Journal of Applied Biological Sciences 10 (2): 35-40, 2016
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Genomic Conformation of Apple Mosaic Virus Turkish Isolates Coat Protein Gene Regions

Filiz ERTUNÇ
Ankara University, Faculty of Agriculture, Department of Plant Protection, Ankara, Turkey

*Corresponding author:
E-mail:ertunc@agri.ankara.edu.tr

Received: March 09, 2016
Accepted: April 22, 2016

Abstract

Apple mosaic virus, apathogen of stone and pome fruits and hazelnut worldwide, shows great variability in its biological, serological and molecular properties. The coat protein sequences of fifteen ApMV variants from different hazelnut varieties and the 'Granny Smith' apple variety were obtained in Turkey. The phylogenetic analyses of the sequences of the Turkish isolates and of additional sequences of other ApMV variants from the NCBI database indicates the existence of different ApMV groups in the world. The sequences obtained from hazelnut revealed slightly different nucleic acid and amino acid composition compared to the sequences obtained from apples in Turkey and from the different locations in the world.

Keywords: Apple mosaic virus, apple, hazelnut, genomic variability

INTRODUCTION

Apple (Malus x domestica) is one of the most widely grown fruit crop of temperate zones in the world. It belongs to the family Rosaceae and it is commercially produced in different climatic zones in Turkey. Turkey is one of the leading countries for apple production in the world with 3,1 million tons [1].

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 499 000 tons, which provides 61,5 % of the world's production [1] . 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 [2]. 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 [3,4]. 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 RNA2 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) [5,6]. The genus Ilarvirus comprises of a large group of plant viruses and woody trees are the primary hosts [7].

Symptoms of ApMV vary widely depending on climatic conditions, virus isolate, host species and plant cultivar [2]. 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 [8].

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) [9,10]. 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 [11].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 [12].

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 GenBank to reveal and determine the genetic variability of ApMV.

MATERIAL and METHOD

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 [4]. Young hazelnut bark tissue scraps were used for RNA extraction using Rott and Jelkman [13] protocol whereas used Menzel et al.'s [14] RNA extraction protocol was used for apple foliage. Extracted RNAs were subjected to one step RT-PCR amplification with primer pair of Menzel et al.'s [14] and the amplified target coat protein gene products were 262 bp long [4].

Sequence determination

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

Phylogenetic analysis

Selected amplicons obtained with ApMV CP gene,s 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 [15,16]. The alignments were used to construct phylogenetic trees using thecomputer programme MEGA 5 [17].

RESULTS

A total of 15 ApMV isolates, 8 hazelnut and 7 apple were selected on the basis of symptom expression, the host and geographic origin, for the coat protein gene sequence characterization (Figure 1 and Table 1). 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 [14] was the only primer amplified the coat protein gene genome of the both hosts in the present research. Nucleotid sequences, obtained from the 15 variants were compiled, trimmed to correspond to coat protein region and deposited in The NCBI-Genbank 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 geneof ApMV. They were analysed together with the other ApMV coat protein gene sequences collected from the NCBI Genebank Database (Table 1). The constructed phylogenetic tree (Figure 1) 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 phylogenic analysis as seen in Figure 3. Turkish apple isolates were with the same cluster of Indian apple isolates but Turkish hazelnut isolates occured in the mixed group.

The concensus 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 subtitutions 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.

DISCUSSION

Systemic mosaic type symptoms have been detected for a long time in most of the hazelnut and apple ochards in Turkey, indicating the existence of widespread of ApMV. In this study, a total of 15 ApMV isolates, 8 hazelnut and 7 apple were selected on the basis of symptom expression, host and also geographic origin for the coat protein gene sequence characterization for this research. The nucleotid 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 suggest that ApMV strains co-evolve with their host plants and that this may result in CP heterogeneity [11]. Recently, two isolates of ApMV collected from Malatya, (Turkey) have been compared and characterized by Korkmaz et al. [18].

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 [11].

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. Futher researchs must be conducted on the strain characterizations of Apple mosaic virus.

Acknowledgements

The authors are grateful to TUBITAK for supporting this research by the project coded 106O447 and Ankara University Biotechnology Institute for providing coat protein gene sequences of Turkish ApMV isolates.

REFERENCES

[1] Dumanoğlu,H, Yeşiloğlu,T , Erdoğan,V, Serçe,S, Demirsoy, H , Akçay E,Yazıcı K, Ernim C, Karabat S, Özüpek, Ö. 2015. Meyve üretiminde değişimler ve yeni arayışlar. Türkiye Ziraat Mühendisliği 8. Teknik Kongresi , Ankara, Bildiriler Kitabı-1: 550-578.

