

Coat protein variability of *Apple mosaic virus* isolates from different plant hostsFiliz Ertunç* 

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*Corresponding author email: ertunc@agri.ankara.edu.tr**Abstract**

Apple mosaic virus, apathogen of stone and pome fruits and hazelnut worldwide, shows great variability in its biological, serological and molecular properties. Apple mosaic virus (ApMV) variants were collected from hazelnut plantations located in Black Sea region of Turkey and the apple isolates were collected from main apple cultivation areas. The infection was present on different local hazelnut varieties but the infection was only detected on Granny Smith apple variety in Turkey. The *Rubus* plantations surrounding the orchards were also infected with ApMV. The coat protein sequences of fifteen ApMV variants from different hazelnut varieties and the 'Granny Smith' apple variety were obtained and the phylogenetic analyses were performed. The sequences obtained from hazelnut revealed slightly different in nucleic acid and amino acid composition compared to the sequences obtained from apples in Turkey. Since the Granny Smith is originated from abroad, the infection might be imported by the infected plant material to Turkey.

Key words: *Apple mosaic virus, apple, hazelnut, coat protein variability*

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Introduction

Apple (*Malus x domestica* Lam) is one of the most widely grown fruit crop of temperate zones in the world. It belongs to the family *Rosaceae* and it is widely produced in different climatic zones in Turkey (Dumanoglu et al. 2015, Taşçı, 2017). Turkey is one of the leading countries in the World with 3625960 tons of apple production (TUIK, 2019).

Hazelnut (*Coryllus avellana* L.) is native to Turkey and it is commercially cultivated along the Black Sea coast. Turkey is the leader of hazelnut production in the world with 515000 tons, which provides 56 % of the world's production (TUIK, 2019). *Apple mosaic virus* (ApMV) occurs worldwide and it is known as the most common virus infection on woody trees of the family *Rosaceae*, including apple, pear, apricot, plum, almond and also rose plants (Nemeth 1986). It has also been determined as the causal agent of the severe mosaic-type of infection and has a significant economical importance in hazelnut production in Turkey and also in Poland (Kobyłko and Nowak 2006; Ertunç et al.2014). This virus is transmitted by vegetative propagation material in woody plants.

ApMV is a species of the genus *Ilarvirus* (subgroup III) in the family Bromoviridae and has a tripartite, positive sense, single stranded RNA genome. RNA1 and RNA 2 code for the proteins involved in virus genome replication while RNA3 is

bicistronic and encodes a movement protein (MP) and a coat protein (CP), the latter being expressed from a subgenomic RNA (RNA4) (Alrefai et al.1995; Shiel et al.1995). The genus *Ilarvirus* comprises of a large group of plant viruses and woody trees are the primary hosts (Van Regenmortel et al.2000).

Symptoms of ApMV vary widely depending on climatic conditions, virus isolate, host species and plant cultivar (Nemeth 1986). They range from symptomless infections to severe systemic mosaic, chlorosis and vein clearing in apples and chlorotic ringspots and severe systemic mosaic in hazelnut. An overall reduction in size of fruits and the trees of both species is also observed (Sutic et al.1999). The development of rapid and reliable detection methods of variants of the virus infection. requires knowledge of sequence variability of the virus variants when designing PCR primers. Gene sequences of Indian isolates were conserved and their comparison revealed a maximum of 96 % homology to a Korean isolate of ApMV (AY125977) (Tombisana et al.2009, Lakshmi et al.2011).

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The phylogenetic analysis of coat protein gene of ApMV discriminated two main clusters of isolates: one cluster Maloidea hosts and the second in all woody plants (Grimova *et al.* 2013).

Coat protein sequences of two Korean isolate of ApMV, K1 and K2 have been investigated and homology of coat proteins were determined as 85.6 % in aminoacid composition. (Lee *et al.* 2002).

The aim and the objective of this study was to enhance the scientific knowledge on the molecular variability of ApMV variants. We sequenced the CP gene of ApMV variants from apple and hazelnut collected from different locations in Turkey and compared them with those of other ApMV variants deposited in Genebank to reveal and determine the genetic variability of ApMV.

Materials and Methods

Virus source and sequences

Apple mosaic virus isolates were collected from symptomatic hazelnut (*Corullus avellana* L.) and apple trees (*Malus x domestica* L.) var. 'Granny Smith', from the Eastern and Western Black Sea coast and major apple production areas in Turkey in 2007-2010 (Ertunc *et al.* 2014). Young hazelnut bark tissue scraps were used for RNA extraction using Rott and Jelkman (2001) protocol whereas used Menzel *et al.* (2002)'s RNA extraction protocol was used for apple foliage. Extracted RNAs were subjected to one step RT-PCR amplification with Menzel *et al.* (2002)'s primer pair and the amplified target coat protein gene products were 262 bp long (Ertunc *et al.* 2014).

