academic Press. Fifth Edition.

ICTV. (2021). Genus: Potyvirus - Potyviridae -Positive-sense RNA Viruses - ICTV. https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rnaviruses/w/potyviridae/572/genus-potyvirus

Jang, Y. W., Jo, Y., Cho, W. K., Choi, H., Yoon, Y. N., Lim, S. M., Lee, Y. H., Bae, J. Y., and Lee, B. C. (2018). First Report of Bean Common Mosaic Necrosis Virus Infecting Soybean in Korea. *Plant Disease*, 102(10), 2051. https://doi.org/10.1094/PDIS-09-17-1474-PDN

Kamboj, R., and Nanda, V. (2018). Proximate composition, nutritional profile and health benefits of legumes – A review. *Legume Research*: An international Journal, 41(3),325–332.

Kanat, Z. (2022). Kuru Fasulye Ürün Raporu 2021.Tarımsal Ekonomi ve Politika Geliştirme Enstitüsü Müdürlüğü, Türkiye. https://arastirma.tarimorman.gov.tr/tepge

Katungi, E., Farrow, A., Chianu, J., Sperling, L. and Beebe, S. (2009). Common bean in Eastern and Southern Africa: a situation and outlook analysis. International Centre for Tropical Agriculture, 61

Kelly, J. D. (1997). A review of varietal response to bean common mosaic potyvirus in *Phaseolus vulgaris*. *Plant Varieties and Seeds* ,10 (1).

Khabbazi, A.D., Bashir, N. S., Khabbazi, S. D., & Ighani, H. (2017). Extraction and molecular detection of viral dsRNA from different infected plants. *Journal of Scientific Agriculture*, 1. https://doi.org/10.25081/jsa.2017.v1.54

Kyle, M. M., and Provvidenti, R. (1993). 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.

Kumar, S., and Pandey, G. (2020). Biofortification of pulses and legumes to enhance nutrition. *Heliyon* , 6 (3). https://doi.org/10.1016/j.eliyon.2020.e03682

Larsen, R. C., Miklas, P. N., Druffel, K. L., and Wyatt, S. D. (2005). NL-3 K strain is a stable and naturally occurring interspecific recombinant derived from Bean common mosaic necrosis virus and Bean common mosaic virus. *Phytopathology*, 95(9), 1037–1042. https://doi.org/10.1094/PHYTO-95-1037

Larsen, R. C., Miklas, P. N., Eastwell, K. C., and Grau, C. R. (2008). A strain of Clover yellow vein virus that causes severe pod necrosis disease in snap bean. *Plant Disease*, 92(7). https://doi.org/10.1094/PDIS-92-7-1026

Laura, E. Y. L., Azize, O., Arlette, A., Joel, A., Joelle, T., Gustave, D., and Alexandre, D. (2018). Morphological characterization of common bean (Phaseolus vulgaris L.) landraces of Central region of Benin Republic. *Journal of Plant Breeding and Crop Science*, 10(11), 304–318. https://doi.org/10.5897/jpbcs2018.0766

Lee, S., Kim, H., Lee, J. Y., and Rho, J. Y. (2017). Development of rapid and highly sensitive detection of Bean common mosaic necrosis virus in leguminous crops using loop-mediated isothermal amplification assay. *Journal of Virological Methods*, 249, 117–120. https://doi.org/10.1016/j.jviromet.2017.08.023

Mangeni, B. C., Were, H. K., Ndong'a, M., and Mukoye, B. (2020). Incidence and severity of bean common mosaic disease and resistance of popular bean cultivars to the disease in western Kenya. *Journal of Phytopathology*, 168 (9), 501–515. https://doi.org/10.1111/jph.12928

Martínez, F., and Daròs, J.-A. (2014). Tobacco etch virus Protein P1 Traffics to the Nucleolus and Associates with the Host 60S Ribosomal Subunits during Infection. *Journal of Virology*, 88(18), 10725–10737. https://doi.org/10.1128/jvi.00928-14