[2] Nemeth M., 1996. Virus, mycoplasma and rickettsia diseases of fruit trees. Dordrecht, Netherlands, Martinus Nijhoff Publishers.

[3] Kobylko T and B. Nowak, 2006. Detection and occurence of Apple mosaic virus in hazelnut in South-East Poland. J Plant Pathol: 122.

[4] Ertunc F, S. Topkaya, A. Sezer, 2014. Distribution and molecular detection of apple mosaic virus in apple and hazelnut in Turkey. Afr J Biotechnol 13: 3144-3149.

[5]Alrefai R, L.L. Domier, C. J. D'Arcy, S. S. Kobran 1995.The complete nucleotide sequence of apple mosaic virus RNA-3. Arch Virol 140: 1247-1256.

[6] Shiel P.J., R.H. Alrefai, L.L. Domier, S.S. Kobran, P.H. Berger, 1995. The complete sequence of Apple mosaic virus RNA-3. Arch Virol 140: 1247-1256.

[7] Van Regenmortel M. H. V., C.M. Fauquet, D.H.L. Bishop, E.B. Cartens, M.K. Estes, M.K. Lemon, J. Maniloff , M.A. Mayo, D.J. McGeoch, C.R. Pringle, R.B. Wickner,

2000. Virus Taxonomy, Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press.

[8] Akbas B and K. Degirmenci 2010. Simultaneous detection of Apple mosaic virus in cultivated hazelnuts by one-tube RT-PCR. Afr J Biotechnol 9: 1753-1757.

[9]Tombisana T, T. Rana, V. Hallan, R. Ram, A.A. Zaidi, 2009. Molecular characterization of the Indian Strain of Apple mosaic virus isolated from apple (Malus domestitica). Phytoparasitica 37: 375-379.

[10] Lakshmi V, V. Hallan, R. Ram, N. Ahmed, A.A. Zaidi, A. Varma, 2011. Diversity of Apple mosaic virus isolates in India based on coat protein and movement protein genes. Indian J Virol 22(1): 44-49.

[11] Lee P G, K.H. Ryu, H.R. Kim, D.W. Lee, J.S. Kim, M.H. Park, Y.M. Noh, S.H. Cho, D.H. Han, C.H. Lee, 2002. Cloning and phylogenetic characterization of coat protein genes of two isolates of Apple mosaic virus from 'Fuji' apple. Plant Pathology J, 18: 259-265.

[12] Grimova L, L. Winkowska, P. Rysanek, P. Svoboda, K. Petrzik, 2013. Reflects the coat protein variability of apple mosaic virus host reference. Virus Genes 47 : 119-125.

[13] Rott M. E. and W. Jelkmann, 2001. Characterization and detection of several filamentous viruses of cherry, adaptation of an alternative cloning method (DOP-PCR and modification of an RNA extraction protocol. Eur J Plant Pathol 107: 411-420.

[14] Menzel N, W. Jelkmann, E. Maiss, 2002. Detection of four apple viruses by multiplex RT-PCR assays with coamplification of plant m-RNA as internal control. J Virol Methods 99: 89-92.

[15] Hall T. A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acid S, 41: 95-98.

[16] Thompson J.D., TJ Gibson, F. Plewniak, F. Jeanmougin , D.G. Higgins, 1997. The

Clustal_ X: Windows interface flexible stratejies for multiple sequence alignment aided by quality analysis tools. Nucl Acid Res, 25 (24): 4876-4882.

[17]Tamura K, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol , 28: 2731-2739.

[18] Korkmaz, G., H.M. Sipahioğlu, M. Usta, 2013. Survey of Apple mosaic virüs in apple-growing provinces of East Anatolia (Malatya and Van) by RNA probe hybridization assay and RT-PCR. Turkish J Agric. Forestry 37: 711-718.

