

Bean Common Mosaic Potyvirus (BCMV) Characterized from Bean (*Phaseolus vulgaris* L.) Crops Affected by Mosaic Disease in Denizli Province, Türkiye

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Abstract: Bean (*Phaseolus vulgaris* L.) is one of the world's oldest crops with both financial and nutritional importance. Bean common mosaic potyvirus (BCMV) is one of the prevalent viral agents that affect beans across the globe. Determining the presence of the agent in the relevant region is critical for minimizing crop losses by implementing appropriate preventive and control measures. In this study, 73 bean leaf samples were collected from bean-growing areas in the Denizli province of Türkiye in 2022. The samples were screened for the presence of viral agents using Polymerase Chain Reaction (PCR) with specific primers targeting the polypeptide gene. 26 of the bean samples were found to be positive for BCMV. The coat protein gene sequences of two randomly selected positive isolates were sequenced and deposited in the GenBank with accession numbers OQ910196 and OQ910197. The nucleotide sequences of isolates were found to have high similarity with those of isolates identified in various regions of the world. Phylogenetic analysis indicated that these isolates from Denizli, Türkiye were closely related to other Turkish isolates. However, since some Turkish isolates in the cluster associated with the Denizli isolates were identified as belonging to the US-5 or NL-6 strain, the current sequences may be related to these strains. Further research is necessary to identify the exact strain of the Denizli isolates, which could be achieved through the use of a strain differentiation set.

Keywords: *Phaseolus vulgaris*, bean common mosaic potyvirus, phylogeny, nucleotide similarity

1. Introduction

Bean common mosaic potyvirus (BCMV) is an RNA virus with a positive polarity and single-stranded genome, which is a member of the Potyvirus genus in the Virgaviridae family (Ivanov et al., 2014). The length of the genome is approximately 4.7 to 5.2 kilobases and contains a leader sequence, open reading frames, non-coding regions, and a poly-A tail at the 5' and 3' ends, respectively (El-Sawy et al., 2013). The three primary open reading frames in the BCMV genome encode critical viral enzymes, including protease, helicase, and RNA polymerase, that are essential for replication and infectivity. Moreover, most open-reading frames encode structural proteins that enable the virus to be recognized by host cells and

facilitate infection progression. The non-coding regions within the BCMV genome harbor regulatory elements that are crucial for viral replication and gene expression. These regions are necessary for the virus to replicate efficiently within host cells (Oana et al., 2009; Hull, 2014).

The symptoms of BCMV infection can vary depending on the host plant species and age, the virus strain, and the environmental conditions. However, typical symptoms include mosaic, mottling, yellowing, and stunting of leaves. The pattern of mosaic symptoms can be irregular or vein-clearing, and the severity of symptoms may increase with age. Infected plants may also exhibit necrosis, distortion, and reduced growth rates (Flores-Estevéz et al., 2003; Çulal Kılıç et al.,

2020). In some host plants, BCMV infection can lead to the production of small, malformed fruits and reduce crop yields (Kelly et al., 1995; Collmer et al., 2000). Furthermore, BCMV can weaken the host plant and make it more susceptible to other pathogens or environmental stresses. However, it is noted that even in asymptomatic infected hosts, these effects can reduce crop yield by up to 50% (Morales, 2006). As a result, the symptoms of BCMV infection can have significant economic impacts on agriculture.

BCMV can infect plants in various ways, including direct contact via leaf aphids a non-persistent manner, seeds, leaves, and other plant materials, as well as through infected plant pollen and contamination from tools or greenhouses (Su, 2013; Tang and Feng, 2022). Aphid-mediated transmission can have a significant impact on the spread of BCMV. Studies conducted during periods of high aphid populations have demonstrated that an initial BCMV seed infection rate of 2-6% can result in 100% plant infection due to transmission by aphids (Galvez and Morales, 1989). It has been reported that BCMV can be transmitted by approximately 36 aphid vector species from 21 genera, including *Macrosiphum solanifolii*, *M. pisi*, *Myzus persicae*, *Aphis gossypii*, *Diuraphis noxia*, and *Rhopalosiphum pseudobrassicae* (Zaunmeyer and Meiners, 1975; Halbert et al., 1994; Jordan and Hammond, 2008; Worrall et al., 2015).