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

Melgarejo, T. A., Lehtonen, M. T., Fribourg, C. E., Rännäli, M., and Valkonen, J. P. T. (2007). Strains of BCMV and BCMNV characterized from lima bean plants affected by deforming mosaic disease in Peru. *Archives of Virology*, 152(10), 1941–1949. https://doi.org/10.1007/s00705-007-1008-z

Miklas, P. N., Hang, A. N., Kelly, J. D., Strausbaugh, C. A., and Forster, R. L. (2002). Registration of Three Kidney Bean Germplasm Lines Resistant to Bean Common Mosaic and Necrosis Potyviruses: USLK 2 Light Red Kidney, USDK 4 Dark Red Kidney, and USWK 6 White Kidney. *Crop Science*, 42(2), 674–675. https://doi.org/10.2135/cropsci2002.6740

Mink GI, Vetten J, Ward CW, Berger PH, Morales F, Myers JR, Silbernagel MJ, Barnett OW (1994) Taxonomy and classification of legume-infecting potyviruses: A proposal from the Potyviridae Study Group ofthe Plant Virus Subcommittee of ITCC. Arch Virol 139: 231–235

Mishra, R., Verma, R. K., Sharma, P., Choudhary, D. K., and Gaur, R. K. (2014). Interaction between viral proteins with the transmission of Potyvirus. *Archives of Phytopathology and Plant Protection*, 47(2), 240–253. https://doi.org/10.1080/03235408.2013.807659

Morales, F. J. (2008). Bean Golden Mosaic Virus. *Encyclopedia of Virology*, 295–301. https://doi.org/10.1016/B978-012374410-4.00695-6

Morales, Francisco José, and Bos, L. (1988). Bean common mosaic virus. *Association of Applied Biology*,England, 337, 73.

Musembi Mutuku, J., Wamonje, F. O., Mukeshimana, G., Njuguna, J., Wamalwa, M., Choi, S. K., Tungadi, T., Djikeng, A., Kelly, K., Entfellner, J. B. D., Ghimire, S. R., Mignouna, H. D., Carr, J. P., and Harvey, J. J. W. (2018). Metagenomic analysis of plant virus occurrence in common bean (Phaseolus vulgaris) in Central Kenya. *Frontiers in Microbiology*, *9*(DEC). https://doi.org/10.3389/fmicb.2018.02939

Mwaipopo, B., Nchimbi-Msolla, S., Njau, P. J. R., Mark, D., and Mbanzibwa, D. R. (2018). 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. https://doi.org/10.1094/pdis-01-18-0198-re

Mwaipopo, B., Rajamaki, M. L., Ngowi, N., Nchimbi-Msolla, S., Njau, P. J. R., Valkonen, J. P. T., and Mbanzibwa, D. R. (2021). Next-generation sequencing-based detection of common bean viruses in wild plants from Tanzania and their mechanical transmission to common bean plants. *Plant Disease*, 105(9). https://doi.org/10.1094/PDIS-07-20-1420-RE

Myers, J. R., and Kmiecik, K. (2017). Common Bean: Economic Importance and Relevance to Biological Science Research (pp. 1–20). Springer, Cham. https://doi.org/10.1007/978-3-319-63526-2_1

Nadeem, M. A., Yeken, M. Z., Shahid, M. Q., Habyarimana, E., Yılmaz, H., Alsaleh, A., Hatipoğlu, R., Çilesiz, Y., Khawar, K. M., Ludidi, N., Ercişli, S., Aasim, M., Karaköy, T., and Baloch, F. S. (2021). Common bean as a potential crop for future food security: an overview of past, current and future contributions in genomics, transcriptomics, transgenics and proteomics. *Biotechnology and Biotechnological Equipment*, 35(1), 758–786. https://doi.org/10.1080/13102818.2021.1920462

Nei M. and Kumar S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

Nigam, D., LaTourrette, K., Souza, P. F. N., and Garcia-Ruiz, H. (2019). Genome-Wide Variation in Potyviruses. *Frontiers in Plant Science*, 10, 1439. https://doi.org/10.3389/FPLS.2019.01439/BIBTEX

Omunyin, M. E., Gathuru, E. M., & Mukunya, D. M. (1995). Pathogenicity groups of bean common mosaic virus isolates in Kenya. *Plant Disease*, 79(10). https://doi.org/10.1094/PD-79-0985

Pasev, G., Kostova, D., and Sofkova, S. (2014). 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. https://doi.org/10.1111/jph.12149

Rabenstein, F., and Kühn-institut, J. (2012). Family - Potyviridae. Virus Taxonomy, February, 1069–1089.