Sequence determination

Following the amplification, the amplified RT-PCR products were purified by Qiaquick PCR purification kit of Qiagen. Sequence determination was performed in Biotechnology Institute Laboratories at Ankara University. Coat protein gene sequences of Turkish ApMV isolates were obtained by Beckman-Coulter sequencer.

Phylogenetic analysis

Selected amplicons obtained with ApMV CP genes were subjected to direct sequencing. The sequences were assembled using Sequencer 4.1 software and compared with selected nucleotide

sequences in the Genebank database, using BLAST (version BLASTN 2.2.18) (NCBI, Bethesda, MD, USA). Sequence alignments were performed, using Clustal X and BioEdit programmes (Hall, 1999; Thompson *et al.* 1997). The alignments were used to construct phylogenetic trees using the computer programme MEGA 5 (Tamura *et al.* 2011).

Results and Discussion

A total of 15 ApMV isolates, 8 hazelnuts and 7 apples were selected on the basis of symptom expression, the host and geographic origin, for the coat protein gene sequence characterization. Symptoms were chlorotic systemic mosaic, oak leaf pattern and diffuse ring spots on hazelnut and severe systemic mosaic and vein clearing on apple foliage. The virus was present and observed only on cv. Granny Smith apples whereas many of the local varieties of hazelnut grown in Black Sea coast were infected with ApMV.

Primer pair of Menzel *et al.* (2002) was the only primer amplified the coat protein gene genome of the both hosts in the present research. Nucleotide sequences, obtained from the 15 variants were compiled, trimmed to correspond to coat protein region and deposited in The NCBI-Genebank Database with the accession numbers GU939596-GU9396610. The obtained sequences were between 200-237 nucleotide long and were corresponding to 3' end of the coat protein gene of ApMV. They were analysed together with the other ApMV coat protein gene sequences collected from the NCBI Genebank Database. The constructed phylogenetic tree (Figure 2) revealed that hazelnut isolates and one apple isolate showed different phylogeny and they were 50 % distinct in nucleotide sequences than the other ApMV isolates. They were close to sequences of Australian hop isolates. Apple variants of Turkish ApMV isolates were quite close to Indian ApMV sequences and were settled in the same cluster. All of the ApMV variants were clustered according to their original host plants and 5 different clusters (apple, pear, prune, hop and mixed) were obtained according to their original host plants in phylogenetic analysis as seen in Figure 1. Turkish apple isolates were with the same cluster of Indian apple isolates but Turkish hazelnut isolates occurred in the mixed group.