Table 1. Listing and geographical location of ApMV variants characterized in this study in Genebank

GQ 131805	ApMV	Apple	Fuji	Brasil
AY854050	ApMV	Strawberry	-	USA
AM403478	ApMV	Apple	KMiL	China
AM490197	ApMV	Apple	-	China
FJ429309	ApMV	Apple	Golden delicious	India
FJ429311	ApMV	Apple	Golden delicious	India
NC_003480	ApMV	Apple	-	USA
FM178274	ApMV	Apple	-	India
N546183	ApMV	Prunus sp.	-	India
AF473580	ApMV	Hop	-	Australia
AF473581	ApMV	Hop	-	Australia
AY054386	ApMV	Plum	-	Czech Republic
AY054387	ApMV	Hop	-	Czech Republic
AY125977	ApMV	Apple	Fuji	Korea
AF548367	ApMV	Apple	Fuji	Korea
AY542540	ApMV	Apple	-	Belgium
AY542541	ApMV	Pear	-	Belgium
AY542542	ApMV	Pear	Iv 10	Czech Republic
AY542543	ApMV	Pear	Kravare	Czech Republic
AY542544	ApMV	Pear	Cerin	Czech Republic
FJ752493	ApMV	Apple	-	Ukraine
AY054385	ApMV	Apple	-	Czech Republic
AY054387	ApMV	Hop	-	Czech Republic
AY054388	ApMV	Almond	-	Czech Republic
AMU03857	ApMV	Apple	-	Spain
GU939596	ApMV	Apple	Granny Smith	Turkey (Isparta-Egirdir)(This research)
GU939597	ApMV	Apple	Granny Smith	Turkey(Ankara-AUZF)(This research)
GU939598	ApMV	Hazelnut	Karafindik	Turkey(Duzce-Akçakoca)(This research)

GU939599	ApMV	Hazelnut	Karafindik	Turkey(Adapazari-Hendek)(This research)
GU939600	ApMV	Hazelnut	Mincane	Turkey (Giresun-FA)(This research)
GU939601	ApMV	Apple	Granny Smith	Turkey (Antalya-Korkuteli)(This research)
GU939602	ApMV	Apple	Granny Smith	Turkey (Tokat-merkez)(This research)
GU939603	ApMV	Apple	Granny Smith	Turkey (Tokat-merkez)(This research)
GU939604	ApMV	Hazelnut	Palaz	Turkey (Ordu-merkez)(This research)
GU939605	ApMV	Hazelnut	Palaz	Turkey (Düzce-merkez)(This research)
GU939606	ApMV	Hazelnut	Karafindik	Turkey (Adapazarı-Kocaali)(This research)
GU939607	ApMV	Hazelnut	-	Turkey(Adapazarı-Ferizli)(This research)
GU939608	ApMV	Apple	Granny Smith	Turkey(Isparta-Gelendost)(This research)
GU939609	ApMV	Hazelnut	Palaz	Turkey (Giresun-Piraziz)(This research)
GU939610	ApMV	Apple	Granny Smith	Turkey (Nevşehir)(This research)

