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ORIGINAL ARTICLE

Prevalence and molecular characterization of important potato viruses in the Tokat province of Turkey

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Abstract

Background It is believed that viruses affect potato yield more than any other pathogens worldwide.

Method and Results We report here on a survey of the four most common potato viruses in the Tokat Province of northern Turkey. Leaf samples were collected from potato plants showing signs of viral diseases in five districts of the province. Over 400 leaf samples were tested using RT-PCR with virus-specific primers. Among the one or more viruses detected in 218 (52%) leaf samples, *Potato virus Y* (PVY) was the most common (47.1%), followed by *potato virus S* (PVS; 16.7%), *potato virus X* (PVX; 6.0%) and *potato leaf roll virus* (PLRV; 5.3%). The most common mixed infections were PVY + PVS (6.9%). A phylogenetic analysis of the gene sequences showed all Turkish PVS isolates to be clustered with the PVS^{O} group, two PVY isolates with the $PVY^{N–WI}$ group and one isolate with the PVY^{NTN} group. Turkish PVX isolates are in the Type X group of the two major PVX isolate groups. The Turkish PLRV isolates were separated into two major groups depending on the results of the phylogenetic analysis, with six cases in Group 1 and one in Group 2.

Conclusions PVY, PVX, PVS and PLRV were detected in potato production areas in Tokat. A phylogenetic comparison of the gene sequences revealed all Turkish isolates to be immigrant members of the world populations of these viruses. Our results emphasize the importance of the strict quarantine control of plant materials entering Turkey.

Keywords Phylogenetic analysis · *Potato leaf roll virus* · *Potato virus S* · *Potato virus X* · *Potato virus Y* · Quarantine

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important human food crops [1–3]. It is grown worldwide, and notably more than half of all potato production is carried out in developing countries [3]. China produced the most potato in 2020, while Turkey ranked 16th in the world with 5,200,000 tons [4], but declined to 5,100,000 tons in Turkey in 2021 [5]. While various fungi, bacteria and other pests and parasites affect potato production, viruses are considered the main factor limiting potato yield [2]. Potato crops were reported to have been infected by 52 viruses and one viroid worldwide in 2020 [3], although only 12 are found worldwide. Most seem to have a limited distribution, with 20 in South America and only 18 in other regions (North America, Asia, Europe, Africa and Oceania). *Potato virus Y* (PVY), *potato leaf roll virus* (PLRV), *potato virus X* (PVX) and *potato virus S* (PVS) are the most frequently reported viruses, causing significant losses in yield [3, 6–9]. All four viruses have single-stranded positive-sense RNA genomes, among which the first three have filamentous virions while those of PLRV are isometric. The viruses also differ in various other characteristics and in their taxonomic placement. PVY, PVX and PVS have filamentous helically constructed virions that are around 11–13 nm in diameter, but of different lengths. All three are transmitted experimentally by sap inoculation, and two – PVY and PVS – are transmitted "non-persistently" by probing aphids, whereas PLRV is transmitted "persistently" by aphids, but not by sap inoculation.

PVY is one of the main viral causes of crop losses in potatoes, and is found everywhere in the world. It is a member of the potyvirus group and has a genome of approximately 9.7 kb and virions 730 nm long [10]. Its genome encodes a single polyprotein, which is cleaved into 10

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mature proteins by three virus-specified proteases [10, 11]. PVY is transmitted non-persistently by over 50 aphid species [12–15]. The symptoms associated with PVY vary, and are genetically controlled as they depend on the strain of the virus and the infected potato variety [16–18]. PVX is common in potato crops worldwide [19], with 515 nm-long virions. It can be transmitted mechanically, by contact between plants, but not by aphids, and has been spread worldwide in infected tubers [20]. PVX, like PVY, infects many solanaceous plants, notably potato, tomato and tobacco [21], and sometimes without obvious symptoms [21, 22]. PVS is a carlavirus with 700 nm filamentous virions that are usually less flexuous than those of PVX and PVY [21]. Like PVY, but not PVX, it is transmitted in nature by probing aphids, especially *M. persicae*, but can be also transmitted by sap inoculation [13]. PVS infection causes a characteristic collapse of the upper surfaces of the leaves and stunting that results in 10–20% yield losses [18, 23–25]. Some PVS strains also cause an irregular yellowing of old leaves and a bronze staining of leaves [16–18].