Although it is believed to have originated in South or East Asia, BCMV has now spread widely and can be found in legume-growing regions across the globe (Gibbs et al., 2008). The virus is particularly prevalent in countries that cultivate economically important bean varieties like dry beans, green beans, and soybeans, as well as weed hosts (Coutts et al., 2011; Worrall et al., 2015). Despite its global prevalence, there has not been a significant number of studies on BCMV conducted in Türkiye. Some of these studies focus on resistance against the virus, while others aim to detect the presence of the virus or determine its strains (Açıköz, 1984; Kutluk Yılmaz et al., 2002; Güzel and Arlı Sökmen, 2003; Deligöz and Arlı Sökmen, 2013; Çulal Kılıç et al., 2015; Çolak Ateş et al., 2017; Yeken et al., 2018; Palacioğlu et al., 2020). However, studies on the phylogenetic relationships of BCMV are quite limited. The aims of this study are to diagnose the presence of the virus in bean (*Phaseolus vulgaris* L.) plants in the Denizli region using molecular methods and to investigate the phylogenetic relationships of the isolate with other isolates worldwide based on the polyprotein gene.

2. Materials and Methods

2.1. Plant material, RNA extraction, and reverse transcription

In 2022, healthy bean leaf samples as well as symptomatic leaves were collected from bean (var. Ayşekadın) fields in Denizli province, Türkiye (73 samples). A silica-based method for total RNA extraction was applied manually as described by Foissac et al. (2001). Complementary DNA methodology (reverse transcription) was performed as reported by Usta and Güller (2020).

2.2. Polymerase chain reaction tests

The presence of BCMV in the collected samples was determined by Polymerase Chain Reaction (PCR) test using CP-specific primer sets. The forward (5'-GGATGCGGAGAATCTGTG-3') and reverse (5'-GATTGACGTCCTTGCAG-3') oligonucleotide primers (Vemulapati and Bhat, 2009) were used to amplify a fragment of about 850 bp. The PCR program and PCR master mix were prepared as described by Usta and Güller (2020). 5 µL of 1 Kb DNA ladder (Fermentas, USA) and 15 µL of the PCR product were loaded into a 0.9% agarose gel containing EtBr in Tris-Acetate EDTA buffer (TAE1X), and electrophoresed at 80 V for 45 min. The gel was then visualized and photographed under ultraviolet light (UV) (Syngene™ UV Transilluminator 2020LM). A previously identified BCMV Van isolate (MK191026) was used as a positive control for virus diagnosis. Genomic RNA from a healthy bean plant served as the negative control.

2.3. Sequencing and bioinformatic analysis

DNA fragments of about 850 bp in size that were PCR amplified were purified from the gel using a commercial kit (Thermo Scientific, USA) and bidirectionally sequenced using the Sanger method (Medsantek, Bağcılar-İstanbul). Two BCMV isolates from Denizli bean crops were recorded in the GenBank. The pairwise identity index amongst the current isolates and the existing BCMV isolates in Genbank was generated using the Sequence Demarcation Tool (Version 1.2). The relationship of coat protein sequences of the Denizli isolates with isolates from different geographic locations was determined using the BLAST and CLC Main Workbench software. Using the neighbor-joining method, a phylogenetic dendrogram was constructed with a bootstrap test of 1000 resampling, and Tomato spotted wilt virus isolate (KP008129) was selected as the outgroup to improve the resolution of the tree.

3. Results and Discussion

3.1. Virus detection

The approximate percentage of product losses in bean production attributed to pathogens is around 10% and the significance of viral infections is increasing day by day due to the lack of effective control against them (Agrios, 1997; Bitocchi et al., 2012). Among over 140 viruses that affect leguminous plants, BCMV takes the first place as the most common host virus for beans (Morales, 2006). In bean production, the yield and quality loss due to BCMV is indirectly related to its effective spread through leafhoppers and seed transmission (Larsen et al., 2002; Nault et al., 2004). Identification of viral agents is crucial for combating viral diseases, and research efforts should primarily focus on this issue. Viral detection in plant samples is often achieved using DNA-based and serological methods (Akdura and Kılıç, 2022; Santosa et al., 2023). In this study, we accomplished the detection of BCMV in the bean production areas in the Denizli region of Türkiye, utilizing the RT-PCR method. Virus symptoms such as mosaic, chlorosis, vein banding, rosetting, and leaf deformation were observed in bean leaves in the survey areas (Figure 1).

