The molecular characterization of the coat protein sequence and differentiation of CMV-subgroup I on tobacco from native flora in Turkey

Mustafa USTA¹*, Abdullah GÜLLER², Abidin GÜNAY¹

¹Van Yuzuncu Yil University, Faculty of Agriculture, Department of Plant Protection, Van, Turkey; mustafauta@yyu.edu.tr (*corresponding author); gunayabidin@hotmail.com
²Bingöl University, Faculty of Agriculture, Department of Plant Protection, Bingöl; Turkey; gullerabdullah@hotmail.com

Abstract

Cucumber mosaic virus (CMV) has a broad plant-host range and a wide ecological zone distribution. Virus-like symptoms were observed on tobacco fields of Adıyaman province (Turkey) showing conspicuous mottling, greenish mosaic patterns and severe malformations of leaves. A total of forty tobacco samples tested positive against CMV by reverse transcription polymerase chain reaction (RT-PCR) using coat protein gene specific primers. Five randomly chosen CMV isolates were cloned into pGEM T-Easy vector and transformed into Escherichia coli JM109 strain. The recombinant bacterial clones containing insert-DNA were further purified and sequenced bidirectionally. In multiplex-RT-PCR studies carried out, it was found that all 40 CMV isolates belong to Subgroup I by resulting a 593 bp long DNA fragments. CMV subgroup IA was found to predominate in 4 out of 5 tobacco samples and CMV subgroup IB was found in 1 out of 5 CMV-positive samples by comparing the isolates with CMV reference isolates in phylogenetic tree. However, no Subgroup II sequences were found by multiplex RT-PCR using discriminating primers. The nucleic acid sequences were analyzed for the investigation of diversity of coat protein (CP) sequences of 5 CMV isolates. The sequence similarity ranged from 94.2-100% with the CMV subgroup I isolates infecting diverse plants in other regions of the world. The evolutionary tree revealed that the CMV IA Adıyaman isolates exhibited a genetic affinity with Australian and Spanish isolates. However, the CMV IB Adıyaman isolate showed a close genetic relationship with only the Australian isolates. To our knowledge, this study shows for the first time the occurrence of CMV IA and IB isolates infecting cultured tobacco plants in Adiyaman province.

Keywords: characterization; cloning; cucumber mosaic virus; phylogeny; subgroup

Introduction

Tobacco (Nicotiana tabacum L.) is one of the substantial economic products and is grown in most countries such as Brazil, Turkey, Canada, USA, and China where it represents primary producing fields (FAO, 2013). Virus-borne diseases frequently lead to reduced quality of product and yield. Tobacco crops are attacked by numerous viruses such as potato Y potyvirus (PYV), tobacco ringspot nepovirus (TRSV), pepper mottle potyvirus (PeMoV), cucumber mosaic cucumovirus (CMV), alfalfa mosaic alfamovirus (AMV), tobacco leaf
curl geminivirus (TbLCV), tobacco etch potyvirus (TEV), tobacco mosaic tobamovirus (TMV), tomato spotted wilt tospovirus (TSWV) and become epidemic in many tobacco cultivated areas (Valand and Muniyappa, 1992; EPPO/CABI, 1996b; Chatzivassiliou, 2008; Chen et al., 2014; Akinwumi et al., 2016).

Adiyaman is traditionally one of the oldest tobacco planting provinces of Turkey which is well known for its local tobacco. Adiyaman’s tobacco is an important source for rural income, ranking 5th with its 8% tobacco production of Turkey (TUIK, 2015). Single, double or multiple virus infections can potentially destroy the tobacco crops, if it is planted on a large scale. *Cucumber mosaic virus* (CMV) is considered important host of tobacco, which has a wide array of hosts capable of making epidemics in more than 1300 plant species (Garcia-Arenal and Palukaitis, 2008).

CMV, belonging to the *Bromoviridae* family, was firstly recorded in cucumber and melon cultivation areas in USA in 1916 (Doolittle, 1916), thereafter recognized in various agricultural and ornamental plants from other numerous countries such as Argentina, India, Korea, China in temperate and tropical regions (Rodríguez Pardina et al., 2013; Nagendran et al., 2018; Park et al., 2018). CMV is a destructive disease in *N. tabacum* with symptoms like mosaic, deformation, dwarfing and sometimes necrosis in infected plants depending on the variety of plant and viral isolate, weather conditions, existence of satellite RNA, pathogenicity, and co-existing infections (Palukaitis et al., 1992). It possesses an isometric-shaped, tripartite (+) ssRNA genome of approximately 29-30 nm diameters, without envelopes. This genome is encapsidated in separate particles, which are RNA-4 (also referred to as subgenomic RNA), RNA-3, RNA-2 and, RNA-1 in increasing length (Palukaitis and Garcia-Arenal, 2003). CMV has been transmitted experimentally by plant sap and non-persistently in more than 75 species of a stylet-borne aphids from plant to plant, especially from *Myzus persicae* and *Aphis gossypii* (Kaplan et al., 1997).