PLRV is a luteovirus with isometric virions of 24 nm, and is probably one of the first viral diseases of potato to have been recognized. It was discovered as a component of 'potato degeneration' disease, attributed to continuous vegetative propagation [16–18]. Symptoms such as those caused by PLRV were first described in the United Kingdom early in the 20th century, although subsequent studies showed mixed infections to be responsible. The mixed infections were separated by tuber core grafting [26] and by sap and aphid transmission [27], and PVY, PVX and PLRV emerged. PLRV, unlike PVY, PVX and PVS, is not transmitted by sap, but transmitted persistently by aphids and by grafting. Its virions are found in the phloem of plants, and it has a narrow host range. The virus has been found to be carried persistently by more than 10 aphid species, especially the green peach aphid (*M. persicae*) [7, 28, 29].

There are few previous studies of potato viruses in the Tokat province. Kutluk Yilmaz et al. [30] investigated viruses using the Enzyme-Linked Immuno Sorbent Assay (ELISA) method, and reported PVY and other viruses in both leaf and tuber samples. The approaches to the identification of viruses using the polymerase chain reaction (PCR) method are more sensitive and specific than earlier methods based on virion protein serology. PCR testing has not been used to date to identify potato viruses in North Central Turkey. In the present study we use a more specific PCR-based method to determine the prevalence of PVY, PVX, PVS and PLRV in the Tokat Province, and also compared the partial gene sequences that were obtained to determine their relationships with isolates in other parts of the world.

Materials and methods

Sample collection

A total of 418 plant samples showing symptoms of viruses were collected from potato-producing areas in the Niksar, Central, Erbaa, Başçiftlik and Artova districts of the Tokat Province in 2018. The collected samples were stored at -20 °C until the time of testing.

RNA isolation

The potato leaf samples were tested for the presence of PVY, PVS, PVX, and PLRV using the RT-PCR method with virus-specific primers. Total RNA was extracted from the potato leaves following the protocol described by Astruc et al. [31]. A two-stage RT-PCR testing protocol was adopted, in which a total of 500 ng RNA was first reverse transcribed to complementary DNA (cDNA) using random primers and Superscript III reverse transcriptase (Thermo Fisher Scientific, Vilnius, Lithuania). For the cDNA synthesis, 2 µl of total RNA, 1µl random hexamer primer (5'-d(NNNNNN)-3'N = G, A, T, C), (10 µM) and 8 µl distilled water were mixed in a tube and incubated for five minutes at 5ºC and then left on ice for three minutes. Then, 5X MMLV buffer (5X), 0.2 mM dNTP (25 mM), 0.5 µl random hexamer primer (10 µM), 0.25 µl RNAse inhibitor (10u/µl), 0.25 µl Reverse transcriptase (20-20u/µl) and distilled water (to 20 µl) were added to tubes, which were incubated at 25 °C for 5 min and 42 °C for 60 min, and then at 85 °C for 5 min.

PCR studies

The cDNA was used as a template for the PCR assays with specific primer pairs to four viruses (Table 1). The RNA was transcribed by PCR in a 25 µl mixture containing 2.5 µl cDNA, 0.2 µl 25mM dNTPs, 2 µl 25 mM $MgCl_2$, 5 µL 5X green reaction buffer, 0.5 µl of each specific primer (50 pmol) (Table 1), 1.25-units *Taq* DNA polymerase (Promega, Madison, USA) and sterile ultra-pure water. The PCR products were electrophoresed in a 1% agarose gel and stained with ethidium bromide.

Phylogenetic analyses

The phylogenetic analyses were carried out using the nucleotide sequences of the CP region of the PVS, PLRV and PVX isolates and the P1 region of the PVY isolates obtained in the study, together with sequences from the GenBank database. The phylogenies were calculated using the neighbor-joining method in MEGAX software [32]. Bootstrap values were calculated using 1,000 replicates.

Table 1 Primer sequences used in the study

Virus	Sequence	Region	Polarity	Product (bp)	Annealing temperature
PVS	5'-TGGGGAATCAGTCCGGCTAGTC-3' 5'-ACTGGACCTGCGCTTAGGCT-3'	Coat protein	Sense Antisense	1,100 bp	62 ºC
PLRV	5'- CGCGCTAACAGAGTTCAGCC-3' 5'-GCAATGGGGGTCCAACTCAT-3'	Coat protein	Sense Antisense	336 bp	62 ºC
PVX	5'-TAGCACAACACAGGCCACAG-3' 5'-GGCAGCATTCATTTCAGCTTC-3'	Coat protein	Sense Antisense	562 bp	62 ºC
PVY	5'-AAGCTTCCATACTCACCCGC-3' 5'-CATTTGTGCCCAATTGCC-3'	P1 protein	Sense Antisense	856 bp	58 ºC

Results

Survey

In the study, a total of 418 leaf samples were tested for the presence of viruses (Table 2). The potato plants exhibited a wide range of symptoms, such as yellowing, stunting, leaf deformation, necrotic spots on the leaves (Fig. 1) and the characteristic "calico" symptoms of alfalfa mosaic virus.