A total of 73 samples, 50 symptomatic and the remaining asymptomatic, were tested for the presence of virus agent using specific primers. Viral agent was detected in 26 of the samples, but not in the rest. Some of the symptomatic samples did not show the presence of the viral agent, which could be due to other viral agents such as bean yellow mosaic virus (BYMV), bean pod mottle virus (BPMV), cucumber mosaic virus (CMV), alfalfa mosaic virus (AMV), bean common mosaic necrosis virus (BCMNV), soybean mosaic virus (SMV), which can produce similar symptoms (Damayanti et al., 2010; Hosseini and Hosseini, 2014; Peñafior et al., 2016; Çulal Kılıç et al., 2020).

The BCMV, which was first identified in an infected bean in the United States by Stewart and Reddic (1917), has since been reported in various hosts with a wide range, particularly in bean species, and in different regions. In Saudi Arabia, Al-Shahwan et al. (2017) tested a total of 1368 symptomatic plants for 11 viruses using DAS-ELISA in various weed and crop plants (cowpea, potato, eggplant, bean, broad bean, winter squash, pepper, and broad bean). The BCMV infection was found in 1.2% of the alfalfa samples, but not in the bean samples. In addition, some of the crop plants (cowpea, broad bean, and potato) and nine weed species were found to be infected with BCMV. In 2019, in Tajikistan, cucumber, mung bean, and tomato samples showing virus-like symptoms were collected and tested using RT-PCR. The BCMV infection rate in mung beans was reported to be 53% (Chan et al., 2022). On the other hand, in Indonesia, only BCMV was reported in Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.)), while the presence of BCMV and CMV was reported in soybean and mungbean plants (Damayanti et al., 2010).

Numerous studies have been conducted in various research fields in Türkiye indicating the prevalence of BCMV. Gümüş et al. (2001) reported the infection rates of BCMV, AMV, and tomato black ring virus (TBRV) in bean seed samples in the Aegean Region as 24.1%, 41.4%, and 13.8%, respectively. In Tokat-Türkiye province, BCMV infection was calculated as 59% in bean seed samples using DAS-ELISA (Kutluk Yılmaz et al., 2002). In Samsun-Türkiye, it was reported that 36% of 499 bean plants were infected with BCMV, 10.8% with CMV, 2.8% with BCMNV, and 2% with BYMV. Additionally, BCMV, BCMNV, and CMV infections were reported as 8.9%, 17%, and 17%, respectively, in tested seeds (Güzel and Arlı Sökmen, 2003). BCMV infection has also been reported in several regions of Türkiye including



Figure 1. Symptoms observed in bean plants associated with BCMV in Denizli province

Van (Güller and Usta, 2018), Antalya (Usta and Güller, 2020), Burdur (Çulal-Kılıç and Yardımcı, 2015), Lakes Region (Kılıç et al., 2020), Adana (Apalak, 2018), Muğla (Kılıç et al., 2013), Western Mediterranean Region (Çulal Kılıç et al., 2015), Antalya, Aydın, Balıkesir, Bursa, Erzincan, Kahramanmaraş, Karaman, Konya, Mersin, Muğla, Niğde, Samsun, and Tokat provinces (Arlı Sökmen et al., 2011).

In Türkiye, some studies have focused on identifying plant resistance genes against BCMV and BCMV strains to minimize economic losses. Regarding this, molecular markers developed in association with some resistance genes for BCMV and BCMNV are widely used in breeding studies (Johnson et al., 1997; Naderpour et al., 2010). A total of 39 bean varieties were tested for BCMV using different molecular markers in Türkiye. The results of this study revealed that 11 of the bean varieties had a single resistance gene, $I+bc-I^2$ genes in 25, and $bc-I^2 + bc-3$ genes in 3 (Palacioğlu et al., 2020). Similarly, 43 different bean varieties were screened for resistance to BCMV using three different genes and four different molecular markers. The study reported that some bean varieties had resistance genes against viruses (Yeken et al., 2018). In a study aimed at identifying BCMV strains, NL-6, NL-4, and RU-1 strains of BCMV were detected in leaf and seed samples collected from Samsun province, Türkiye (Deligoz and Arli-Sokmen, 2008). In addition, Arli-Sokmen et al. (2016) examined BCMV pathogroups with 367 bean seed and leaf samples from 15 provinces. The study reported that most BCMV isolates belonged to pathogroup VII and that there were a wide variety of BCMV pathogroups in Türkiye.