Rzhetsky A. and Nei M. (1992). A simple method for estimating and testing minimum evolution trees. *Molecular Biology and Evolution* **9**:945-967.

Saitou, N. and Nei, M. (1987). The neighbour-joining method:A new method for reconstructing Trees. *Molecular Biology and Evolution*, 4, 406–425.

Semba, R. D., Ramsing, R., Rahman, N., Kraemer, K., & Bloem, M. W. (2021). Legumes as a sustainable source of protein in human diets. *Global Food Security*, 28. https://doi.org/10.1016/j.gfs.2021.100520

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

Sharma, P., Sharma, P. N., Kapil, R., Sharma, S. K., and Sharma, O. P. (2011). Analysis of 3′-terminal region of Bean common mosaic virus strains infecting common bean in India. *Indian Journal of Virology*, 22(1), 37–43. https://doi.org/10.1007/s13337-011-0038-z

Shen, W., Shi, Y., Dai, Z., and Wang, A. (2020). The RNA-Dependent RNA Polymerase NIb of Potyviruses Plays Multifunctional, Contrasting Roles during Viral Infection. *Viruses*, *12*(1). https://doi.org/10.3390/V12010077

Shukla, D. D., Frcnkel, M. J., and Ward, C. W. (1991). Structure and function of the potyvirus genome with special reference to the coat protein coding region. *Canadian Journal of Plant Pathology*, 13(2), 178–191. https://doi.org/10.1080/07060669109500953

Shukla, D. D., and Ward, C. W. (1989). Structure of Potyvirus Coat Proteins and its Application in the Taxonomy of the Potyvirus Group. *Advances in Virus Research*, 36(C), 273–314. https://doi.org/10.1016/S0065-3527(08)60588-6

Silbernagel, M. J., Mink, G. I., Zhao, R. L., & Zheng, G. Y. (2001). Phenotypic recombination between bean common mosaic and bean common mosaic necrosis potyviruses in vivo. *Archives of Virology*, 146(5), 1007–1020. https://doi.org/10.1007/s007050170132

Simonsen, M., Mailund, T., and Pedersen, C. N. S. (2011). Inference of large phylogenies using neighbor-joining. *Communications in Computer and Information Science*, 127 CCIS. https://doi.org/10.1007/978-3-642-18472-7_26

Smýkal, P., von Wettberg, E. J. B., and McPhee, K. (2020). Legume genetics and biology: From mendel's pea to legume genomics. *International Journal of Molecular Sciences* ,21(9). https://doi.org/10.3390/ijms21093336

Soler-Garzón, A., McClean, P. E., and Miklas, P. N. (2021). Genome-Wide Association Mapping of bc-1 and bc-u Reveals Candidate Genes and New Adjustments to the Host-Pathogen Interaction for Resistance to Bean Common Mosaic Necrosis Virus in Common Bean. *Frontiers in Plant Science*, 12. https://doi.org/10.3389/fpls.2021.699569

Sousa, G. (2019). The World's Top Dry Bean Producing Countries. World Atlas. https://www.worldatlas.com/articles/the-world-s-top-dry-bean-producingcountries.html

Strausbaugh, C. A., Miklas, P. N., Singh, S. P., Myers, J. R., and Forster, R. L. (2003a). 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. https://doi.org/10.1094/PHYTO.2003.93.6.683

Strausbaugh, C. A., Miklas, P. N., Singh, S. P., Myers, J. R., & Forster, R. L. (2003b). Genetics and Resistance 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).

