

Molecular characterisation of Alfalfa mosaic virus isolates in potato from the Tokat province, Türkiye

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ABSTRACT

The *Alfalfa mosaic virus* (AMV) was detected in potato fields in the Tokat province. The coat protein (CP) sequences of AMV isolates from the Tokat province were determined and compared with sequences of reference AMV isolates from GenBank. Total nucleic acid (TNA) was extracted from plants with positive results according to serological test results. Then, reverse-transcription polymerase chain reaction (RT-PCR) was performed using primer pair specific to partial the coat protein region, and positive PCR products were sent for sequence analysis in both directions. Two Turkish AMV isolates (AMV-PN3-5 and AMV-PN3-6) had a 96-99% nt homology amongst themselves, according to nucleotides (nt) sequence analysis. Based on the phylogenetic tree obtained from 24 AMV isolates from GenBank for both sequences, the two Turkish AMV isolates were clustered in subgroup I containing Iranian, Canadian, Turkish, Korean, and Serbian isolates, at the nucleotide level. Sequence comparison showed that these two isolates of AMV shared 96% to 99.7% sequence similarity with the twenty-six reported isolates of AMV obtained from GenBank. This is the first report on the genetic variability of AMV isolates infecting potato crops in the Tokat province.

1. Introduction

Potato (*Solanum tuberosum* L.), is one of the most important horticulture crops in the world. Turkey is one of the important potato producers in the Mediterranean region and potatoes can be grown almost anywhere in the country (Yardımcı et al. 2015). Turkey produced 5100674 tons of potatoes in 1389415 decares (da) in 2021 (TUIK 2020). The Tokat province produced 50514 tons of potatoes in a cultivation area of 20291 da.

Multiple virus infections lead to a decrease in yield and tuber quality in potato plants (Kolychikhina et al 2021). Potato is infected by more than 40 viruses such as *Potato virus Y* (Potyvirus, PVY), *Potato virus X* (Potexvirus, PVX), *Potato virus S* (Carlavirus, PVS), *Potato virus A* (Potyvirus, PVA), *Potato virus M* (Carlavirus, PVM), AMV (Alfamovirus), and *Potato leaf roll virus* (Polerovirus, PLRV) and 2 viroids such as *Potato spindle tuber viroid* (PSTVd). These viruses cause significant yield losses in potato crops (Hameed et al 2014, Kolychikhina et al 2021).

The *Alfalfa mosaic virus* (AMV) is a type species of the *Alfamovirus* genus in the *Bromoviridae* family within plant viruses. It has a worldwide distribution and infects more than 600 plant species belonging to the *Solanaceae*, *Fabaceae*, *Umbelliferae*, and *Compositae* families and several vegetables such as potato, tomato, alfalfa, pepper, eggplant, tobacco, clover, legumes, and woody crops (Brunt et al. 1990; Bol 2008). Potato plants infected with AMV show symptoms in the form of bright yellow areas on the leaves called calico and tuber necrosis in tubers (Nie et al. 2015). It has been reported that AMV causes tuber necrosis in potato tubers in Canada (Nie et al. 2015). In recent years, studies conducted by Nie et al (2020) have also

shown that the necrosis seen in AMV-infected tubers is dependent on the potato variety rather than the AMV strain/haplotype. The virus is easily transmitted by a minimum of 14 aphid species non-persistently, primarily *Myzus persicae* (Ragsdale et al. 2001). AMV is also transmitted by seeds, pollen, and *Cuscuta* spp. (Bailliss and Offei 1990, Hemmati and McLean 1977).

The genome of AMV is composed of three single strands of positive polarity (+ssRNA) particles. RNA1 (P1 protein) and RNA2 (P2 protein) segments are responsible for viral replicase proteins. RNA 3 encodes the coat protein (CP) gene and the viral movement protein (MP), both of which are required for infection (Tenllado and Bol 2000; Bol 2003, 2008). Furthermore, the P1 and P2 sequences encoded by the AMV genome have low genetic diversity, whereas the CP and MP gene regions have high genetic diversity (Bergua et al. 2014).