RNAs were extracted and tested by RT-PCR, as described, to determine the presence of potato viruses (PVY, PLRV, PVX, and PVS). The tests revealed a total of 218 leaf samples to be infected with one or more viruses. The rates of infection were 47.1, 16.7, 6.0 and 5.3% for PVY, PVS, PVX and PLRV, respectively (Table 2). The virus incidence rates of each district are presented in Table 2. PVY and PVS were the most common potato viruses in the Tokat province.

The numbers of double and triple infections in potatoes from different centers are presented in Table 3. Only three samples were found to be infected with all four viruses.

A summary of the single and mixed infections in potatoes from different centers is presented in Table 4. While 148 of the tested leaf samples had a single infection, 70 samples had mixed infections at different rates. The majority of mixed infections were detected in the Başçiftlik and Artova districts. The highest rate of PVY infection was found in the Central, Niksar and Erbaa districts, while no PVX or PLRV infections were identified. PVX infections were mostly detected in the Başçiftlik and Artova districts.

Phylogenetic analyses

To determine the evolutionary relationship of viruses, three samples of PVY, three of PVX, eight of PVS and seven of PLRV were selected. The RT-PCR products of the selected isolates were sequenced bidirectionally using the specific primers.

The sequence data of the three Turkish PVY isolates were obtained and submitted to GenBank, where they were given Accession Codes: MW699160 (PM1-6), MW699161 (PA1-1), MW699162 (PE2-6). The two PVY sequences had 99% nucleotide similarity with the homologous regions of isolates from Poland, Germany and the United States, and the third isolate showed a 99% similarity with isolates from India, the United Kingdom and China. The phylogenetic tree, based on the partial sequences of the P1 gene of the Turkish PVY isolates and 12 reference isolates obtained from GenBank, formed several clusters (Fig. 2). Two PVY isolates clustered with isolates in pathogroup $PVY^{N–WI}$ from the United States, Germany and Poland, and one isolate was clustered with $PVYN^{NTN}$ isolates from China, India, the United Kingdom and the United States.

The sequences of the CP genes of the eight Turkish PVS isolates were compared with reference PVS isolates obtained from GenBank. These isolates were submitted to GenBank and were given Accession Codes: MW699152 (PA3-2), MW699153 (PB5-4), MW699154 (PB6-2), MW699155 (PB7-1), MW699156 (PN3-6), MW699157 (PA3-3), MW699158 (PN5-2) and MW699159 (PN14-3). The PVS sequences formed two major clusters, one of which included PVS ordinary strains (PVS^{O}), and the other, PVS Andean strains (PVS^{A}). All Turkish PVS sequences were grouped in the PVS^{O} cluster (Fig. 3), and four isolates (PA3-3, PN3-6, PN5-2, PN14-3) obtained in the study were clustered with the Yunnan isolate from China and the Sam-24 isolate from the United Kingdom, while the other four isolates (PB71-1, PB5-4, PA3-2, PB6-2) were grouped with the Irena, Valery and Alex isolates from Ukraine and the Sam-13-O7 isolate from the United Kingdom. In genetically defined PVS^{O}, the nucleotide identity ranged from 92.6 to 100%. Cox and Jones [33] reported that all PVS^{O} isolates could be classified into seven subclusters, and PVS^{A} isolates could be classified into three groups. In our study, the PVS isolates were separated into three subclusters (Fig. 3).

The Accession Codes of the three Turkish PVX isolates were MW699163 (PA1-5), MW699164 (PA3-3) and MW699165 (PB7-6). The PVX isolates were clustered into two groups, as reported earlier by Santa Cruz and Baulcombe [34]. Group 1 comprised isolates from the United Kingdom, Asia and Europe, whereas Group 2 comprised isolates from the United States. The Turkish PVX isolates were in Group I and were type X (Fig. 4). Two of the PVX isolates showed a 98% nucleotide similarity with isolates from the United Kingdom and China, and one isolate (PA1-5) showed a



Fig. 1 Symptoms of the potato leaves that were found to be infected with: a: PLRV; b, d: PVY; c: AMV