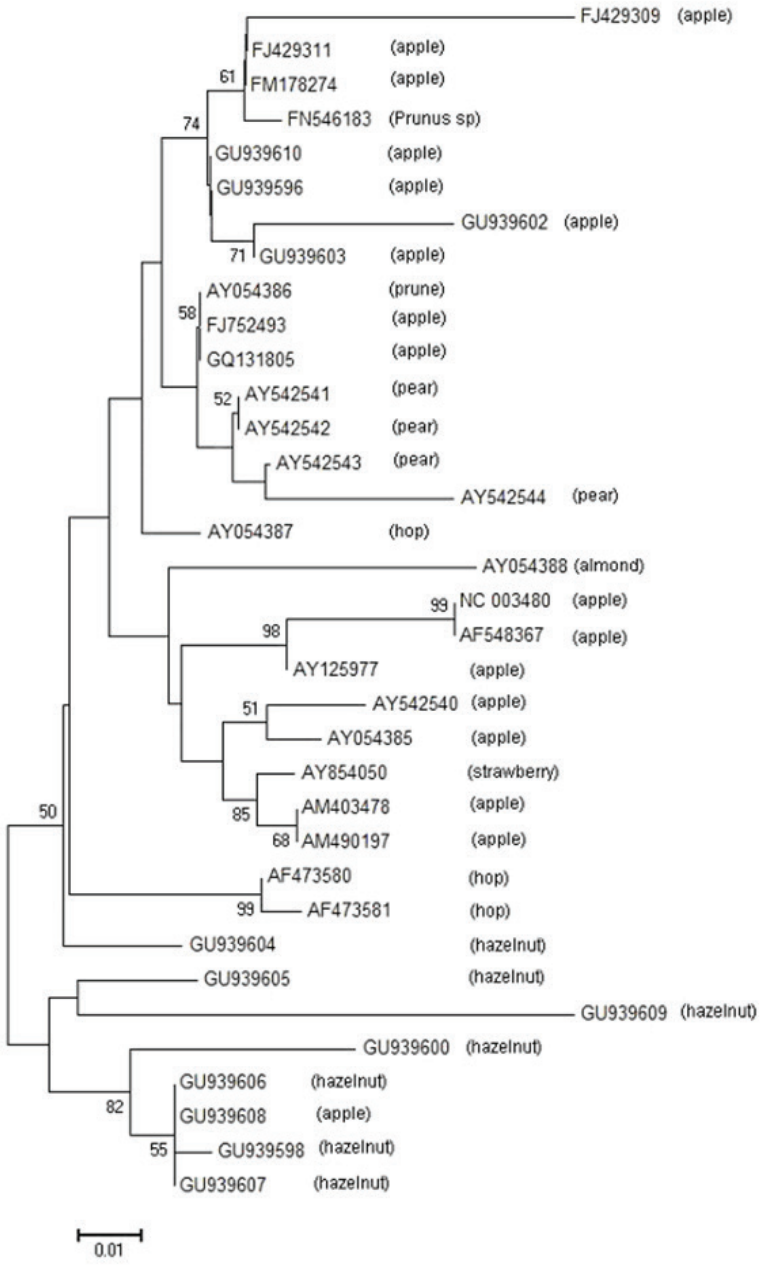

Figure 1. Phylogenetic analysis of nucleotide sequences of coat protein of ApMV isolates. Phylogenetic analysis was obtained using the computer programme of Mega 5 (Tamura et al. 2011). The numbers near nodes were determined by bootstrap analysis (100 replicates)