Tamura, K., Stecher, G., and Kumar, S. (2021). Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*.

Tan, M. and Yolcu, H. (2021). Current status of forage crops cultivation and strategies for the future in Turkey: a review. *Journal of Agricultural Sciences*, *27*(2), 114-121.

Tavert-Roudet, G., Anne, A., Barra, A., Chovin, A., Demaille, C., and Michon, T. (2017). The potyvirus particle recruits the plant translation initiation factor eif4e by means of the vpg covalently linked to the viral RNA. *Molecular Plant-Microbe Interactions*, 30(9), 754–762. https://doi.org/10.1094/MPMI-04-17-0091R/ASSET/IMAGES/LARGE/MPMI-04-17-0091-R_F6.JPEG

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

Tongbram, K., Singh, Y. C., and Singh, O. K. (2021). A Study on Production and Marketing Constraints of French Bean (*Phaseolus vulgaris* L.) Growers in Bishnupur District of Manipur. *Asian Journal of Agricultural Extension, Economics & Sociology*, 39(9), 37–41. https://doi.org/10.9734/ajaees/2021/v39i930639

Trott, A. R., Welch, T. C., Hundy, G. F., Trott, A. R., Welch, T. C., and Qadri, B. (2016). Chapter 4. Refrigeration and Air Conditioning, 6 (December 2015), 59–87.

TurkStat (2021). Turkey Statistical Institute, Crop Production Statistics. https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1

TurkStat (2022). Turkey Statistical Institute, Crop Production Statistics. https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-2.Tahmini-2022-37248

Valli, A. A., Gallo, A., Rodamilans, B., López-Moya, J. J., and García, J. A. (2018). The HCPro from the Potyviridae family: an enviable multitasking Helper Component that every virus would like to have. *Molecular Plant Pathology*,19(3),744–763. https://doi.org/10.1111/mpp.12553

Vetten, H. J., and Allen, D. J. (1991). Recent progress in the identification of viruses of Phaseolus vulgaris in Africa. Annual Report (USA).34:3-4.

Vetten, H. J., Lesemann, D. E., and Maiss, E. (1992). Serotype A and B strains of bean common mosaic virus are two distinct potyviruses. *Potyvirus Taxonomy*. (pp 415-431). Springer,Vienna.

Wainaina, J. M., Kubatko, L., Harvey, J., Ateka, E., Makori, T., Karanja, D., Boykin, L. M., and Kehoe, M. A. (2019). Evolutionary insights of Bean common mosaic necrosis virus and Cowpea aphid-borne mosaic virus. *Peer Journal*:6297 . https://doi.org/10.7717/peerj.6297

Wamonje, F. O., Tungadi, T. D., Murphy, A. M., Pate, A. E., Woodcock, C., Caulfield, J. C., Mutuku, J. M., Cunniffe, N. J., Bruce, T. J. A., Gilligan, C. A., Pickett, J. A., and Carr, J. P. (2020). Three Aphid-Transmitted Viruses Encourage Vector Migration from Infected Common Bean (*Phaseolus vulgaris*) Plants Through a Combination of Volatile and Surface Cues. *Frontiers in Plant Science*, 11, 1981. https://doi.org/10.3389/FPLS.2020.613772/BIBTEX

Wilker, J., Navabi, A., Rajcan, I., Marsolais, F., Hill, B., Torkamaneh, D., and Pauls, K. P. (2019). Agronomic Performance and Nitrogen Fixation of Heirloom and Conventional Dry Bean Varieties Under Low-Nitrogen Field Conditions. *Frontiers in Plant Science*, 10, 952. https://doi.org/10.3389/fpls.2019.00952

Worrall, E. A., Wamonje, F. O., Mukeshimana, G., Harvey, J. J. W., Carr, J. P., and Mitter, N. (2015). Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus: Relationships, Biology, and Prospects for Control. *Advances in Virus Research*, 93,1–46. Academic Press Inc. https://doi.org/10.1016/bs.aivir.2015.04.002