CMV isolates are principally categorized into two parts as subgroup I and subgroup II (SI and SII) based on serological tests, triplet mapping of coat protein (CP) gene, RT-PCR followed by RFLP analyses, nucleic acid hybridization, and nucleotide sequence similarity. Furthermore, SI is subdivided into the subgroup IA (SI-A) and subgroup IB (SI-B) depending on the analysis of open reading frame gene, untranslated region sequences of RNA3 and a cladistic assay of the CP gene. Such a classification has been reported and supported by various researchers (Palukaitis et al., 1992; Roossinck, 2002; Lin et al., 2003).

The presence of CMV and its subgroups has been reported in many international and national studies with different tests in different hosts (Kaplan et al., 1997; Rodríguez Pardina et al., 2013). However, studies on subgroup discrimination of this virus nationwide are limited. Although it is a widespread virus in many crops, little is known about the molecular features of Turkish tobacco isolates and their subgroups they belong. This research has addressed the categorization and molecular analysis of 5 CMV strains at the genomic level in symptomatic tobacco plants from Adiyaman province of Turkey.

**Materials and Methods**

**CMV isolates**

A total of 40 CMV isolates included in this paper were obtained from our previous tobacco field survey performed during the period from August to September 2018 in Adiyaman province, located in southeast region of Turkey (Günay, 2019). Plant samples and their RNA preparations were maintained at -70 °C until processed. The all isolates were subjected to CMV subgroup discrimination assays resulting two fragments of 593 and 704 bp, specific to CMV subgroup I and CMV subgroup II, respectively (Chen et al., 2011). Five randomly selected Subgroup I CMV isolates were further characterized by molecular cloning.

Before the discrimination subgroup, the cultures of CMV isolates were maintained on *N. tabacum*. Mechanical inoculation was carried out in two cotyledon leaf stages by rub procedure using phosphate buffer (PB), nicotine (2.5%) and carborundum powder as an abrasive. The systemically infected test plants kept in
the climate chamber at room temperature and daylight situations for further investigations. The symptomless tobacco plants were used as a negative control during all experimental processes.

**Total RNA extraction, primer design, and cDNA synthesis**

For all specimens, total RNA extractions were performed from about 0.1 g frozen leaf tissues, following the protocol described by Foissac *et al.* (2001) with minor modifications. As given in Table 1, specific upstream and downstream primer sets were adopted from previous studies (Nakazono-Nagaoka *et al.*, 2005; Chen *et al.*, 2011), which targeted the CP gene to detect and differentiate CMV SI and SII in tobacco. In all cDNA syntheses, random hexamer primers were used instead of oligo-dT as complementary to mRNA because the CMV nucleic acid was not polyadenylated.

The extracted RNAs were used in the first-strand cDNA synthesis (complementary DNA). In brief, into a nuclease-free microfuge tube, 1 μl of random hexamer primer (20 pmol/μl), 2 μl of extracted RNA as a template and 1 μl of dNTP (10 mM) were put then completed to 12 μl with nuclease free water. The mixture was held at 65 °C for 5 min and chilled on ice.

Four μl of 5X RT Reaction buffer, 2 μl of 0.1M DTT, 1 μl of RNAse inhibitor and 1 μl of reverse transcriptase (RT) enzyme (Thermo Scientific, USA) were added to complete the reaction mixture to a final volume of 20 μl. The reverse transcription reaction was performed at 42 °C for 50 min. To inactivate the RT enzyme, the mixture was incubated at 70 °C for 15 min. The cDNAs were maintained at -20 °C until processed.

**Identification of CMV SI and SII isolates by Multiplex-RT-PCR (M-RT-PCR) assays**

The cDNAs were served as template in M-RT-PCR assay. For CMV SI and SII, the reagents were adjusted empirically in a total volume 50 μl, consisting of 34.6 μl of sterile distilled water, 2 μl of cDNA, 3 μl of MgCl2 (25 mM), 1 μl of dNTPs (10 mM), 1 μl of downstream and upstream primers (20 pmol), 5 μl of 10X Taq buffer, 0.4 μl of Taq DNA polymerase (5 U/μl) (Thermo Scientific, USA) enzyme. The temperature cycles of M-RT-PCR reaction were as follows: initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, elongation at 72 °C for 45s. Final elongation occurred at 72 °C for 10 minutes.