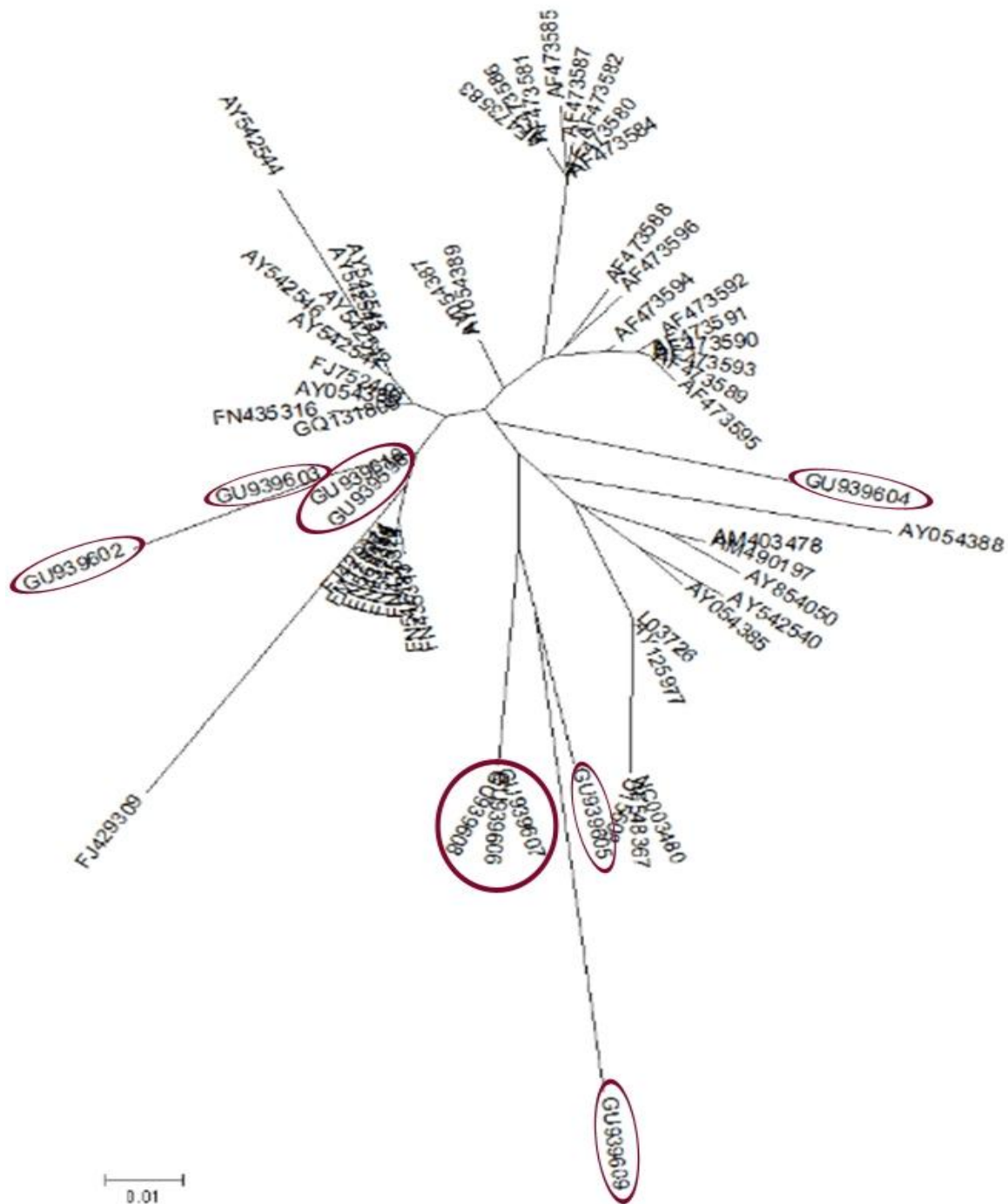


Figure 1. Phylogenetic analysis of nucleotide sequences of coat protein gene of ApMV isolates.

The consensus CP nucleotide sequences of the compared ApMV isolates showed more than 88-99% identity with all the ApMV variants at the nucleotide level. Putative translation products deduced from the corresponding CPs was 245 aminoacids with percentages of identity between 87-99 % among all the isolates.

Multi-alignment with other sequences at the coat protein composition level showed quite similarity between the Turkish apple and hazelnut isolates (Figure 2) although there were minor substitutions of amino acids unlikely to the great differences on

nucleotide sequences coat protein gene. Two Turkish hazelnut isolates (GU 939607 Adapazari-Ferizli and GU 939609 Giresun) showed minor changes at the amino acid composition level, hazelnut at amino acid positions 145 (R instead of I), 147-148 (TT instead of LV), 152 (S instead of D), 178-179 (SF instead of EA) and 218 (L instead of Y). Amino acid composition of Turkish ApMV apple isolates were in great homology (100%) with other apple ApMV variants present in the world but homology ratio was 84% between the amino acid composition of Turkish hazelnut and apple isolates of ApMV.

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AF473580
DLKVYTFHIRVNSLSSNGWIGLVEDYDESNPKGPNMMDRKGFKKQDP
RGWQWEAPPNTTF
AF473581 .....I.....187
NC_003480 .....GM.....187
AY054385 .....187
AY054386 .....Y...Q.....187
AY054387 .....187
AY054388 N.....L.....187
AY125977 .....187
AF548360 .....GM.....187
AY542540 .....187
AY542541 .....187
AY542542 .....187
AY542543 .....187
AY542544 .....LS.....187
AY854050 ...I.....G...C.....187
AM403478 ...I.....187
AM490197 ...I.....187
FM178274 .....187
FJ429309 .....QLL...AS...N.....L...T.....187
FJ429311 .....187
FJ752493 .....187
GQ131805 .....187
FN546183 .....187
GU939596 .....187(A)
GU939607 .....R.TT...S.....187(H)
GU939608 .....S.....187(A)
GU939609 .....SF.....187(H)
GU939610 .....187(A)
AF473580
DDFVKKFRLVLEFKTNFAAGAKVFMRDLYVITSELPPVQIPMNVLLID
ERFVRIMKLG
AF473581 .....I.....245
NC_003480 ...R.....T.....DLEL---241
AY054385 ...R.....V.....T.....DLEL---241
AY054386 ...R.....L...LA.DLEL---241
AY054387 .....LS...V..DLEL---241
AY054388 ...R.....T.....S.....DLEL---241
AY125977 ...R.....T.....DLEL---241
AF548360 ...R.....T.....DLEL---241
AY542540 .....TS.....DLEL---241
AY542541 ...R.....T.A...DLEL---241
AY542542 ...R.....T.....DLEL---241
AY542543 .....T.....DLEL---241
AY542544 .....LSD...DLEL---241
AY854050 ...R.....I.....D.....DM.DL---241
AM403478 ...R.....214
AM490197 ...R.....R...D...DMLDL---241
FM178274 ...R.....T.....DLL---239
FJ429309 ...R.S...N...S...L...EY.T...DLL---239
FJ429311 ...R.....T.....DLLGL---241
FJ752493 ...R.....220
GQ131805 ...R.....T.....DLEL---241
FN546183 ...R.....T.....T.....DLEL---241
GU939596 ...R.....D.....DM.DL---217(A)
GU939607 .....Y.....218(H)
GU939608 .....RV.....216(A)
GU939609 .....217(H)
GU939610 ...R.....H.....219(A)
AF473580
DLKVYTFHIRVNSLSSNGWIGLVEDYDESNPKGPNMMDRKGFKKQDP
RGWQWEAPPNTTF
AF473581 .....187
NC_003480 .....GM.....187
AY054385 .....187
AY054386 .....Y...Q.....187
AY054387 .....187
AY054388 N.....L.....187
AY125977 .....187
AF548360 .....GM.....187
AY542540 .....187
AY542541 .....187
AY542542 .....187
AY542543 .....187
AY542544 .....LS.....187
AY854050 ...I.....G...C.....187
AM403478 ...I.....187
AM490197 ...I.....187
FM178274 .....187
FJ429309 .....QLL...AS...N.....L...T.....187
FJ429311 .....187
FJ752493 .....187
GQ131805 .....187
FN546183 .....187
GU939596 .....187(A)
GU939607 .....R.TT...S.....187(H)
GU939608 .....S.....187(A)
GU939609 .....SF.....187(H)
GU939610 .....187(A)
AF473580