3.2. Sequence analysis and phylogenetic relations of Denizli-BCMV isolates

The approximately 850 bp fragments amplified by PCR from BCMV (Figure 2) were purified from the gel and sequenced molecularly. After confirmation of the sequences using BLAST

analysis on the National Centre for Biotechnology Information (NCBI) website, the 850 bp CP gene sequences of Denizli BCMV isolates were deposited in the GenBank under accession numbers OQ910196 and OQ910197. The BLAST query revealed that the Denizli isolates showed 96-100% nucleotide similarity with other BCMV isolates worldwide, in line with the pairwise identity index created with the Sequence Demarcation Tool software (Muhire et al., 2014) (Figure 3).

The BCMV isolates were divided into 4 groups based on the phylogenetic tree constructed using Table 1, and it should be noted that the grouping of isolates was not dependent on region or host. Based on the available phylogenetic tree, it appears that the BCMV isolates obtained from Denizli province were found to be most closely related to the Antalya (MN104840) and Samsun (KT766180) isolates, which were also isolated from bean plants in Türkiye. Additionally, the Denizli isolates were found to be closely related to isolates from bean plants in England (AY112735), India (EU713858, FJ157246), as well as from *M. atropurpureum* in Australia (EU761198), albeit to a lesser extent. Interestingly, BCMV isolates from Türkiye, including Antalya (MN104839), Van (MK191026), Samsun (KT766179), and Tokat (KT766181), formed a separate clade apart from the other isolates (Figure 4). However, given factors such as the close proximity of the Denizli and Antalya provinces, the suitability of the exchange of plant material such as seeds between regions, and the ease of vector-borne viral transmission, it would have been expected that all BCMV isolates from this area would be in the same cluster. Indeed, the fact that all isolates from the same region are not in the same cluster is likely related to the presence of different strains or pathogroups of BCMV. Based on the presence of pathogenicity genes and the reactions of host varieties in the strain differentiation set (11 bean varieties), BCMV strains are divided into 6 pathogenicity groups [PG (1), PG (2), PG (4), PG (5), PG (6), and PG (7)] (Silbernagel et al., 2001).

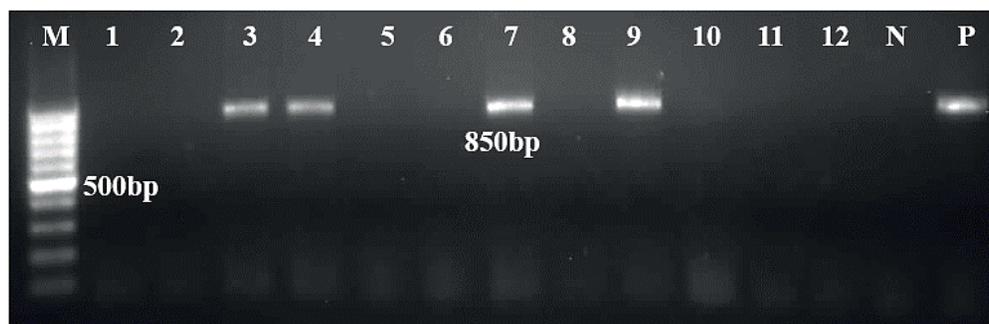


Figure 2. Agarose gel image of PCR-amplified DNA bands of coat protein gene of BCMV isolates
M: 1kb DNA marker (Thermo Scientific), P: Positive control, N: Negative control, Lane 3, 4, 7, 9: BCMV-infected bean samples.