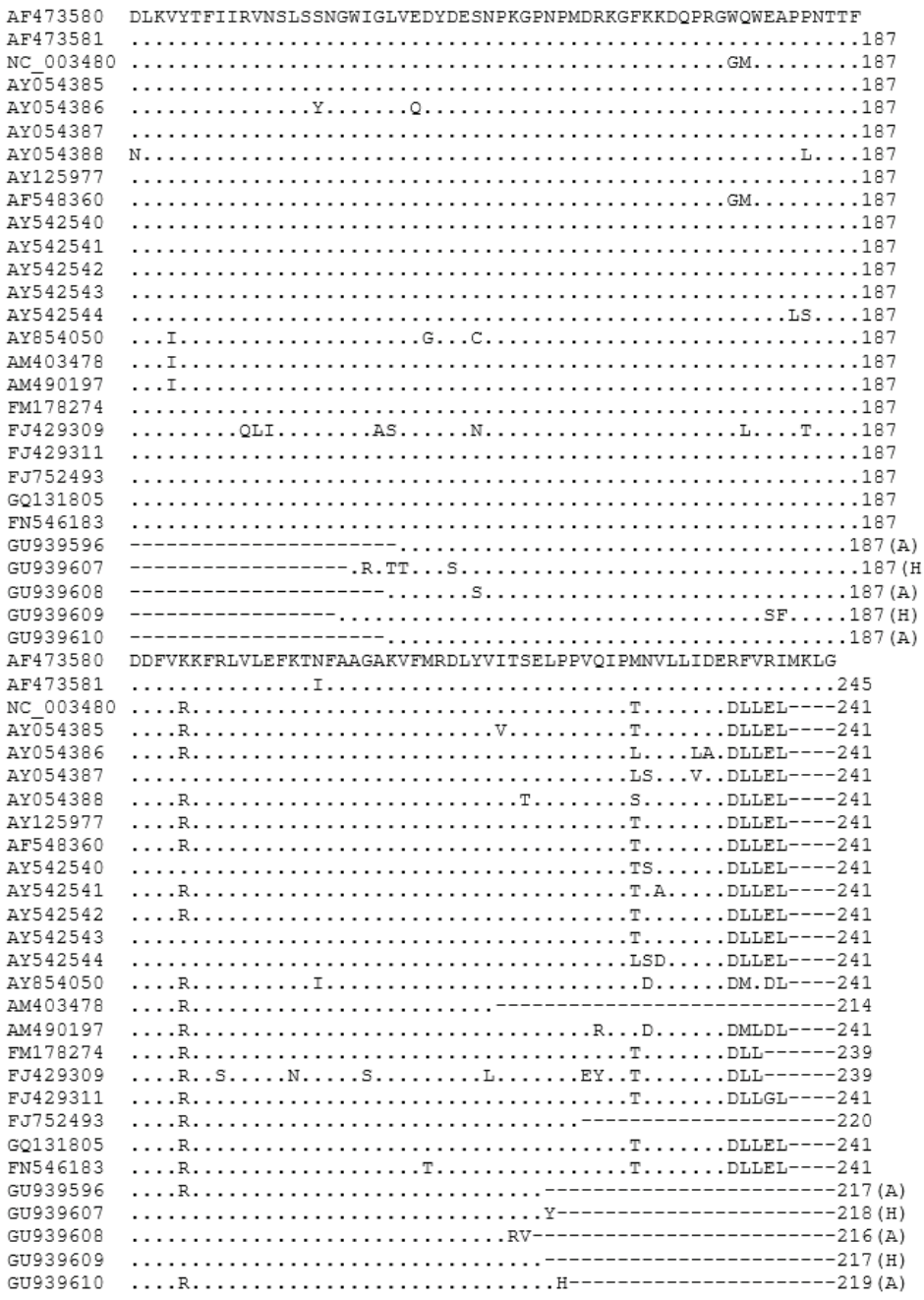

Figure 2. Multiple amino acid sequence alignment of the coat protein of ApMV Turkish isolates deposited in the GeneBank and also those sequences available from GeneBank. Dots indicated identical amino acides and dashes indicates the ends, (A) is apple origin and (H) hazelnut origin. Reference isolate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

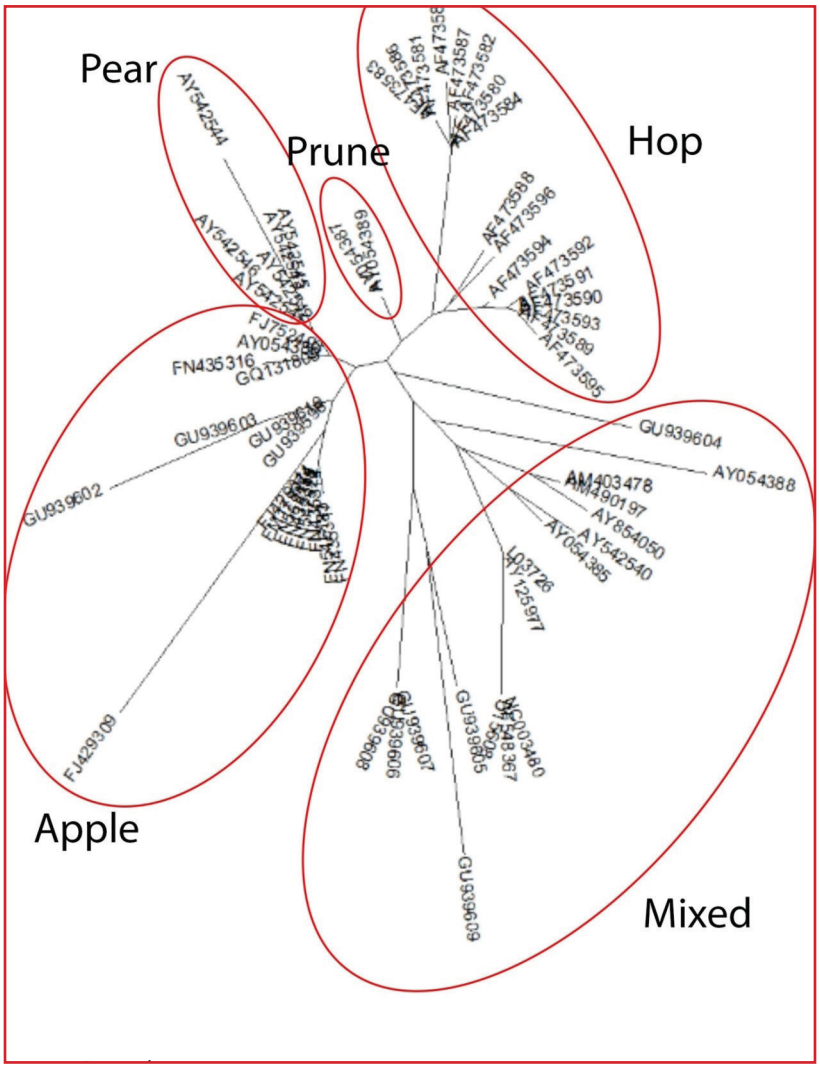

Figure 3. Phylogenetic analysis of nucleotide sequences of coat protein gene of ApMV isolates