Table 2 The occurrence rates of detected viruses

District	Number of samples	Number of infected samples and % infection	Number of samples infected with each virus and % samples infected			
			PVY	**PVS**	**PVX**	**PLRV**
Center	116	63 (53.3)	63 (54.3)	1 (0.9)	-	-
Niksar	92	46 (50)	38 (41.3)	8 (8.7)	-	1
Erbaa	101	14 (13.9)	14 (13.9)	-	-	-
Başçiftlik	65	60 (92)	52 (80)	47 (72.3)	3 (4.6)	18 (27.7)
Artova	44	35 (79.5)	30 (68.2)	14 (31.8)	22 (50)	3 (6.8)
Total	**418**	**218 (52.6)**	**197 (47.1)**	**70 (16.7)**	**25 (6.0)**	**22 (5.3)**

Table 3 Number of mixed virus infections

District	Number of collected sample	Number of infected sample	Number of infected samples and % infection							
			PVY + PVS	**PVY + PVX**	**PVY + PLRV**	**PVS + PVX**	**PVS + PLRV**	**PVY + PVS + PVX**	**PVY + PVS + PLRV**	**PVY + PVS + PVX + PLRV**
Center	116	63	1(0.8)	-	-	-	-	-	-	-
Niksar	92	46	1(1.1)	-	-	-	-	-	-	-
Erbaa	101	14	-	-	-	-	-	-	-	-
Başçiftlik	65	60	26(38.4)	-	1(1.5)	-	4 (7.6)	1(1.5)	10(15.3)	2(3.07)
Artova	44	35	2(4.5)	9(20.4)		2(4.5)	-	9(20.4)	-	1(2.2)
Total	**418**	**220**	**29(6.9)**	**9(2.2)**	**1(0.2)**	**2(0.5)**	**5(1.2)**	**10(2.4)**	**10(2.4)**	**3(0.7)**

Table 4 Single and mixed infections rates

District	Number of collected samples	Number of infected samples	Number of infected samples and % infection													
			Single infection				**Double infections**						**Triple infections**			**Quadruple infections**
			PVY	**PVS**	**PVX**	**PLRV**										
Center	116	63	62(53.4)	-	-	-	1(0.8)	-	-	-	-	-	-	-	-	-
Niksar	92	46	37(40.2)	7 (7.6)	-	1(1.08)	1(1.0)	-	-	-	-	-	-	-	-	-
Erbaa	101	14	14(13.9)	-	-	-	-	-	-	-	-	-	-	-	-	-
	65	60	12(18.5)	3(4.6)	-	-	26(40)	-	1(1.5)	-	4(6.1)	-	1(1.5)	10(15.3)	-	2(3.07)
Artova	44	35	9(20.45)	-	1(2.3)	2(4.5)	2(4.5)	9(20.4)	-	2(4.5)	-	-	9(20.4)	-	-	1(2.2)
Total	**418**	**218**	**134 (32.05)**	**10(2.4)**	**1(0.23)**	**3(0.17)**	**30(6.9)**	**9(2.2)**	**1(0.2)**	**2(0.5)**	**5(1.2)**	0	**10(2.4)**	**10(2.4)**	**0**	**3(0.7)**

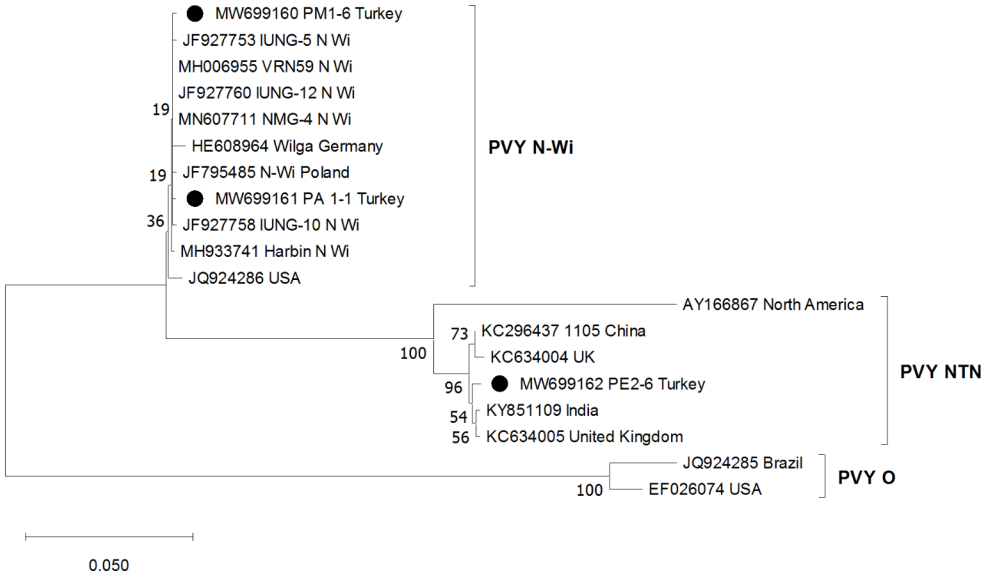


Fig. 2 Phylogeny of selected PVY isolates based on their P1 gene sequences. The phylogeny was constructed using the Neighbor-Joining method in MEGA software. Bootstrap supports above 70% (n = 1000 bootstraps) are indicated for each node. The black discs indicate the isolates obtained in this study