In Turkey, AMV infection has been reported in different crops using different detection approaches including mechanical inoculation (biological indexing) and Double Sandwich Enzyme-linked Immunosorbent Assay (DAS-ELISA), and RT-PCR methods (Sertkaya et al. (2017), Özdemir et al. 2011). Viral pathogen were recorded in eggplant in the Manisa province (Özdemir et al. 2011), in bean in the Burdur province, West Mediterranean (Çulal Kılıç and Yardımcı 2015), in pepper in Tokat (unpublished), in alfalfa in the Van and Bingöl provinces (Usta and Güller 2020, Güller et al. 2022), and in other provinces (Arlı-Sökmen et al. 2005; Demir 2005; Buzkan et al. 2006; Özdemir and Erilmez 2007; Çetinkıran and Baloğlu 2011).

PVY, AMV, PVS, and PLRV viral agents that cause infections in potato plants have been reported from the Tokat province (Topkaya 2020). Symptoms that may be caused by the AMV agent have been observed in potato plants during field surveys conducted in potato-growing areas. The main aim of this study was to molecularly determine the AMV isolates in potato plants from the Tokat province and compare sequence identities between the Turkish AMV potato isolates and reference AMV isolates reported in GenBank.

2. Material and Method

2.1. Virus source

In 2019, AMV was determined serologically and molecularly in potato growing areas in Tokat by Topkaya (2020). Two samples were chosen for further molecular characterisation.

2.2. Total Nucleic acid (TNA) extraction and complementary DNA (cDNA) synthesis

The total nucleic acid (TNA) extraction of isolates was done using leaves of potato plants according to Astruc et al. (1996) with minor modifications. The isolated TNAs were stored at -20°C until the cDNA synthesis. Total RNAs were used as a template for RT-PCR by using the random hexamer primer (5'-NNNNNN-3'). cDNAs were synthesized in a total reaction volume of 10 µl to be used as a template in amplification studies. For cDNA synthesis, the 2.5 µl of extracted TNA was used as a template and added to a PCR tube, and incubated for 5 min at 65°C. Then the reaction mix containing 1 µl 10X reaction buffer (WizScript™, Republic of Korea), 0.5 µl 20X dNTP (2.5 mM), 1 µl random hexamer primer, 0.5 µl reverse transcriptase enzyme ((WizScript™, Republic of Korea), 0.25 µl RNase inhibitor, 1.75 of distilled water added to the tube. RT incubation was performed with min incubation at 25°C, followed by at 37°C for two hours and 85°C for 5 min.

2.3. Polymerase Chain Reactions (PCR)

RT-PCR was performed using the specific primers AMV-CP F (5'-GTGGTGGGAAAGCTGGTAAA-3') and AMV-CP R (5'-CACCCAGTGGAGGTCAGCATT3') (Martínez-Priego et al. 2004) for partial coat protein gene sequence of AMV. Amplification was performed in a final volume of 25 µl containing 2.5 µl of 10× Taq Buffer, 2 µl of MgCl₂, 2 µl of cDNA, 0.5 µl of each dNTP (10 mM) mix and, 10 pmol forward and reverse primers with 0.25 µl of Taq DNA polymerase (Fermentas, USA), 18.25 µl of distilled water. After then, PCR products (about 700bp) were electrophoresed in 1.5% agarose gel including ethidium bromide.

2.4. Phylogenetic analysis

The sequence data of the CP gene was subjected to a Blast Nucleotide search (Basic Local Alignment Search Tool) for comparison with references AMV isolates from GenBank. The phylogenetic tree was generated with two Tokat AMV isolates and references AMV isolates derived from the GenBank. The evolutionary relationship was calculated with the maximum likelihood (ML) method of the MEGA7 (Kumar et al. 2016) and Sequence Demarcation Tool Version 1.2 (SDTv1.2) (Muhire et al. 2014) software. The bootstrap values were performed with 1000 replications.