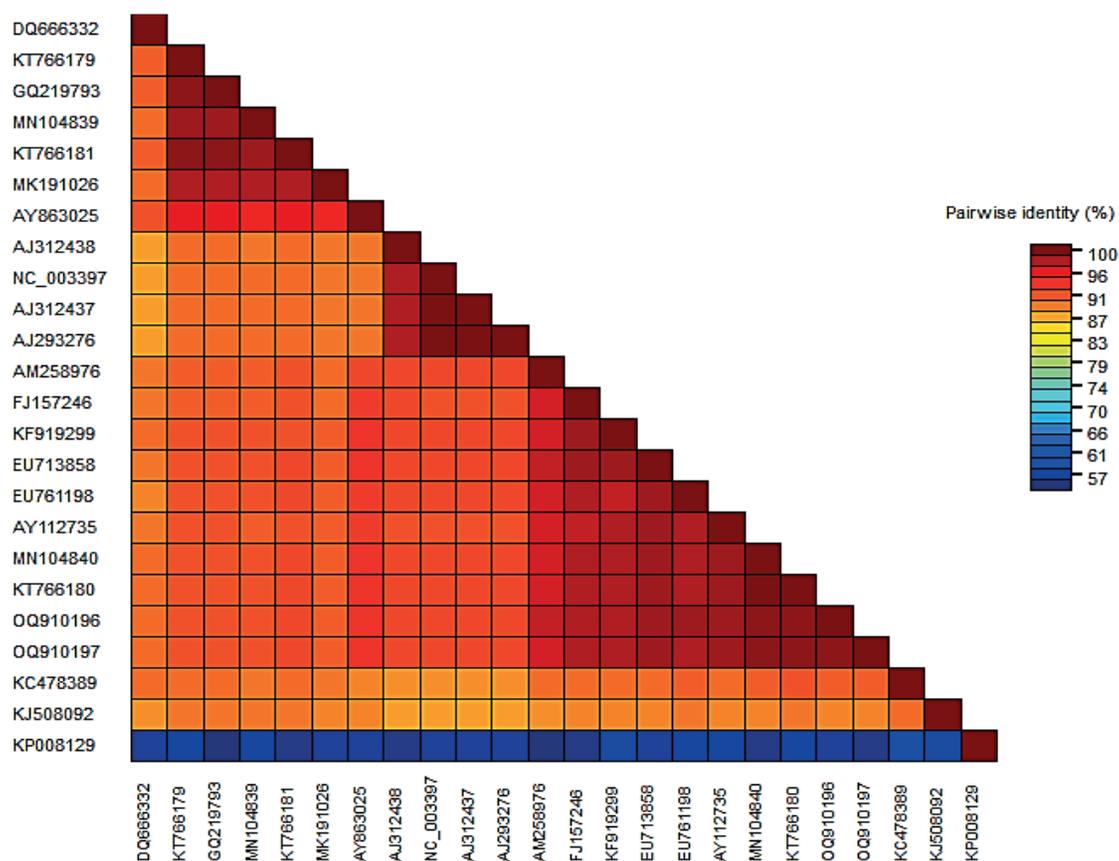


Figure 3. Pyramid matrix showing nucleotide similarity of Denizli BCMV isolates with other isolates in the world using Sequence Demarcation Tool program
 OQ910196 and OQ910197 are the accession numbers of Denizli-BCMV isolates. Acc. No. KP008129: Outgroup (TSWV isolate)

Table 1. Data associated with BCMV sequences from the genbank to generate a phylogenetic dendrogram

No	Accession number	Virus origin	Virus's host
1	AJ312437	China	Cowpea
2	AJ293276	China	Cowpea
3	NC003397	China	Cowpea
4	AJ312438	China	Cowpea
5	EU713858	India	Common bean
6	KF919299	-	Common bean
7	AY863025	Russia	<i>Phaseolus vulgaris</i>
8	AM258976	Peru	Lima bean
9	FJ157246	India	<i>Phaseolus vulgaris</i>
10	EU761198	Australia	<i>Macroptilium atropurpureum</i>
11	AY112735	England	<i>Phaseolus vulgaris</i>
12	MN104840	Türkiye (Antalya)	<i>Phaseolus vulgaris</i>
13	MN104839	Türkiye (Antalya)	<i>Phaseolus vulgaris</i>
14	MK191026	Türkiye (Van)	<i>Phaseolus vulgaris</i>
15	KT766179	Türkiye (Samsun)	Common bean
16	KT766181	Türkiye (Tokat)	<i>Phaseolus vulgaris</i>
17	OQ910196	Türkiye (Denizli)	<i>Phaseolus vulgaris</i>
18	OQ910197	Türkiye (Denizli)	<i>Phaseolus vulgaris</i>
19	KT766180	Türkiye (Samsun)	<i>Phaseolus vulgaris</i>
20	GQ219793	USA	<i>Phaseolus vulgaris</i>
21	KJ508092	South Korea	<i>Glycine max</i>
22	KC478389	China	Hyacinth bean
23	DQ666332	Colombia	<i>Phaseolus vulgaris</i>