Fifteen μl amplified target fragments were photographed under UV light after electrophoresis on 1.5% agarose gel containing ethidium bromide (EtBr). Healthy tobacco plants were used as negative control. A CMV SI isolate confirmed by previous sequence analysis was used as positive control.

**Cloning, sequencing and cladistic analyses**

Amplified-DNA fragments were separately excised from agarose gel and the amplicons were recovered using GeneJET Gel Extraction Kit (Thermo Scientific, USA) following the manufacturer’s instructions. Five randomly selected CMV isolates were cloned into the pGEM T- Easy vector (Promega, USA) following the manufacturer's instructions and transformed into *E. coli* JM 109 strain. Recombinant plasmids were purified and sequenced bidirectionally in an automated sequencer (Sentebiolab Company, Turkey). Insert sequences were trimmed from raw sequences using CLC Main Workbench program (Version 6.7.1) and recorded to NCBI Database (www.ncbi.nlm.nih.gov).

As presented in Table 2, the cladistic analysis was performed with representative 16 CMV isolates belonging to Subgroup IA, IB, and Subgroup II published in the NCBI. The phylogenetic tree was constructed using Mega 7 program bootstrapped 100 times using the neighbor-joining algorithm (NJA) (Kumar *et al.*, 2016). The nucleic acid sequence alignments were generated using CLC Main Workbench program (Version 6.7.1) to estimate similarity scores. Tomato aspermy virus (EF153735) sequence was used as an outgroup to root the phylogenetic tree.
Results

Symptoms produced by CMV isolates
Symptom development of CMV isolates was examined experimentally. The symptoms developed on *N. tabacum* in experimental studies were similar to the natural diseased *N. tabacum*, but were more evident and severe (Figure 1).

![Symptoms of CMV-infected tobacco plants. Panel A and B: Foliar mosaic, leaf puckering in experimental tobacco plants in climate chamber, Panel C and D: Greenish mottle, leaf distortion in field detected tobacco plants.](image)

Identification of CMV subgroup I
Forty samples of nucleic acids were amplified by M-RT-PCR using discrimination primers (Table 1). The resulted amplicons were about 593 bp length indicating that the all isolates were belong to CMV Subgroup I. However, no 704 bp DNA fragments were observed when the Subgroup (SII) primers were used to investigate CMV Subgroup II members (Figure 2).

BLAST analyses and multiple alignments
Amplification products of five CMV Subgroup I, representing full length of coat protein gene of (657 bp), were successfully cloned into the pGEM T-Easy vector. The BLAST analyses confirmed the coat protein origin of five cloned sequences. The CMV-Subgroup I tobacco isolates were named TR41, TR54, TR93, TR128, and TR131 and submitted to GenBank with accession numbers of MK89142, MK890143, MK890144, MK890145, and MK890146, respectively. Multiple alignments revealed a high homology between Adiyaman and another Subgroup I isolate in the GenBank. Full-length CP gene sequences of Adiyaman CMV-Subgroup I isolates from tobacco growing region exhibited 94.2 to 100.0% indentity between five isolates. Multiple sequence alignments indicated that five CMV Subgoup I Adiyaman isolates and the isolates from other geographic origins had 75.1 to 99.7% identity at the nucleotide level (Table 3).

Cladistic analysis
Based on molecular relationships and nucleotide sequence similarity, five CMV isolates were classified into two major subgroups. The four isolates (MK890144, MK890146, MK890143, and MK890142) were clustered in SI-A and one isolate (MK890145) in SI-B, fortified by supporting values as shown in Figure 3.
Table 1. Product size and nucleotide sequences of primers utilized in RT-PCR and M-RT-PCR for detection and discrediting the subgroups of CMV

| CMV primer types | Upstream          | Downstream                     | Amp. size |
|------------------|-------------------|--------------------------------|-----------|
| Characterization | ATGGGACAAATCTGAATCAAC | TCAGACTGGGAGCACTCCAG          | 657 bp    |
| Subgroup I (SI)  | GCCACCCCAAAATAGACCG | ATCTGCTGGCGTGAGATTCTTCT        | 593 bp    |
| Subgroup (SII)   | CTACGTATTATCTTCC   | AACCAGTGATTTACCATCGC           | 704 bp    |