98% similarity with isolates from the United Kingdom and Colombia. Santa Cruz and Baulcombe [34] reported that the distinction between the type B and type X coat proteins was correlated with the ability or inability, respectively, of the different strains to overcome Nx-mediated resistance.

The Accession Codes of the seven Turkish PLRV isolates were MW699145 (PA3-4), MW699146 (PA4-4), MW699147 (PA4-10), MW699148 (PB2-1), MW699149 (PB4-2), MW699150 (PB5-1), MW699151 (PB5-9). A comparison of the CP region sequences of PLRV isolates revealed the Turkish PLRV isolates to share 97–100% nucleotide identity with isolates from other parts of the world. The phylogeny was calculated from the partial CP gene sequences of the Turkish PLRV isolates and 26 reference isolates from GenBank representing different clusters (Fig. 5). The Turkish PLRV isolates were divided into two clusters in the phylogeny defined by Ristic et al. [35], with six isolates in Group 1 and one in Group 2. The Group 1 Turkish isolates were divided into three subgroups, and six showed high homology with isolates from China, Bangladesh, Iran and India, and one isolate (PB5-1) grouped with isolates from Serbia, China and India. The PB2-1 isolate showed 100% similarity with the isolates from Saudi Arabia (KC875235) and China (KR051194), and four isolates (PA3-4, PA4-4, PB4-2, PB4-10) showed a 99% similarity with French and Chinese isolates and were grouped together (Fig. 5).

Discussion

Viral diseases are among the most common problems in plants, and potyviruses are the leading threat to potato production all over the world. Several studies of potato viruses have been carried out in Turkey (36–41), but not specifically in the Tokat province, and not using RT-PCR and gene sequence comparisons.

Our study showed viruses to be common in the potato crops of Tokat Province, with more than half of the collected plant samples being infected by one or more viruses identified for the first time using RT-PCR and viral genes sequencing (especially CP), although the presence of these viruses has been previously reported serologically and biologically. The CP gene is the most common molecular marker used in investigations of genetic diversity and molecular evolution in plant viruses [36, 37], and so the CP regions of the viruses were sequenced in our study, other than for PVY. It has been suggested that the P1 gene region of the virus is the least conserved among the potyviruses and also among the PVY strains [38], and so the P1 region was used in our study. In summary, our analyses revealed the potato viruses of Tokat to be close to those found in other countries. The Tokat PVY isolates shared a maximum 99% nucleotide similarity with $PVY^{N–WI}$ and PVY^{NTN} from Germany, Poland, India and the United Kingdom; and PLRV shared a maximum 99–100% nucleotide identity with the isolates from China, Saudi Arabia, Bangladesh and Ireland. The identity of the PVS isolates ranged from 92 to 99%, and PVX had 98–99% nucleotide similarity with the Chinese and UK isolates. The value of gene sequencing is that it is more informative than serological tests. While PVY isolates can be identified serologically, serological tests do not effectively discriminate among the different PVY strains [39–44].

The most common virus was PVY with a prevalence rate of 47.12%, followed by PVS (16.74%) in the study. In a previous study, Carpar and Sertkaya (2016) [45] reported the most common virus in the potato production areas of the Hatay province in Turley in 2013–2014 to be PVY.