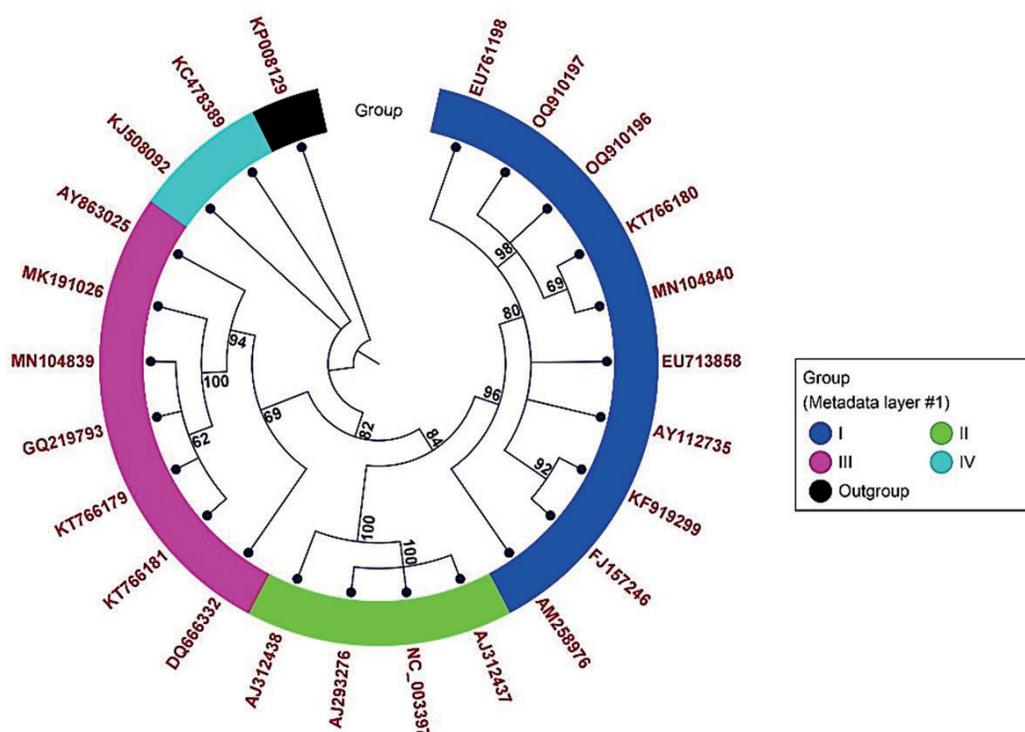


Figure 4. Phylogenetic dendrogram generated by the CLC Main Workbench program using worldwide identified BCMV isolates

A phylogenetic dendrogram was constructed using the neighbor-joining algorithm, with bootstrapping values of 100 replications displayed as scores within the nodes.

Drijfhout (1978) also reported that NL-1 (US-1), NL-2, NL-4 (US-6), NL-6 (US-4), NL-7, US-2, and US-5 are the races of BCMV. Additionally, the RU-1 strain was first identified in bean seeds from Russia by USDA in 1985 and classified under pathogroup 6 [PG (6)] based on the reaction in the strain differentiation set (Silbernagel et al., 2001). Some studies related to BCMV strains have identified NL-1, NL-6, and RU-1-like strains in Tanzania (Lillian and Msuku, 2001) and NL-1 strains in Western Australia and India (Saqib et al., 2005; Sharma et al., 2006).

The GenBank database shows that the Turkish-Samsun (KT766180) isolates in the cluster containing the Denizli isolates represent pathotype IVb. Furthermore, other isolates that were included in the same cluster (shown in blue) are the NL-1 strain (EU713858 and AY112735), and NL-4 strain (KF919299) which is closely related to the and PG(7). In this case, the Denizli isolates likely belong to either the US-5 or NL-6 strains, which are included in PG (IV) due to their more fine clustering. On the other hand, the other Turkish isolates [Antalya (MN104839), Van (MK191026), Samsun (KT766179), and Tokat (KT766181)] used clustered with the RU1 strains from Russia and the USA. It can be concluded that the phylogenetic

divergence is related to the BCMV strains. Further research is necessary to identify the exact strain of the Denizli isolates, this could be achieved through the use of a strain differentiation set.

4. Conclusions

In this study, bean samples collected from Denizli (Türkiye) were tested for BCMV infection. Out of 73 samples, 26 yielded 850 bp DNA fragments corresponding to the viral polyprotein gene, indicating a 35.6% infection rate. The gene sequences of two randomly selected BCMV isolates were recorded in GenBank with accession numbers OQ910196 and OQ910197. The sequences showed high nucleotide similarity with those of isolates identified worldwide. Phylogenetic analyses revealed that the Denizli isolates were closely related to other Turkish isolates, however, based on some members within the Denizli isolate group, the current isolates may belong to the US-5 or NL-6 strain. More research is required, using a race differentiation set, to confirm the precise strain of the Denizli isolates.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they

have seen/read and approved the final version of the article ready for publication.

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Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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