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Research Article

Molecular Characterization of Polyprotein genes of Two BCMV (*Bean common mosaic potyvirus*) isolates in Antalya (Turkey) and Their Genomic Divergence

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Abstract

Common bean (*Phaseolus vulgaris* L.) is regarded as one of the most important crops of the Fabaceae family throughout the world. Diseases caused by viruses are the most important factor limiting the production of beans. Bean specimens with classic virus-like symptoms were collected from bean fields in Antalya (Turkey) in July and August, 2018. BCMV was examined by RT-PCR test (Reverse Transcriptase -Polymerase Chain Reaction) using appropriate primer pairs directed to the partial NIb and the capsid protein (CP) gene which was devised to identify and to characterize the viral agent. The PCR test produced approximately 850 bp amplicon of expected lengths in 11 out of 20 fresh leaf tissues, indicating the presence of BCMV. Two of them were randomly selected and molecularly cloned into a congruent plasmid vector to reveal the CP sequences of interested isolates. Obtained recombinant clones consisting of insert genes were bidirectionally sequenced and both of the sequences were registered in the GenBank with MN104839 and MN104840 accession number. The provided BCMV partial CP gene sequences comprised 823 bp coding for 274 amino acid residues. The CP gene of these isolates was aligned with those of 17 isolates deposited in the GenBank database from different geographical location and its phylogenetic relationships were determined. Molecular analysis of the CP gene sequences of Antalya isolates showed the highest identity rates between 91.22 % and 94.71 %, at the nucleotide level. Moreover, phylogenetic analyses revealed that BCMV-Antalya 1 and Antalya 10 are best clustered with the Turkish isolate (KT766179) and England isolate (AY112735), respectively. By this study, the genetic difference of BCMV isolates have been determined in the bean plant from Antalya province of Turkey.

Keywords: BCMV (*Bean common mosaic potyvirus*), RT-PCR, Characterization, Phylogenetic relationship

Antalya (Türkiye) 'da İki Fasulye Adi Mozaik Virüs (BCMV) İzolatlarının Polyprotein Genlerinin Moleküler Karakterizasyonu ve Genomik Farklılıkları

Özet

Fasulye (Phaseolus vulgaris L.), dünya çapında Fabaceae familyasının en önemli ürünlerinden biri olarak kabul edilmektedir. Virüslerin sebep olduğu infeksiyonlar, fasulye üretimini sınırlayan en önemli faktördür. Antalya ilinde 2018 yılı Temmuz ve Ağustos ayında fasulye ekilen tarlalardan virüs benzeri simptomları gösteren fasulye örnekleri toplanmıştır. Toplanan örnekler, *Bean common mosaic potyvirus* (BCMV) virüsünü araştırmak ve viral ajanının kapsid proteinini (CP) karakterize etmek amacıyla dizayn edilen primerler yardımıyla RT-PCR testi ile testlenmiştir. 20 taze yaprak örneğinin 11'inde beklenen büyüklükte (850 bp) fragment elde edilmiştir. Rastgele iki pozitif izolat seçilmiş ve kısmi CP gen dizisinin ortaya çıkarılması amacıyla uygun bir plazmid vektöre klonlanmıştır. İnsert gen içeren rekombinant klonlar çift yönlü olarak dizilenmiş ve elde edilen her iki izolatın sekansı MN104839 ve MN104840 erişim numarası ile Gen Bankasına kaydedilmiştir. Antalya izolatlarının kısmi CP gen dizisinin 823 bp'den oluştuğu ve 274 amino asit parçasını kodladığı belirlenmiştir. BCMV Antalya izolatlarının CP geni, GenBankası veri tabanında ve farklı coğrafi bölgelerde belirlenen 17 izolat ile çoklu karşılaştırma yapılmış ve filogenetik ilişkileri belirlenmiştir. Antalya izolatlarının CP gen sekansları, nükleotit seviyesinde diğer izolatlar ile %91.22 ve % 94.71 arasındaki en yüksek benzerlik oranlarını göstermiştir. Ayrıca, filogenetik analizler, BCMV-Antalya izolatlarının en iyi şekilde Türkiye (KT766179) ve İngiltere izolatı (AY112735) ile kümelendiğini ortaya koymuştur. Bu çalışma ile Antalya (Türkiye) ili fasulye alanlarında belirlenen iki BCMV izolatının genetik farklılıkları ortaya konmuştur.