APPENDICES

Appendix-1: The Mean ELISA Absorbance Values of Differential Common Bean Cultivars Tested by Using BCMNV Polyclonal Antiserum

a) The Isolate NL-5-like

Bean Cultivar/HG	$A_{405\ nm}$*	Assessment
Redland Greenleaf C /3	1.648	Positive
IVT 7214 /7	0.145	**Negative**
IVT 7233 /11	0.133	**Negative**
Negative Control	**0.159**	

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

b) The NWS Isolate

Bean Cultivar/HG	$A_{405\ nm}$*	Assessment
Stringless Green Refugee/1	3.298	Positive
UI-36 Red Max /2	1.000	Positive
Redland Greenleaf B /3	3.308	Positive
UI-34 Red Max /4	3.391	Positive
Pinto UI-114 /5	3.375	Positive
Othello/6	0.254	**Negative**
Negative Control	**0.168**	

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

Appendix-2: The Mean ELISA Absorbance Values of Soybean Cultivars Tested Against BCMNV Polyclonal Antiserum

No.	Soybean Cultivar	$A_{405\ nm}$*	Assessment
1	Adasoy	0.880	Positive
2	Arisoy	0.650	Positive
3	Ataem	0.700	Positive
4	Atakisi	0.810	Positive
5	Atlas 3616	0.260	Positive
6	Blaze	0.850	Positive
7	Cinsoy	0.340	Positive
8	Gapsoy-16	0.120	**Negative**
9	Ilksoy	0.410	Positive
10	May5451	1.290	Positive
11	Mona	0.180	**Negative**
12	Nazlican	0.210	**Negative**
13	Ohio	1.400	Positive
14	Prota-Y	0.130	**Negative**
15	SA-88	0.860	Positive
16	Safir	0.140	**Negative**
17	Samsoy	1.010	Positive
18	Soy Anam	0.140	**Negative**
19	Srebrina	0.150	**Negative**
20	Umut-2002	0.118	Negative
	Negative Control	**0.120**	

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

Appendix-3: The partial nucleotide and amino acid sequences of NIb+ Coat Protein regions of the NWS isolate of BCMNV

 # Nucleotide Sequence (519 base)

↓ Coding Start

GGAAAAGCACCATACATAGCAGAAACAGCCCTTCGCAAGCTCTATACGGAC
AAAGATGCCAAAATGGAGGAAATGCAAGAGTACCTGAAACAGCTTGAATTT
GATTCTGATGATGAGGTGTATGAATCCGTGTCAACACAATCCAGCAAGAAA
GAAGAAGAGAAAGACGCTGGGGCCGATGAGAGAGAGAAGGACAAAGGCAA
AGGCCCAGCGGATAAAGACGTTGGAGCTGGCTCAAAAGGAAAAGTAGTGCC
AAGATTGCAGAAAATCACCAAAAAGATGAATTTGCCTATGGTTGGCGGTAG
GATGATTCTAAACTTGGACCACCTAATTGAGTACAAACCGCAGCAGACGGA
CTTGTACAACACAAGAGCTACCAAGGCACAATTTGAAAGATGGTACGAAGC
AGTCAAGACTGAATATGAGCTTAATGACCAGCAAATGGGAGTAGTAATGAA
TGGCTTCATGGTGTGGTGCATCGATAATGGGACATCTCCCGATGTGAATGGA
GTGTGGGT

 # Amino acid Sequence (172 aa)

GKAPYIAETALRKLYTDKDAKMEEMQEYLKQLEFDSDDEVYESVSTQSSKKEE
EKDAGADEREKDKGKGPADKDVGAGSKGKVVPRLQKITKKMNLPMVGGRMI
LNLDHLIEYKPQQTDLYNTRATKAQFERWYEAVKTEYELNDQQMGVVMNGF
MVWCIDNGTSPDVNGVW

Appendix-4: The partial nucleotide and amino acid sequences of NIb+ Coat Protein regions of the NL-5-like isolate of BCMNV