3. Results and Discussion

AMV infects more than 600 plant species worldwide and is transmitted mechanically, by seed/weed seeds, and by aphids in a non-persistent manner (Bol 2003). AMV infection on potato plants has been reported in different countries such as Egypt, Canada (Xu and Nie 2006), Korea (Jung et al. 2000), Iran, and Turkey (Çarpar and Sertkaya 2016, Topkaya 2020) Saudi Arabia (Al-Saleh et al. 2014). In previous studies in Turkey, AMV was reported on various hosts and at different infection rates. AMV was detected in the Hatay province by Sertkaya et al. (2017) at 5.4% and 4.6% rates during the potato production in 2014 and 2015 years, respectively, and 15.3% rates of AMV in *Physalis angulata*. AMV was observed in potato plants in the Tokat province. In a previous study conducted by Topkaya (2020), it was detected at 1.38% rate in the tested samples. Although this infection rate seemed to be lower, it has the possibility to increase because it easily spreads such as by non-persistent transmission with aphids and mechanically. The variation in the rate of AMV from year to year has also been reported among different authors (Wang et al. 2012; Milošević 2013; Rusevski et al. 2011, 2013; Stanković et al. 2014).

As a result of RT-PCR tests with AMV-specific primers, expected bands of around 700 bp were obtained from two samples. PCR products were sequenced by the Sanger method (Atlas Biotechnology- Ankara). Obtained sequence data were analysed with MEGAX software and compared with reference isolates (Table 1) and the obtained phylogenetic tree. Based on the phylogenetic analysis, all AMV strains were clustered in two main groups (Subgroup I and Subgroup II). Subgroup I contained Iranian, Canadian, Turkish, Korean, and Serbian isolates, whereas subgroup II contained strains from France and England. In the phylogenetic tree, two Turkish AMV isolates (PN 3-5 and PN 3-6) were clustered with subgroup I including Chinese, Serbian AMV isolate, and two Turkish İğdir isolates (Figure 1). In previous studies, AMV isolates were separated into two groups of I and II by Parrella et al. (2010) and then further divided the second group into IIA and IIB subgroups by Parrella et al. (2011) Later on, the AMV isolates were grouped into four or more different groups based on sequence information of coat protein region by Stanković et al. (2014). Based on CP sequence comparisons, AMV potato isolates under this study were 92 to 99% identical at the nucleotide level (Figure 2) and 95 to 100% identical at the amino acid level.

Parrella et al. (2000) grouped the AMV isolates based on amino acid sequences as subgroups I and II. In this study, the same changes were obtained (Table 2). The amino acid sequence changes were observed at positions for which variability has already been reported (Parrella et al. 2000). Differences in the CP amino acid sequences are shown in Table 2.

Parrella et al. (2000) divided AMV isolates into two groups and suggested that this distinction may either be due to geographical differences or to variations in the amino acid sequence of their CPs, which may be related to the structural features of the virus particles. Later on, AMV isolates have been reported in different groups regardless of regional distribution by Xu and Nie (2006) and Stanković et al. (2014). Abdel Aleem et al. (2018) reported that the Egyptian AMV isolates formed a new group. Recently in a study, Nie et al. (2020) reported that RNA1 and RNA3 segments of AMV have been grouped into three major clades and RNA2 segments have been grouped into two groups. The isolates were divided into 3 groups in the phylogenetic tree formed based on the full genome sequence of the RNA3

segments containing the CP and MP protein regions. In this study, based on the CP region sequences, the AMV Tokat isolates were grouped into three major groups as reported by Nie et al. (2020). In major group I, the two Turkish AMV isolates were clustered with Serbian and Turkish isolates which was previously reported. Group II has also a common feature in all these groupings. In this study, Turkish AMV isolates showed the same similarity with subgroup I isolate. Group III includes only Egyptian isolate.