Anahtar Sözcükler: Fasulye Adi Mozaik Virus (BCMV), RT-PCR, Karakterizasyon, Filogenetik ilişki

Introduction

Bean (*Phaseolus vulgaris* L.), descending from the Mesoamerica, is a basic nutrient both for fresh and dry consumption (Bitocchi et al., 2012). It belongs to the family of Fabaceae which contains 700 genera and 18000 species and is cultivated in more than 26 million hectares in 126 countries. Turkey's share in this area is about 90 thousand hectares (TUIK, 2018; Balkaya and Yanmaz, 1999) and it ranks fourth with 614 965 tons in all over the world (FAO, 2012). The province of Antalya, where the study is carried out, is the second after Samsun in terms of bean production in Turkey (Arli-Sokmen et al., 2016).

Regardless of the single or double strand, the viral pathogens more than thirty with RNA or DNA genomes were naturally recorded in infected beans. In particular, *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV), *Cowpea aphid-borne mosaic virus* (CABMV), *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV) and *Tomato yellow leaf curl virus* (TYLCV) are severely destructive worldwide and cause economic damage in varying proportions by damaging the bean crops (DPV, 2012). In Turkey, the presence of CMV, AMV, BCMV, CABMV, BCMNV, BYMV, and TBRV *(Tobacco black ring virus)* was demonstrated reported by various researchers (Guzel and Arlı-Sokmen, 2003; Acikgoz, 1984; Yılmaz and Ozaslan, 1987; Fidan and Yorgancı, 1990; Gumus et al., 2001; Arli-Sokmen et al., 2016).

In 1917, BCMV was first recognized from an infected bean (*Phaseolus vulgaris* L.) in the USA by Stewart and Reddic (1917). It is believed that BCMV is of South or East Asia origin and is one of the world's earliest reported plant virus disease, which has a historical back of roughly 100 years (Gibbs et al., 2008; El-kady et al., 2014). It extensively known as a serious pathogen of common bean, cultivated plants and, occasionally wild legumens, and has a potential threat to bean production financially which can cause 100% yield loss, causing an epidemic throughout the world (Stewart and Reddick, 1917; Worrall et al., 2015). BCMV is readily transmitted by seeds and pollens depending on host species and developmental stage, virus strain, phase of the disease, and environment conditions (Kapil et al., 2011; Medina and Grogan, 1961). It can also be naturally transmitted by insects like primarily *Acyrthosiphon pisum, Aphis fabae, Myzus persicae, Aphis craccivora* in non-persistent fashion (requires only a few seconds of stilet penetration for virus acquisition and transmission) (Sastry, 2013; Powell, 2004; Biddle and Cattlin, 2007). Infected bean seeds and susceptible bean varieties are the primary sources of inoculum for this virus. Even if the BCMV rate is low in infectious seeds, this disease can develop rapidly when the population of aphids is high. Plants that develop from infected seeds are often stunted and may led to sterility. BCMV-contaminated seeds are capable of infection for 30 years (Loebenstein et al., 2009; Arli-Sokmen et al., 2016; Mavrič and Šustar-Vozlič, 2004).

BCMV was formerly named with various names such as *Bean virus 1*, *Bean mosaic virus* and *Phaseolus virus 1* (Morales and Bos, 1988). In 1934, it was named as *Bean common mosaic virus* (Pierce, 1934). From 1917 to 1943, all BCMV strains were considered pathologically identical (Drijfhout et al., 1978). After this date, when different pathogenic groups were discovered, BCMV was classified into 7 pathogenic groups in terms of the symptoms in 10 different bean varieties. Also, BCMV strains were divided into two serotypes A and B, based on the serology and restriction analysis of the capsid protein, and the simptomatological responses of diverse cultivars. The A and B serotypes were consequently reclassified and officially categorized into discrete virus agent in 1992, called as BCMNV and BCMV, respectively (Drijfhout et al., 1978; Vetten et al., 1992; Berger et al., 1997).