Table 2. CP gene nucleotide sequences of various CMV strains used for phylogenetic tree

| No  | Origin | Accession no | Strain names | Subgroup | Host              |
|-----|--------|--------------|--------------|----------|-------------------|
| 1   | Hungary| L15336       | trk7         | SII      | -                 |
| 2   | Australia| M21464     | Q            | SII      | -                 |
| 3   | USA    | AF127976     | LS           | SII      | -                 |
| 4   | Japan  | AB006813     | m2           | SII      | -                 |
| 5   | India  | AJ585086     | Indian       | SII      | Lilium            |
| 6   | Australia| U22821      | Ny           | SIA      | -                 |
| 7   | Hungary| AJ517802     | Rs           | SIA      | *Raphanus sativus*|
| 8   | Spain  | AM183119     | Ri-8         | SIA      | Tomato            |
| 10  | Israel | U66094       | Sny          | SIA      | *Cucurbita pepo*  |
| 11  | Italy  | Y16926       | Tfn          | SIB      | Tomato            |
| 12  | Taiwan | D28780       | NT9          | SIB      | Tomato            |
| 13  | USA    | D00462       | C            | SIA      | -                 |
| 14  | Japan  | D42079       | C7-2         | SIB      | -                 |
| 15  | India  | AF281864     | -            | SIB      | *Datura innoxia*  |
| 16  | Turkey | KY474380     | CWP17        | SIB      | Cowpea            |
| 17  | India  | EF153735     | Kolkata      | TAV      | *Chrysanthemum morifolium* |

Figure 2. Analysis of CMV-Subgroup I from individually infected tobacco plants using multiplex RT-PCR; Lane M, 1-kb DNA ladder; PC, Positive control; NC, Negative control; Lane 1-10, CMV Adiyaman isolates
**Table 3. Chart showing multiple alignment analysis of CMV isolates generated with CLC Main Workbench program**

|    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| L6 | 4   | 1   | 99  | 85  | 99  | 70  | 99  | 54  | 99  | 43  | 99  | 39  | 99  | 39  | 99  | 24  | 98  | 95  | 96  | 93  | 95  |
| L2 | 3   | 2   | 99  | 85  | 99  | 70  | 99  | 54  | 99  | 43  | 99  | 39  | 99  | 39  | 99  | 24  | 98  | 95  | 96  | 93  | 95  |
| A  | 1   | 3   | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 4   | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 5   | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 6   | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 7   | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 8   | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 9   | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 10  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 11  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 12  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 13  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 14  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 15  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 16  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 17  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 18  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 19  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 20  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
| A  | 1   | 21  | 99  | 54  | 99  | 24  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  | 99  |
Figure 3. Phylogenetic dendrogram constructed with CMV-Adiyaman isolates and the isolates, retrieved from GenBank, created by the neighbour-joining algorithm. The sequence of tomato aspermy virus isolate (EF153735) was used as an outgroup to root the tree. Bootstrap values are shown on each branch.

Discussion

Identification of CMV isolates is important to elucidate the ancestor of this virus and develop a control strategy. Although ELISA and RFLP assays are used (Haase et al., 1989; Shevchenko et al., 2015), Multiplex RT-PCR is a practical method for the simultaneous differentiation of CMV subgroup I and II (Rizos et al., 1992; Yu et al., 2005; Eyvazi et al., 2015). To differentiate CMV subgroups, various primers have been developed by various researchers based on the conserved sequence within the viral genomic segment. Primers used by Chen et al. (2011) produced 593 bp amplicons in RT-PCR tests, consistent with the present study.

SI-A, SI-B and SII groups of CMV have been reported in literature from numerous countries such as USA (D10538, IA), Japan (D16405, IA), South Korea (AJ27648, IA), Indonesia (AB042294, IB), Philippines (U20219, IB), Spain (AM18319, IA), Taiwan (D28780, IB), Italy (Y16926, IB), USA (AF127976, II), Australia (AF198103, II), South Africa (U37227, II), and Hungary (L15336, II) (Rodríguez Pardina et al., 2013; Arafati et al., 2013). Furthermore, historical records denoted that S-IA members were distributed throughout the world, while SI-B members were mostly reported in eastern Asia, considered to be the origin of this subgroup, although some have been found in Mediterranean region, Iran, California, Brazil, Australia and Greece (Sclavounos et al., 2006; Farzadfar et al., 2013). In this study, it was determined that one isolate classified in CMV SI-B and the others (four isolates) were in CMV SI-A based on the phylogenetic assay. None
of the isolates was classified in CMV subgroup II. The widespread of CMV subgroup I isolates in this region may be due to its severity and abundancy worldwide, compatible with previous reports (Singh et al., 1995; Tian et al., 2009). The absence of CMV SII isolates in our tests could probably be explained by inappropriate climate conditions in the surveyed areas and probably because of its tropical climate’s adoption (Hord et al., 2001; Kumari et al., 2013).