4. Conclusion

AMV was determined in potato-growing areas in the Tokat province. In this study, molecular characterisation of AMV isolates was performed. The Tokat AMV CP isolates (PN3-5 and

PN3-6) showed high nt (92-99%) and aa homology (95-100%) with other world AMV isolates. Based on the phylogenetic tree, two isolates from Tokat were clustered in group I, together with isolates from France, Serbia, Saudi Arabia, and England. CP regions of AMV pepper isolate from Tokat were previously studied and were not included in this study because they were 333 bases long. This is the first potato isolate of AMV to be identified at the molecular level in the Tokat province. This information will contribute to further analysis of AMV on potatoes and other host plants in Turkey.

Table 1. Information about reference isolates used in the study

Accession number	Host	Isolate name	Country
KX710198	<i>Capsicum annuum</i>	R236	Bosnia and Herzegovina
MT210178	<i>Medicago sativa</i>	Alakoy Y1	Turkey
MT210179	<i>Medicago sativa</i>	Alakoy Y9	Turkey
MW962976	<i>Medicago sativa</i>	Bingol A8	Turkey
MW882261	<i>Medicago sativa</i>	Igdir 1	Turkey
KF147805	Tomato	258-11	Serbia
MG600289	<i>Trifolium pratense</i> L.	AMV-PV1	Czech Republic
MW882262	<i>Medicago sativa</i>	Igdir 9	Turkey
MT669393	<i>Glycine max</i>	IA-4-2018	USA
MG922819	<i>Solanum lycopersicum</i> (tomato)	219-14"	Serbia
JQ685860	<i>S. tuberosum</i>	Ke.Ba.Po	Iran
HQ288892	<i>S. tuberosum</i>	-	Egypt
AF294432	<i>S. tuberosum</i>	KR1	Korea
AF294433	<i>S. tuberosum</i>	KR2	Korea
DQ314755	<i>S.tuberosum</i>	Ca518	Canada
DQ314753	<i>S. tuberosum</i>	Ca401	Canada
AF015717	<i>Garden lupin</i>	VRU	England
AF015716	<i>Garden lupin</i>	15/64	England
AJ130708	Carrot	Dac-16	France
L00162	Clover	425 L	USA
KC182568	<i>Capsicum annuum</i>	P-27-09	Serbia
MZ221779	<i>Medicago sativa</i>	Yuanyang_2/H3	China
MZ221776	<i>Medicago sativa</i>	China_Yangling/S	China
MN846751	<i>Acyrtosiphon pisum</i>	BJAp1	China
KX535507	Potato	Es.Fa.Po	Iran