BCMV belongs to the genus Potyvirus, the largest genus in the Potyviridae family, which contains approximately 146 virus species (ICTV, 2013; Ivanov et al., 2014). BCMV has a one-part, 10 kb-sized ss(+) RNA genome which is 750 nm long and 11-13 nm wide, as Potyviruses. The genome is capable of infecting and has both the RNA and the mRNA function that is straightly translated into protein by host ribosomes. BCMV creates a special inclusion body in infested plant cells. The viral RNA molecule possesses a poly (A) tail at 3' end and a genome-linked viral protein (VPg) at 5' end (Hull, 2014; El-Sawy et al., 2013). The infection triggers the formation of cylindrical “pinwhell” inclusion bodies in the infected cells of sensitive bean plants (Morales and Bos 1988).

The six families (Amaranthaceae, Chenopodiaceae, Leguminosae-Caesalpinioideae, Leguminosae-Papilionoideae, Solanaceae, and Tetragoniaceae), particularly cultivated plants such as *Phaseolus* species (predominately *P. vulgaris*), *Vicia faba* (horse bean), *Arachis hypogaea* (peanut), and *Vigna unguiculata* (cowpea) are more sensitive to BCMV infection (Hosseini and Hosseini, 2014). Nowadays, this virus can be naturally detected and isolated from various planted beans (comon bean, horse bean, peanut, cowpea) and from wild legume hosts in numerous countries such as United States, India, Mexico, Peru, China, Netherlands, Taiwan, Iran, Colombia, Thailand, New Zealand, and Turkey (Hosseini and Hosseini, 2014). Also, the indicator plants including *Chenopodium quinoa*, *Macroptilium lathyroides*, *Phaseolus vulgaris*, *Pisum sativum*, *Vicia*

faba (vulnerable) and *Cucumis sativus*, *Medicago sativa*, *Nicotiana tabacum*, *Nicotiana glutinosa*, *Pisum sativum* (nonvulnerable) are experimental hosts used in laboratory diagnosis for BCMV (Worrall et al., 2015; Bos and Gibbs, 1995; Morales and Bos, 1988).

The characteristic manifestations of BCMV-infecting bean plants are a superficial mosaic figure from yellow to green on leaves, mostly associated with irregular wrinkling, malformation, and curling of the leaves. These symptoms are the leading cause of product loss. There may even be infections that do not show any symptoms, leading to product losses of up to 50% (Flores-Estėvez et al., 2003; Morales, 2006).

BCMV infection has been previously reported by some researchers in Turkey's different provinces (Gumus et al., 2001; Acikgoz, 1984; Fidan and Yorganci, 1990, Arli-Sökmen et al., 2016). In this study, RT-PCR assay was set up to amplify partial the capsid protein gene (CP) of BCMV in bean specimens collected in Antalya province. The CP nucleotide sequences of two isolates were randomly selected and phylogenic analyses of these were established with other recorded BCMV nucleotide sequences in NCBI GenBank. The occurrence and phylogenetic relationships of BCMV isolates in bean growing areas of Antalya in Turkey were studied in 2018 and reported in this article.

Material and Methods

Viral source and visual assessment

Mature bean leaves showing virus symptoms like mosaic motive, leaf malformation, downward leaf curling, superficial mottling were collected from a bean field in Antalya-Turkey in 2018.Twenty specimens were collected and placed in the plastic bag in the cold chain during transport, were kept at -80 °C during experimental procedures.

Viral detection

Total RNA (TNA) was obtained from about 0.50 g infected and non-infected leaf tissue according to the silica-based method as a detailed method previously with a few modifications (Foissac et al., 2001). The extracted TNA was suspended in 100 µl of RNase- free water and preserved at -80^{0}C until use.