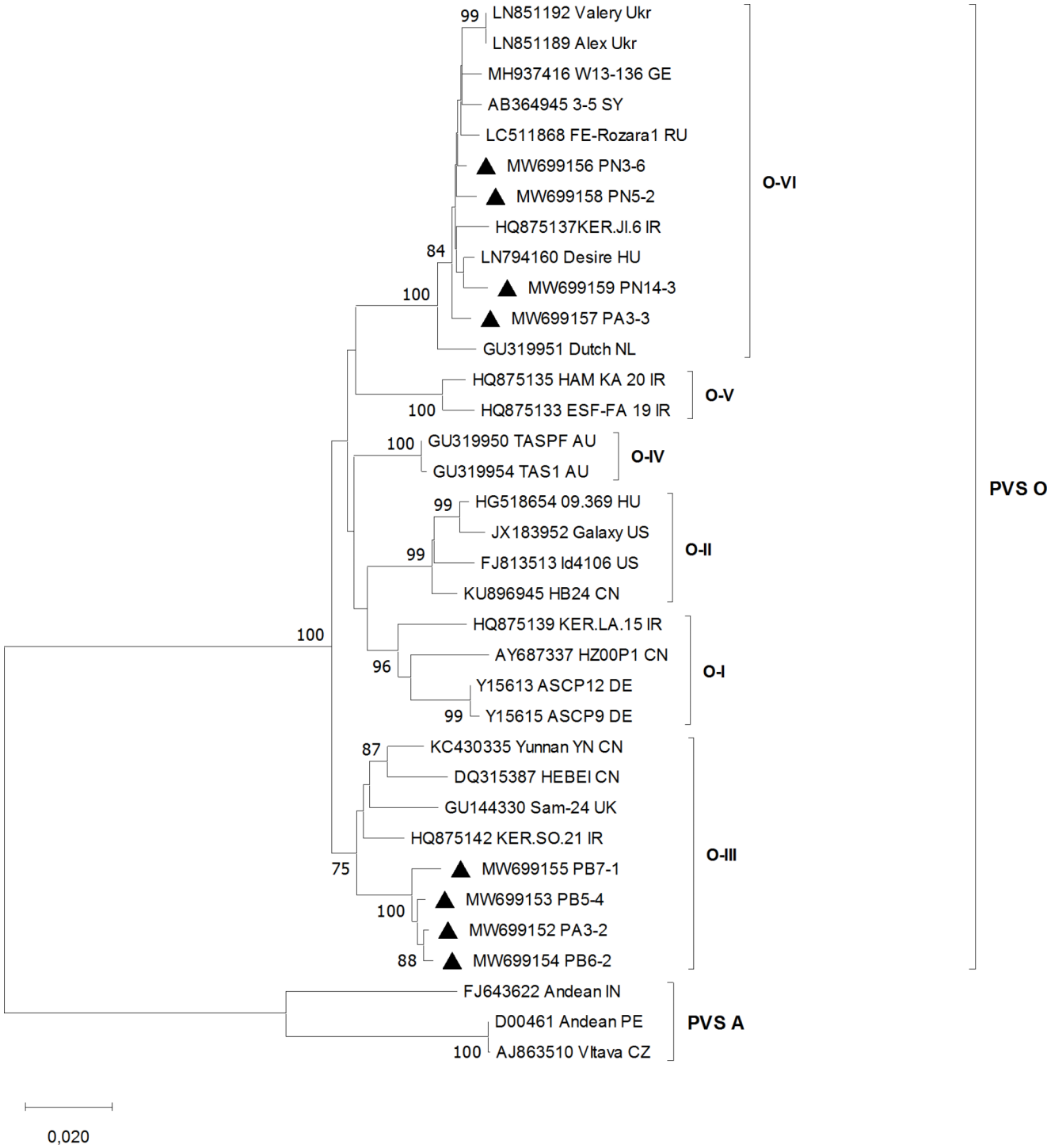


Fig. 3 Phylogeny of PVS isolates based on CP gene sequences and selected isolates from the NCBI database. Phylogeny was calculated using the Neighbor-Joining method. Bootstrap supports above 70% (n = 1000 bootstraps) are indicated for each node. The black triangles indicate the new isolates obtained in the present study

Furthermore, in the Isparta province of Turkey, a 44.23% prevalence was reported for PVY in the potato-growing areas [46]. Topkaya [47] examined PVY, PLRV and AMV infections serologically and molecularly in leaf samples from potatoes in Tokat, and reported a prevalence of 50%, 5.5% and 1.38%, respectively. PVY was also reported to be the most common virus (26%) in other solanaceous plants, such as in Buzkan et al.'s study of pepper [48].

Similar results have been reported in other studies conducted around the world. In a study conducted in Pakistan