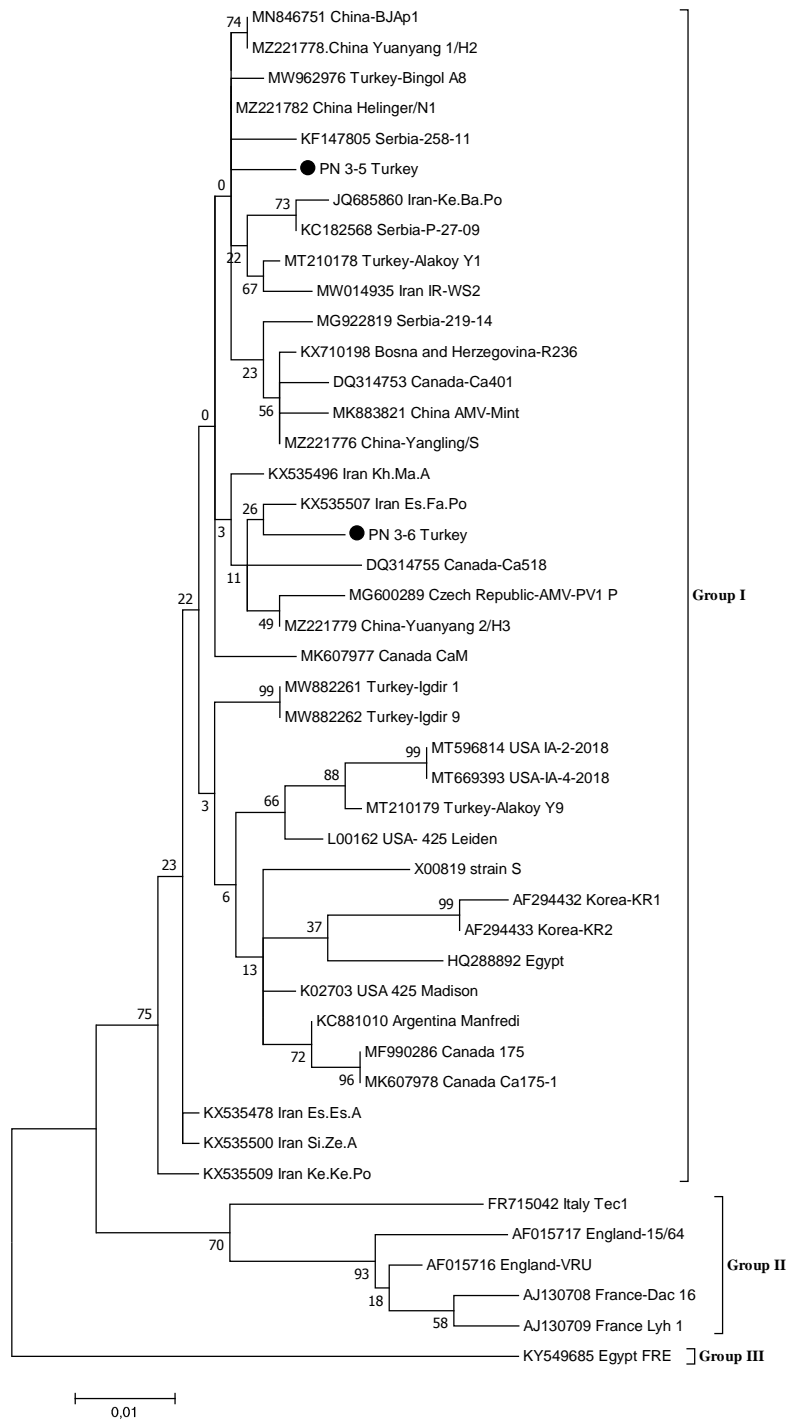


Figure 1. Phylogenetic tree constructed based on the partial nucleotide sequences of the CP gene of two new AMV isolates and references AMV isolates using the maximum likelihood (ML) method of MEGA7 (Kumar et al. 2016). Turkish isolates are indicated using a black-filled circle.