Non-symptomatic bean leaves were utilized as a negative control in the molecular assays (examined negatively in RT-PCR assays). The obtained TNA was utilized for the synthesis of complementary DNA (cDNA).The two primer pairs (reverse and forward) devised according to 3' end of NIb to 3' end of CP gene by Vemulapati and Bhat, (2009) based on the EJ712783 accession number, were used for both cDNA and PCR process (Polymerase chain reaction). The cDNA process was prepared in a volume of 20 microliters using 5 µl of extracted RNA (as a template) for the first stage with the following parameters: 1 µl of dNTP (10 mM) mix, 1 µl of the reverse primer (BCMV-R) and 5 µl of RNase free water for a final volume of 12 µl. The mixture was heated at 65 °C for 5 min and then chilled on ice for 5 min. In the second stage, 4 µl of 5X RT buffer (Fermentas, USA), 2 µl of DTT (DithioThreitol), 1 µl of RNase inhibitor and 1 µl of Reverse Transcriptase enzyme were introduced onto the obtained mixture and kept at 42 °C for 50 min. At last, the mixture was permitted to hold at 70 °C for 15 min to cease the process.

To determine the presence of the BCMV, symptom-indicating leaves were screened by PCR test using capsid protein (CP) specific primer sets. The primers utilized are BF- 5'-GGATGCGGAGAATCTGTG -3' as the forward primer and BR-5'-GATTGACGTCCCTTGCAG -3' as the reverse primer, producing a CP gene sequence about to 850 bp fragment. The PCR regimen was regulated in thermocycler apparatus as follows: 3 min at 94 °C (pre-denaturation), 40 cycles of 30 s at 94 °C (denaturation), 1 min at 62 °C (annealing), and 45 s at 72 °C (extension), 10 min at 72 °C for final extension, finally kept at 4 °C. 5 µL of cDNA were submitted to RT-PCR test in a total volume of 50 µl: 31.6 µl RNase free water, 5µl 10X PCR Buffer, 3µl 25mM $MgCl_2$, 1µl 10mM dNTP, 1µl 20µM of each primer pairs and using the proofreading 0.4 µl *Pfu* DNA polymerase enzyme (5U/µl) (Thermo, USA).

Twenty-five microliters of amplified PCR yields and 1 Kb DNA marker (Fermentas, USA) were loaded into each well and progressed on 1.0 % agarose gel added with EtBr utilizing Tris- Acetate EDTA buffer (TAE1X). The displayed DNA bands were captured by UV light (Syngene™ UV Transilluminator 2020LM) and interpreted by photographing with the gel documentation unit. The BCMV isolate (MK191026) which maintained from the previous study in our laboratory was used as a positive control (Guller and Usta, 2018), and healthy plants were used as a negative control for analysis of the BCMV in molecular tests.

Molecular cloning and sequence analysis

Two randomly isolates of the expected size were extracted from agarose gels by GeneJET Gel Extraction Kit (Thermo Scientific, USA) and inserted into the pGEM T-Easy vector T-A cloning kit (Promega, USA). 5 µl of 2X ligation buffer was put into an Eppendorf tube contained l µl of pGEM T-Easy vector, 2 µl of insert DNA, l µl of T4 ligase enzyme, and 1 µl of µl RNase free water in a total volume of 10 µl. The ligation protocol was carried out overnight at +4 °C. The resulting recombinant plasmids were transferred

into the *E. coli* JM109 competent strain (Promega) by electric shock.

Transformed bacteria were planted in solid LB medium and transformed white colonies including insert DNA were selected after overnight and planted in liquid LB medium including ampicillin. Finally, recombinant plasmids, bearing the cloned viral CP gene, were purified using the ISOLATE II Plasmid Mini Kit (Bioline, Germany), sent to the Sentebiolab company (Ankara/Turkey) for sequencing and sequences were recorded in the GenBank.

Phylogenetic relationships of CP genes of BCMV isolates

The sequences of the Antalya isolates were collated with those in GenBank using the BLAST analyses (Basic Local Alignment Search Tool) facility at the NCBI platform. To determine genetic diversity, multiple alignments were performed to newly obtained gen sequences using the CLC Main Workbench program 6.7.1 and a phylogenetic tree was created by the neighbor-joining method concerning 100 replicates bootstrap test. The historical distances were calculated utilizing the Tamura-Nei methods by MEGA7 package (Molecular Evolutionary Genetics Analysis) (Kumar et al., 2016). For better separation of the phylogenetic tree, *Barley yellow dwarf virus*-PAV (KC900900) was chosen as the outgroup.