 # Nucleotide Sequence (519 base)

⬇ Coding Start

TGGAAAAGCACCATACATAGCAGAAACAGCCCTTCGCAAGCTCTATACGGA
CAAAGATGCCAAAATGGAGGAAATGCAAGAGTACCTGAAACAGCTTGAATT
TGATTCTGATGATGAGGTGTATGAATCCGTGTCAACACAATCCAGCAAGAAA
GAAGAAGAGAAAGACGCTGGGGCCGATGAGAGAGAGAAGGACAAAGGCAA
AGGCCCAGCGGATAAAGACGTTGGAGCTGGCTCAAAAGGAAAAGTAGTGCC
AAGATTGCAGAAAATCACCAAAAAGATGAATTTGCCTATGGTTGGCGGTAG
GATGATTCTAAACTTGGACCACCTAATTGAGTACAAACCGCAGCAGACGGA
CTTGTACAACACAAGAGCTACCAAGGCACAATTTGAAAGATGGTACGAAGC
AGTCAAGACTGAATATGAGCTTAATGACCAGCAAATGGGAGTAGTAATGAA
TGGCTTCATGGTGTGGTGCATCGATAATGGGACATCTCCCGATGTGAATGGA
GTGTGGGT

 # Amino acid Sequence (172 aa)

GKAPYIAETALRKLYTDKDAKMEEMQEYLKQLEFDSDDEVYESVSTQSSKKEE
EKDAGADEREKDKGKGPADKDVGAGSKGKVVPRLQKITKKMNLPMVGGRMI
LNLDHLIEYKPQQTDLYNTRATKAQFERWYEAVKTEYELNDQQMGVVMNGF
MVWCIDNGTSPDVNGVW

Appendix-5: The partial nucleotide and amino acid sequences of NIb+ Coat Protein regions of BCMV isolate (Reference Isolate for Sequence Comparison)

 # Nucleotide Sequence (658 base)

↓ Coding Start

AGGAGCCCCATACATAGCAGAGTCAGCACTTAAAACTCTTTACACAAACAA
GAGAGCAAAGATTGAAGAATTGGCAAAATATCTTGAAGTGCTCGATTTTAA
CTATGAGGTAGGATGCGGAGAATCTGTGCACCTACAGTCAGGAGCTGGACA
ACCACCACCACCAGTAGTGGACGCTGGTGTGGACACTGGGAAGGACAAGAA
AGACAAGGGCAGTAAAGGAAAGGACCCTGAAAGCAAGGAAGGGATAAGAA
CTAACAGCCGCGGAACTGAGAGTTCAACAATGAGGGACAAGGATGTAAATG
CTGGTTCCAAAGGAAAAGTTGTTCCTCGGCTTCAAAGGATCACAAAAAGAA
TGAATTTACCCATGGTGAAAGGAAATGTGATCTTGAATTTAGATCATCTGTT
GGATTACAAGCCAGAACAAACTGACCTCTTTAACACAAGAGCAACAAAGAT
GCAGTTTGAAATGTGGTACAATGCTGTGAAGGGCGAGTATGAGATAGATGA
TGAACAAATGTCAATTGTAATGAATGGCTTTATGGTATGGTGTATTGACAAT
GGCACTTCACCGGACGTGAATGGCACATGGGTAATGATGGATGGAGACGAG
CAAGTGGAATACCCGCTCAAGCCAATGGTTGAAAATGCAAAGCCA

 # Amino Acid Sequence (219 aa)

GAPYIAESALKTLYTNKRAKIEELAKYLEVLDFNYEVGCGESVHLQSGAGQPPP
PVVDAGVDTGKDKKDKGSKGKDPESKEGIRTNSRGTESSTMRDKDVNAGSKG
KVVPRLQRITKRMNLPMVKGNVILNLDHLLDYKPEQTDLFNTRATKMQFEMW
YNAVKGEYEIDDEQMSIVMNGFMVWCIDNGTSPDVNGTWVMMDGDEQVEYP
LKPMVENAKP

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