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DDFVKKFRLVLEFKTNFAAGAKVFMRDLYVITSELPPVQIPMNVLLID
ERFVRIMKLG
AF473581 .....I.....245
NC_003480 ...R.....T.....DLEL---241
AY054385 ...R.....V.....T.....DLEL---241
AY054386 ...R.....L...LA.DLEL---241
AY054387 .....LS...V..DLEL---241
AY054388 ...R.....T.....S.....DLEL---241
AY125977 ...R.....T.....DLEL---241
AF548360 ...R.....T.....DLEL---241
AY542540 .....TS.....DLEL---241
AY542541 ...R.....T.A...DLEL---241
AY542542 ...R.....T.....DLEL---241
AY542543 .....T.....DLEL---241
AY542544 .....LSD...DLEL---241
AY854050 ...R.....I.....D.....DM.DL---241
AM403478 ...R.....214
AM490197 ...R.....R...D...DMLDL---241
FM178274 ...R.....T.....DLL---239
FJ429309 ...R.S...N...S...L...EY.T...DLL---239
FJ429311 ...R.....T.....DLLGL---241
FJ752493 ...R.....220
GQ131805 ...R.....T.....DLEL---241
FN546183 ...R.....T.....T.....DLEL---241
GU939596 ...R.....D.....DM.DL---217(A)
GU939607 .....Y.....218(H)
GU939608 .....RV.....216(A)
GU939609 .....217(H)
GU939610 ...R.....H.....219(A)

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Figure 2. Multiple amino acid sequence alignment of the coat protein of ApMV Turkish isolates deposited in the GeneBank and also those sequences available from GeneBank. Dots indicated identical amino acids and dashes indicates the ends, (A) is apple origin and (H) hazelnut origin. Reference isolate is an coat protein gene of Australian hop strain of ApMV.

The nucleotide sequences of hazelnut isolates were almost 50 % different than the other ApMV variants whereas the apple isolates were 74 % in homology with the other ApMV variants. Nucleotide sequences of some hazelnut isolates were located in the mixed group whereas nucleotide sequences of Turkish apple isolates were close to Indian apple isolates. Previously, Korean ApMV isolates of apple (ApMV-K1 and ApMV-K2) were investigated and according to the phylogenetic analysis, it was concluded that all the stains of ApMV can be classified into three groups according to their host plants. Therefore these results suggested that ApMV strains co-evolve with their host plants and that this may result in CP heterogeneity (Lee *et al.*2002). Recently, two isolates of ApMV collected from Malatya, (Turkey) have been compared and characterized by Korkmaz *et al.*(2013). Comparison of amino acid sequence of the CP gene of Indian isolate revealed that it was 96 % in homology with Korean isolate and clustered most closely with a pear isolate originating from the Czech Republic (Tombisana *et al.*2009).

According to the results of this research, hazelnut isolates were completely distinct from the other strains of ApMV. This data clearly shows that ApMV has many strains on the world according to geographical origin and the infected host plants.

Further researches must be conducted on the strain characterization of the virus.

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