Results

Detection and symptoms of BCMV

In the bean plants inspected in the home garden from Antalya province (Kaş district), the specific infection symptoms concerning BCMV disease consisting of mosaic patterns ranging from light green to dark green, vein banding, downward curl in leaves, rugosity, yellowing and deformations in leaves were observed (Fig 1).

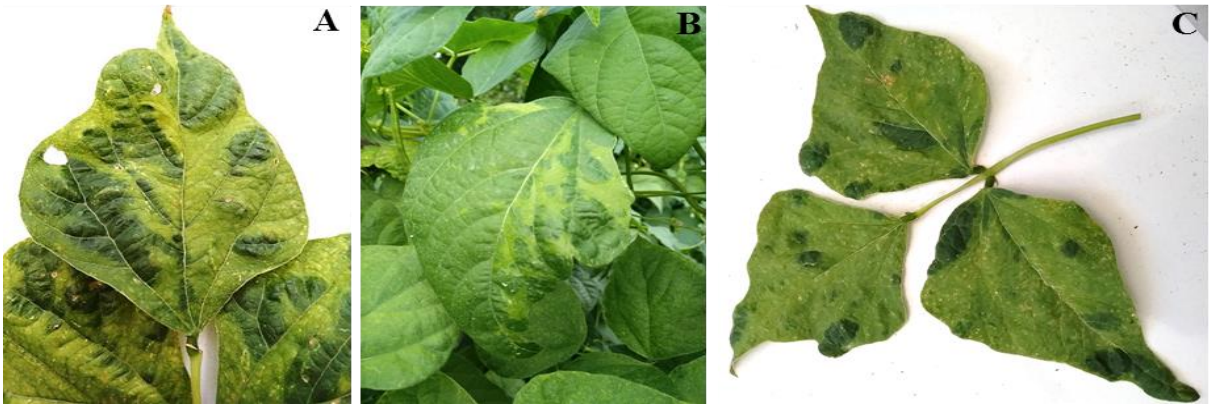

Figure 1. Characteristic symptoms occuring naturally as a reaction in bean leaves against BCMV infections. A: Leaf puckering and diffuse zones of light and dark green B: Leaf distortion, vein banding, and downward at leave margin C: Foliar mosaic rugosity, and mottle symptoms.

For 20 specimens collected, the RT-PCR test was run to multiply the nucleotide sequence encompassing the CP gene from BCMV using pairs of primers. In experimental trials**,** eleven bean specimens reacted positive results by revealing single amplified about 850 bp band, corresponding to CP gene fragments of BCMV. This band was utilized for all subsequent cloning studies. No band was generated from healthy plant tissue and remained nine specimens (Fig 2), namely the BCMV-free.

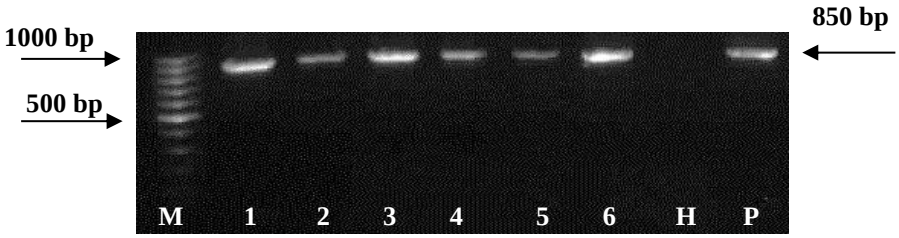

Figure 2. The pattern of RT-PCR yields of the capsid protein (CP) genes of BCMV from independently bean leaves using appropriate primer pairs, after electrophoresis, in a 1.0% agarose gel. Row M: 1 kb DNA marker, Row 1-6: BCMV isolate, Row H: Healthy bean control, Row P: BCMV- positive control.