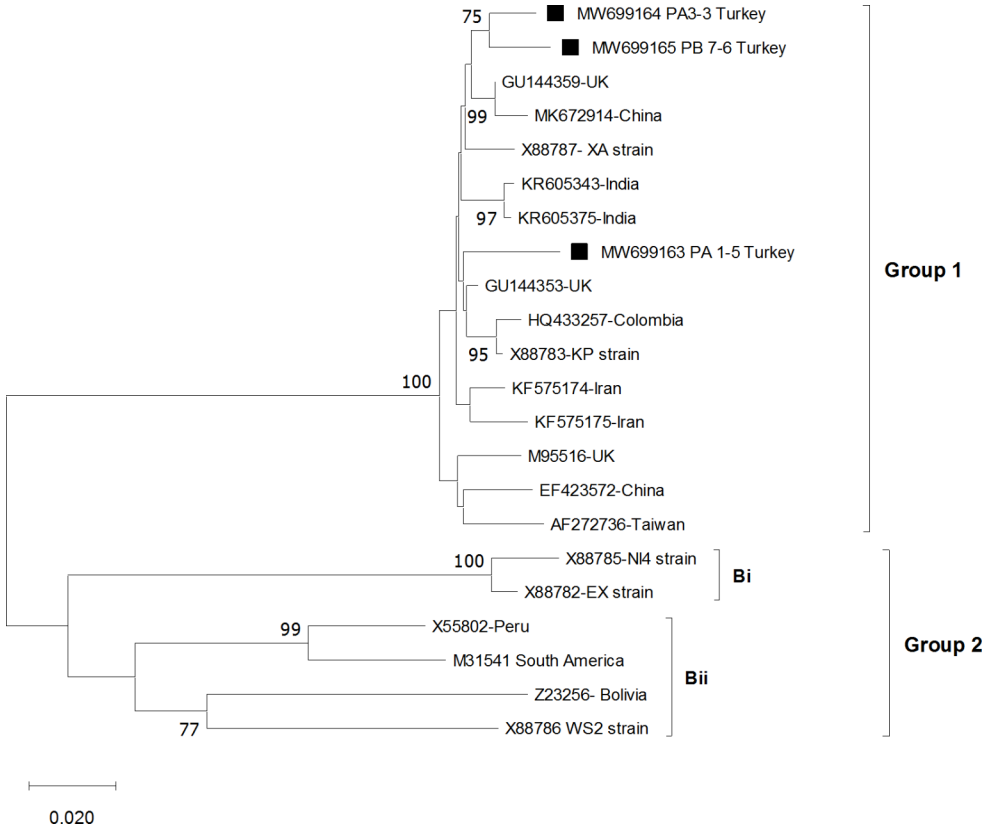


Fig. 4 Phylogeny of PVX isolates based on their CP gene sequences, and compared with different isolates selected from the NCBI database. The phylogeny was calculated using the Neighbor-Joining method. Bootstrap supports above 70% (n = 1000 bootstraps) are indicated for each node. The isolates obtained in this study are shown as black-filled squares

using ELISA tests, PVY was reported to be the most predominant virus, followed by PVX, PVA, PVS, *Potato virus M* (PVM) and PLRV [49]. The presence of potato viruses has previously reported serologically and biologically in the different provinces in Turkey. In one such serological study, PVX was reported as prevalent in the Tokat province by Çitir et al. [50]. PVX has also been determined in different species of Solanaceae in Turkey, such as in tomato in the Samsun province [51] and pepper in the Eastern Mediterranean [48]. PVX was identified in 10.17% of a tomato sample in the Samsun province in serological tests conducted by Arlı Sokmen and Sevik [51], with ToMV + PVX and TSWV + PVX mixed infections reported in 4.9% and 0.5%, respectively. Similarly, Yardımcı et al. [52] reported PVY + PVS double infections to be common in potatoes in the Afyon province.

In 2003, the most leading viral disease affecting potato production in the Niğde province was reported to be PVY + PVS (29.03%) by Guner and Yorganci [53]. The easy transmission of these two viruses through.

mechanical transmission, transmission by tubers and non-persistent transmission by aphids is one of the most important factors in their prevalence. Gul et al. [54] reported PVY and PVS to be dominant in the Swat district in Pakistan.

The number of double infections of different viruses in our samples was also examined for more subtle interactions by converting part of Table X to a "pseudo contingency" table. It was found that although PVY + PVS infections were most numerous, they were close to the expected number. However, PVY + PLRV infections were about three times less common than expected, while PVS + PLRV infections were three times more common than expected. Triple and quadruple infections were also found to be much more common than expected, and all involved PVY and PVS. These findings may reflect differences in aphid transmissions in single and mixed infections, but could also be attributable to multiple-infected plants with more prominent symptoms attracting samplers.

Single infections of PVA or PVS are symptomless and difficult to characterize, while mixed infections of PVY, PVS, PLRV and PVX may cause more severe symptoms, such as rugosity, mottling of the leaves and stunting [52]. PVY and PLRV were reported molecularly by a previous study in Tokat [47], while in the present study PVX and PVS are determined in this region for the first time using RT-PCR methods. We found viruses to be widespread in the potato fields in Tokat province, and that mixed infections result in greater losses.