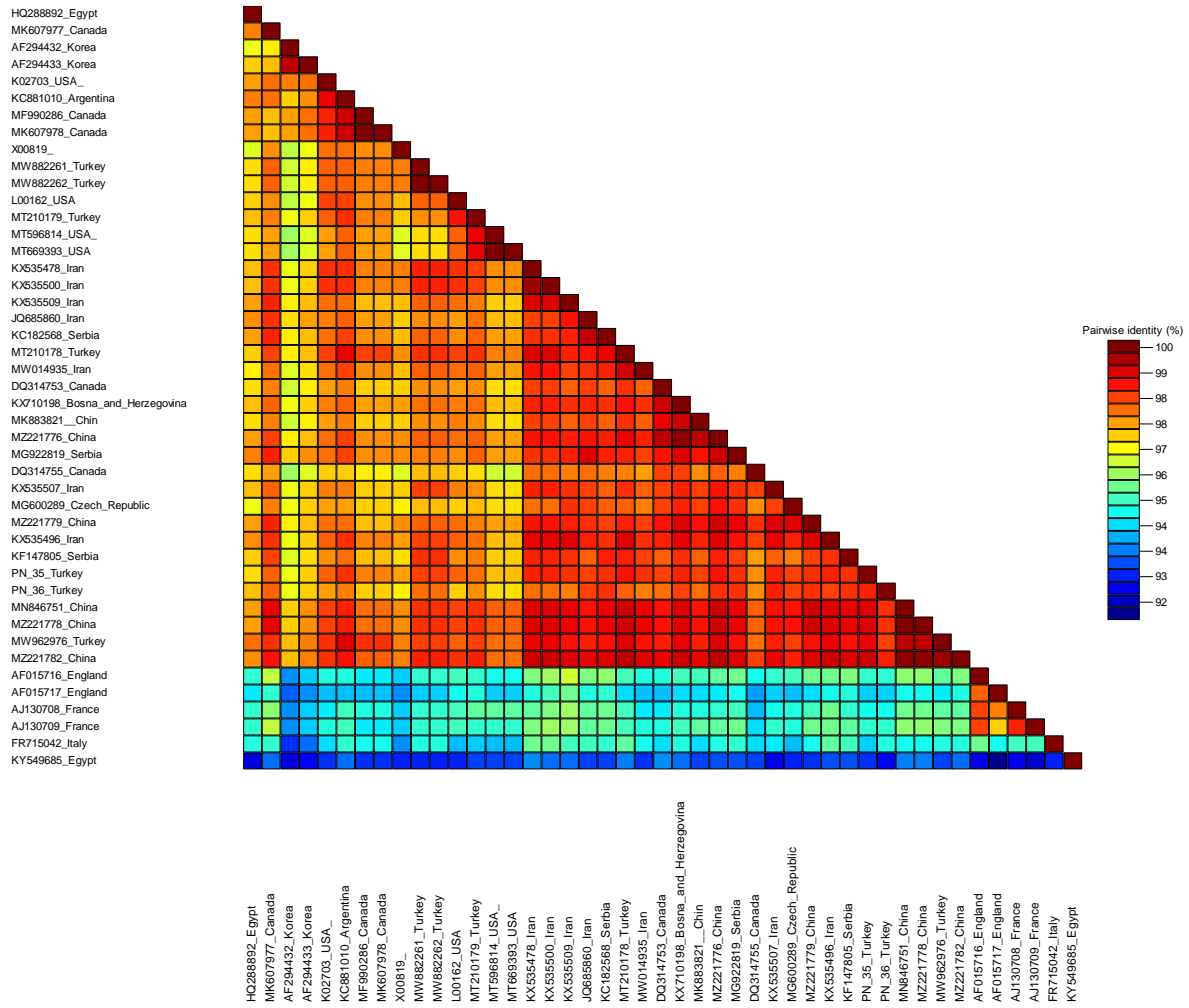


Figure 2. Similarity rates of Turkey AMV isolates with reference isolates.

Table 2. Differences in the CP amino acid sequences

Subgroup I	67	84	94	176	214
KX710198	F	G	Y	Q	E
MT210178	F	G	Y	Q	E
MT210179	F	G	Y	Q	E
MW962976	F	G	Y	Q	E
MW882261	F	G	Y	Q	E
KF147805	F	G	Y	Q	E
MG600289	F	G	Y	Q	E
MW882262	F	G	Y	Q	E
MT669393	F	G	Y	Q	E
MG922819	F	G	Y	Q	E
JQ685860	F	G	Y	Q	E
AF294432	F	G	Y	Q	E
AF294433	F	G	Y	Q	E
DQ314755	F	G	Y	H	E
DQ314753	F	G	Y	H	E
L00162	F	G	Y	Q	E
KC182568	F	G	Y	Q	E
MZ221779	F	G	Y	Q	E
MZ221776	F	G	Y	Q	E
MN846751	F	G	Y	Q	E
HQ288892	F	G	Y	Q	E

Table 2 (continued). Differences in the CP amino acid sequences

Subgroup I	67	84	94	176	214
PN 3-5	F	G	Y	Q	E
PN 3-6	F	G	Y	Q	E
Subgroup II					
AF015716	S	A	F	L	D
AF015717	S	A	F	L	D
AJ130708	S	A	F	L	D

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