Phylogenetic Analyses and Genetic Diversity of BCMV- Antalya isolates

Sequences of viral cDNA were found to be 850 bp in length by sequencing in both directions, designated as Antalya 1 and Antalya 10 isolates and deposited to the NCBI under the accession numbers MN1048389 and MN104840. Phylogenetic interrelation was constructed with a sequence of both isolates and 17 genetically various isolates available in NCBI. The phylogenetic tree created by the neighbor-joining method revealed four basic clades (Fig 3). It indicated that isolates of BCMV Antalya take place in distinct groups, each with an isolate from another country. The partial CP sequences of these isolates were aligned with corresponding CP region sequences of representative sequences of BCMV from Turkey and different regions of the world. Nucleotide sequences of BCMV infecting bean shared 81.41- 94.71% nucleotide identities with other BCMV isolate sequences retrieved for comparison. Antalya 1 and Antalya 10 isolate showed the highest nucleotide sequence similarity of 94.71 with bean seed (TR-243-2) and % 91.22 with bean (NL1) isolates of BCMV, respectively.

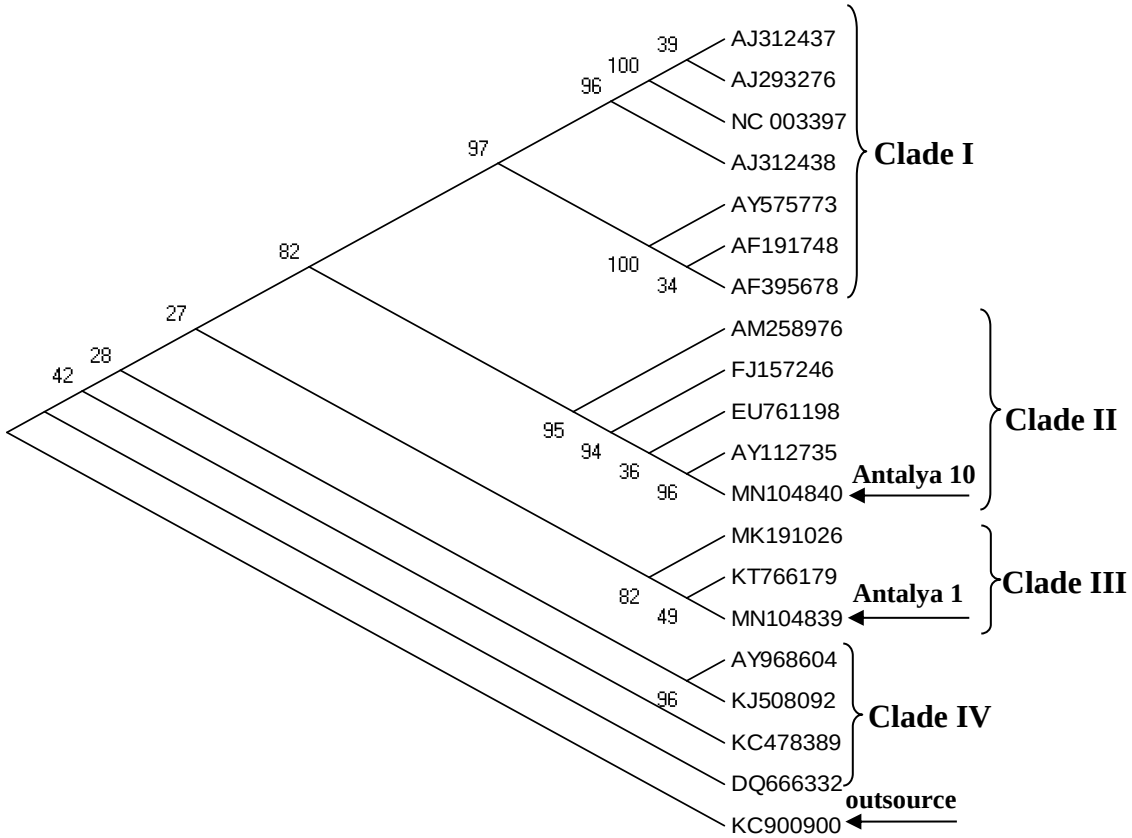

Figure 3. Dendrogram constructed with a neighbor-joining algorithm of the partial CP gene sequences of BCMV and BYDV-PAV was taken as the outgroup to branch the tree. Bootstrap scores for 100 replicates are given next to the roots. The arrows show the status of Antalya isolates. Sequences for BCMV were recovered from Genbank. AJ312437: isolate R (China); AJ293276: isolate Hangzhou, (China); NC003397: isolate R (China); AJ312438: isolate Y (China); AY575773: isolate Taiwan (Taiwan); AF191748: no isolate name (Thailand); AF395678: no isolate name (Taiwan); AM258976: no isolate name (Peru); FJ157246: isolate K2 (India); EU761198: isolate MS1 (Australia); AY112735: isolate NL1 (England): MK191026; isolate Van 1 (Turkey); KT766179: isolate TR-243-2 (Turkey); AY968604: no isolate name (Taiwan); KJ508092: isolate Habin1 (South Korea); KC478389: isolate HB (China); DQ666332: isolate NL4 (Colombia); MN1048389 and MN104840 (Antalya 1 and Antalya 10, this publication).

Discussion

BCMV is the commonest legume-infecting potyvirus and has been recognized as a major restrictive on geographical distributions of the bean. The beans came to Anatolia about 250 years ago and gained a very widespread area (Sehirali, 1988). Bean is affected negatively by many bacterial, fungal and viral disease factors as well as abiotic circumstances. About 30 virus diseases within 12 genera have been identified that lead to significant yield losses in bean production areas (Loebenstein and Thottappilly, 2004). The virus-introduced beans show symptoms such as mosaic,