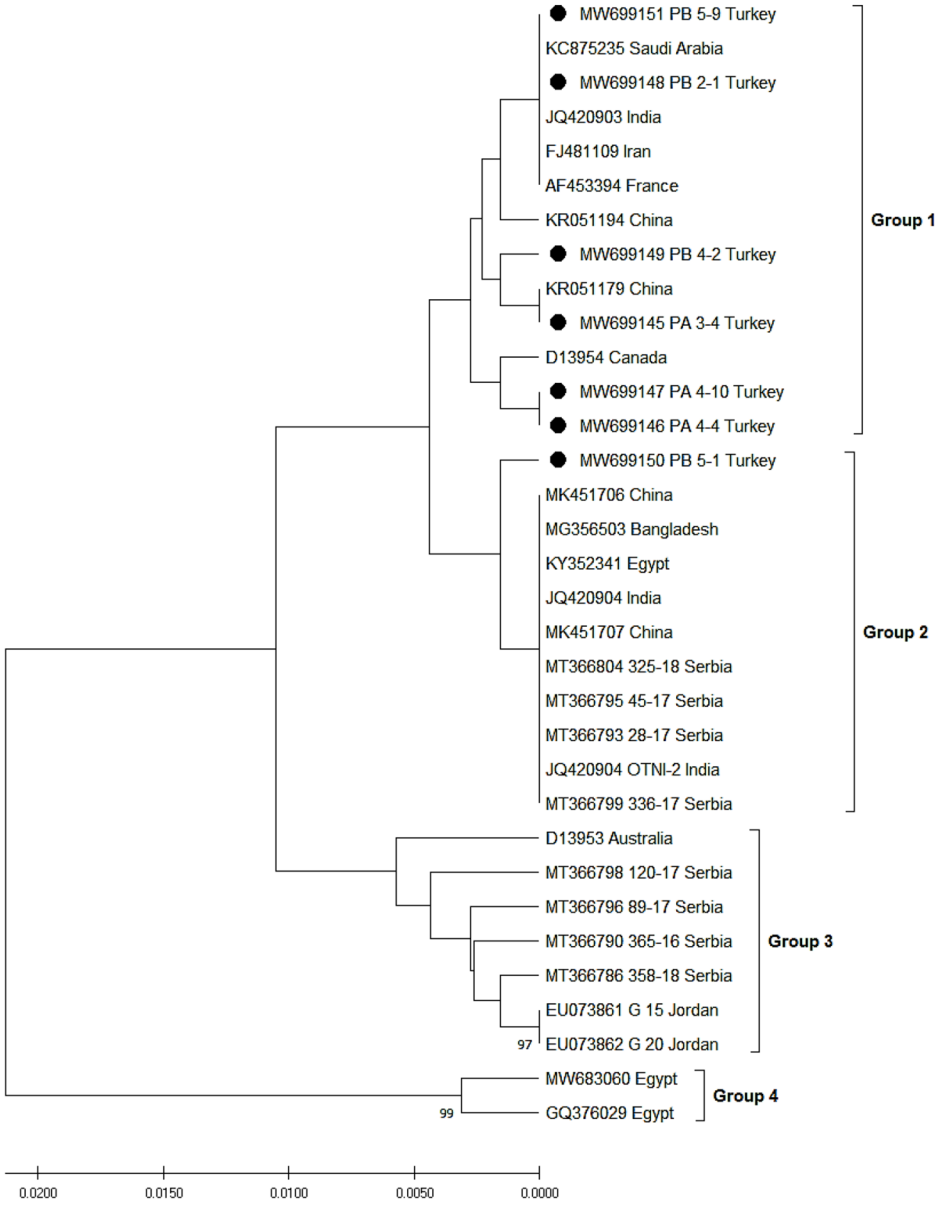


Fig. 5 UPGMA tree based on partial coat protein gene sequence of the selected isolates from the NCBI database generated with MEGA X. Bootstrap supports above 70% (n = 1000 bootstraps) are indicated for each node. The black circles indicate the new PLRV isolates obtained in this study

Conclusion

Viruses are considered a major threat to potato crops worldwide due to their ability to reduce potato yields significantly, and are the most important limiting factor in potato planting areas. In this study we have investigated the prevalence and genetic diversity of PVY, PVX, PVS and PLRV in potato growing areas of Tokat province. To the best of our knowledge, this is the first molecular study of PVX and PVS in Turkey, and we believe it could serve as a base for further studies of the phylogenetic features of the above-mentioned viruses.

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Author contributions AME conducted the laboratory experiments and wrote the original thesis. ST analyzed the data and wrote the manuscript.

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Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article contains no studies involving humans or animals performed by any of the authors.

Consent to participate Not applicable.

Consent for publication All authors give consent for the publication of this manuscript.

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