CMV SI isolates have been perfectly adapted to various plants such as cucumber, tomato, pepper, tobacco, pumpkin, bean, celery, musa crops, peanut, yam, and weeds from different locations in world, confirmed by serological and molecular tests (Eni et al., 2013; Ayo-John and Hughes, 2014; Zhu et al., 2018). The presence of CMV in tobacco has been reported by several researchers as both natural hosts (Chatzivasiliou et al., 2004; Dai et al., 2012; Zhang et al., 2013) and experimental host (Tian et al., 2009; Chikh Ali et al., 2012). It has a nationwide host range with the exception of tobacco, which includes: Myrtle leaf milkwort, globe artichoke, parsley, mint, broccoli, squash, olives, cabbage, peppers, lettuce, spinach, cowpea, gladiolus, tomatoes, zucchini, cucumber, ornamental plants (daffodils, hyacinths, lilies), bean and spinach (Gümüs et al., 2004; Beler and Acıkgöz, 2005; Sevik and Akcura, 2011; Culal Kılıc and Yardımcı, 2012; Erkan et al., 2013; Ergün et al., 2013; Culal Kılıc et al., 2015; Uzunoğulları and Gümüs, 2015; Sertkaya, 2015; Karanfil et al., 2016; Gökdag et al., 2016; Güngör et al., 2017; Karanfil and Korkmaz, 2017; Koc and Fidan, 2017; Sevik, 2012, 2017). Since its transmission to tobacco is not seed-borne (De Bokx and Huttinga, 1981), its presence and prevalence can stem from other virus sources like weeds, the suitable climate environment for active aphid populations throughout tobacco production season and acquired from other cultivated plants grown from CMV-infected seeds (Kaplan et al., 1997; Tsitsipis et al., 2001).

CMV exhibited characteristic symptoms more severely such as mottle, mosaic and leaf malformation in experimental transmission studies on tobacco. These symptoms were similar to those mentioned by Chikh Ali et al. (2012), Sclavounos et al. (2006) and Arafati et al. (2013) on tobacco leaves and on many hosts both native flora and experimental studies (Zhang et al., 1994; Jalender et al., 2017).

Analysis of phylogenetic tree showed that Turkish tobacco SI-A isolates (MK89142, MK890143, MK890144, MK890146) were clustered in the same branch, and well differentiated from the members of SI-B isolates. Within SI-A, one isolate (MK890144) was closely related to the Israeli-squash (U66094) isolate, one isolate (MK890146) was related to the Spanish-tomato (AM183119) isolate, the other two isolates (MK890142 and MK890143) were closely related to each other, which formed a separate cluster. The Adiyaman SI-B isolate (MK890145) was phylogenetically grouped with the Turkish cowpea SI-B isolate (KY474380). Multiple alignments further revealed that the sequence of CP of CMV SI-B isolate showed approximately 94% sequence similarity, whereas CMV SI-A isolates shared the maximum identity of 94.22-100% among themselves at the CP gene level (Table 2). These incidences coincide with the literature reported by Palukaitis and Garcia-Arenal, (2003). The highest homology (100%) was shared between MK890142 and MK890143 isolates (subgroup I-A), while the lowest homology (94.22%) was shared between MK890146 (subgroup I-A) and MK890145 (subgroup I-B) isolates. This variation among CMV I isolates can likely be due to reassortment occurring within the CP gene demonstrating the event of new strains due to proceeding evolvement (Moury, 2004).

Based on previous studies carried out in Turkey, CMV SI-A has been found in pepper, tomato and watermelon (Caglar, 2006), CMV SI and SII in pepper, tomato, cucumber, watermelon and melon (Sarı, 2015), CMV SI-B in cowpea (Karanfil and Korkmaz, 2017) and, CMV SI-A in spinach (Kurtoglu and Korkmaz, 2018). Even though CMV has been detected on a wide range of hosts in Turkey, subgroup categories and their classification of most of them were not currently completed at the nucleotide levels. Further molecular analyses are needed for classifying the CMV isolates into subgroups on diverse crops. The present study is the first report on the presence of CMV subgroup I-A and I-B in infected tobacco and their molecular characterizations in cultured tobaccos in Adiyaman province.
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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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