typically downwardly curled leaf, longer and narrower leaves than usual, vein banding, and leaf distortion in both natural and empirical conditions reported by various investigators (Mangeni et al., 2014; Melgarejo et al., 2007; Deligoz and Sokmen, 2013).

In this study, 20 different bean leaf tissues showing symptoms were collected and tested by RT-PCR using BCMV- CP specific primers. Eleven out of 20 were found to be infected with this virus by producing 850 bp DNA fragments in agarose gel. The rest specimens were BCMV- uninfected. Interestingly, in some specimens (5) were BCMV-negative despite exhibiting characteristic of viral symptoms. This situation may be due to other viruses inducing similiar symptoms such as AMV, TLYCV, BCMNV, CMV and, TSWV in bean (Morales ve Bos, 1988; Jalali and Rastgou, 2017).

Serological and molecular methods, especially ELISA and PCR are widely employed in the detection of plant viruses in experimental traits across the world (Boonham et al., 2014). ELISA-derived methods were utilized by several authors (Peyambari et al., 2006; Davis and Tsatsia, 2008; Dizadji and Shahreaeen, 2011; El- Kady, 2014), whereas the RT-PCR-based methods were performed with specific primers designed to target the CP gene of this virus in other studies. In the present work, the 850 bp fragment (amplified viral cDNA), was obtained in agarose gel test that confirmed the presence of BCMV. This amplified fragment was in line with those of obtained results by other researchers (Bhadramurthy and Bhat, 2009; Colak Ates et al, 2017).

There is not enough data on BCMV at the molecular characterization manner in Turkey. In a study conducted in Samsun between 2002 and 2003, it was determined that 18.9% of 53 bean seed specimens from producers and seed dealers were infected with BCMV. They determined that 36% of the collected 499 leaf samples were infected with BCMV (Guzel and Arlı-Sokmen, 2003). In the another study conducted from Samsun in 2006, a total of 9 BCMV and 3 BCMNV isolates were obtained from the samples determined to be infected with BCMV and BCMNV in the bean-growing region (Deligoz and Arli Sokmen, 2008).

In Izmir, bean plants produced from 70 bean seeds were tested and BCMV was detected in 61.43 % of the specimens by DAS-ELISA and RT-PCR (Saracoglu and Erkan, 2016). Culal Kilic and Yardımcı (2014), 102 specimens from the areas of bean cultivation in Burdur province were tested by RT-PCR assay and BCMV was detected in 24 specimens (23.52%) by IC-RT-PCR method and DAS- ELISA. Similarly, the infection rate of BCMV in 112 bean plants from Mugla province was determined as %17.85 (20 specimens) by DAS-ELISA (Culal Kilic et al., 2013).

It has also been reported by Arli-Sokmen et al. (2016) that 367 bean leaves and seed specimens were tested against BCMV infection by ELISA in 15 distinct provinces (Erzincan, Balıkesir, Bursa, İzmir, Aydın, Kahramanmaras, Mugla, Antalya, Konya, Karaman, Mersin, Nigde, Samsun, Tokat, Hatay) and 67 of them (18.2%) were found with BCMV infected (No infection was found in İzmir, Hatay, Mugla). Besides, the BCMV-associated infection rate was registered as 59% in bean seeds specimens by DAS-ELISA in Tokat (Kutluk Yilmaz et al., 2002).

Of the eleven BCMV-positive, two randomly were successfully cloned and sequenced for the determination of nucleotide structure. The partial CP gene nt sequences of two BCMV isolates consist of 823 bp nucleotide encoded for 274 amino acid residues. Sequence analyses clarified the possible origins of the MN104839 and MN104840 sequences. Bioinformatics analysis revealed that Antalya 1 has a highest similarity with the Turkish isolate (KT766179) by 94.71% and has lowest similarity with the Taiwan isolate (AY968604) by 81.64% , while Antalya 10 isolate has the highest similarity with the England isolate (AY112735) by 91.22% and has the lowest similarity with the Turkish isolate (MK191026) by 81.41%. Interestingly, the two isolates showed homology by 91.41% between themselves, although both shared the same location. Nucleotide sequence difference in Samsun (KT766179), Van (MK191026), and Antalya isolates (MN104840 and MN104839) suggesting that there is high sequence variability within BCMV isolates in Turkey. In further analysis, the gene sequences of both Antalya isolates showed significant genetic differences between each other and the other isolates. Point mutations at nucleotide levels were genetically determined at 27 positions in MN104840 (Antalya 10) and 43 nucleotides for MN104839 (Antalya 1) based on the multiple alignment results performed by CLC Main Workbench program (version 7). This genetic variation and varying similarity rates may likely be due to point mutations occurring in the CP gene region, which shows the development of new strains/isolates due to ongoing evolution (Vallejoes et al., 2006).

Antalya sequences in common bean were further characterized for a genetical relationship using the Tamura-Nei method by MEGA 7 program. Roughly 4 clades appeared through the phylogram created from partial CP gene sequences of isolates chosen from the GenBank. Both isolates were clustered on the distinct branch supported by bootstrap scores of a hundred. This may be because the Antalya isolates in this work are different

isolates/strain of BCMV in biologically different classes, as reported by Drijfhout et al. (1978). Considering only Antalya isolates, Antalya 1 isolate took place in the same group with Peru, India, Australia, and England isolates (Clade II), while Antalya 10 isolate shared the same group along with Turkish isolates only in the Clade III as depicted in Fig 3. The phylogram clearly showed that two BCMV isolates along with other isolates from GenBank were in different clades, suggesting that this situation does not depend on the host or geographic area. BCMV can easily pass through seeds, and transporting BCMV- infected seeds for over long distances can easily spread this virus. It can be concluded that Antalya isolates are in the same group with other isolates from different countries and have high sequence similarity.

BCMV is one of the most financially significant viruses that can be present in all bean growing areas. The absence of chemical solution against plant viruses increases the importance of cultural control. The most effective way to control this virus is to use resistant varieties and certified seeds. Also, the destruction of vectors and weeds, and the removal of annual plant residues are important for inhibiting the spread of the virus (Mink et al., 1994).

Conclusions

We found the two BCMV isolates on bean from Antalya. The characterizations suggest that the Antalya isolates found in different clades, with 94.71 similarities Antalya 1 has the highest similarity with the Turkish isolate (KT766179) and, with 91. 22 similarities Antalya 10 isolate has the highest similarity with the England isolate (AY112735).

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