Research Square

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Occurrence and Molecular Characterization of Some Important Potato Viruses in Tokat, Turkey

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Research Article

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Abstract

Background Potato production is affected by various abiotic and biotic factors such as viruses, fungi, and bacteria. Viruses are considered the main limiting factor in potato production areas.

Methods and Results Leaf samples were collected from potato plants showing signs of viral diseases in five districts of Tokat province in Turkey. To detect the presence of potato viruses, 418 leaf samples were subjected to an RT-PCR test using virus-specific primers. One or more viruses were detected in 220 (52.63%) leaf samples tested. PVY was the most prevalent (47.12%), followed by PVS (16.74%), PVX (5.98%), and PLRV (5.26%). The PVX and PLRV infections were found to be few. The most common multiple infections were PVY+PVS with 6.9% rates. Phylogenetic analysis based on sequence analysis of some positive isolates revealed that all Turkish PVS isolates were grouped with the PVS^{O} cluster. Two PVY isolates were clustered with Group PVY^{N-WI}, and one isolates clustered with $PVYN^{NTN}$ isolates in the phylogenetic tree. Regarding nucleotide sequence similarity of the CP region, PVX isolates were divided into two major groups (Group I and Group II). Turkish PVX isolates were grouped within Group I that including isolates referred to as type X. According to phylogenetic analysis of PLRV isolates, Turkish isolates were clustered into two major groups. Six PLRV isolates belong to Group 1 and one isolate was clustered Group 2.

Conclusion The results indicate that these viruses were detected at different rates in potato fields. This is the first report about the molecular characterization of PVX and PVS based on CP sequences in Turkey. These reports will provide a base for further research on the phylogenetic features of the above-mentioned viruses, including the effect.

Introduction

Potato (*Solanum tuberosum* L.), which is in the Solanaceae family, is the most consumed food for humans after wheat, rice, and maize [1, 2]. Potato is produced in more than 100 countries in the world. Among the potato-producing countries, China holds the first position and Turkey ranks 22nd in the world in potato production in 2019. Potato production is affected by various abiotic and biotic factors such as viruses, fungi, and bacteria. Viruses are considered the main limiting factor in potato production areas [3]. Over 50 viruses and one viroid naturally infect the potato generally in limited areas, but some are common in all areas where potatoes are grown [4]. Only *Potato Y virus* (PVY), *Potato leaf curl virus* (PLRV), *Potato X virus* (PVX), and *Potato S virus* (PVS) have been recorded to cause severe infections worldwide and cause significant losses in yield [4, 5, 6, 7]. Plants are always under infection pressure by some of these viruses as long as there is a source of inoculum in the field [8].

The *Potato Y virus* is one of the major viral diseases that cause crop loss in potatoes and is found everywhere in the world. PVY contains a single-stranded positive-sense RNA genome of approximately 9.7 kb of 730x11 nm in size and is included in the Potyvirus group with filamentous helix particles [9]. Like all potyviruses, the PVY genome has a poly(A) tail at the 3' terminus and a covalently linked VPg protein at the 5' terminus. The virus has a genomic RNA encoding a single polyprotein, which is cleaved into 10 mature proteins by three virus-specific proteases [9, 10]. PVY can be transported non-persistently with over 50-winged aphid species and mechanically by plant sap [11, 12, 13, 14]. Although the symptoms caused by the infection of PVY vary, the signs of curliness and spots on leaves vary depending on the relationships between the virus race and the potato variety [15, 16, 17].

Another viral agent is the *Potato X virus* which is very common in potato-growing countries [18]. PVX has positive sensitive single-stranded RNA (ssRNA) of 515x13 nm in size and the form of a flexible rod. PVX can be transported mechanically and by vectors. The virus causes systemic infections in many plant species including potpotatoesomato, and tobacco [19]. No distinct symptom was formed in some varieties [19, 20].

Potato S virus is within the Carlavirus group, containing positive sense ssRNA and 700x13 nm in size [19]. The most important vector of the virus, like PVY, is *M. persicae* and can be also transported mechanically [12]. As a result of PVS infection in potatoes, initial symptoms are observed on the upper surface of the leaves, the veins collapse or deepen, and a 10–20% product loss is caused by the virus [17, 21, 22, 23]. The tubers and green parts of the plant remain smaller than healthy plants. Some PVS strains cause irregular yellowing of old leaves and bronze staining on leaves [15, 16, 17].

Potato leafroll virus is in the Luteovirus group, has a 24 nm diameter, an isometric particle containing a positive ssRNA, and is one of the first viral diseases studied on the potato plant [15, 16, 17]. PLRV was first described in 1916 and observed in all countries

where potatoes were produced. PLRV is densely found in the phloem tissue of the plant and is a virus that is not transmitted mechanically and has a narrow host environment. The virus was carried persistently with more than 10 aphid species, especially with green peach aphid (*M. persicae*) [6, 24, 25]. The symptoms of viruses may vary depending on environmental conditions, plant variety, biological period of the plant, and coexistence with other viruses.

There are few previous studies about the detection of viral agents in potatoes in Tokat province. Kutluk Yilmaz et al. [26] investigated viruses that infect potato leaves and tubers by using ELISA (Enzyme-Linked ImmunoSorbent Assay) method in 2003 and PVY and mixed infection was determined in both leaf and tuber samples. This study aimed to determine the prevalence of viral agents of PVY, PVX, PVS, and PLRV with PCR-based methods, differently from the ELISA method, in potato production areas in Tokat province. Also, we compared partial sequences of viruses to determine their evolutionary relationship.

Materials And Methods

Sample collection

A total of 418 plant samples showing virus symptoms were collected from the potato-producing areas in Niksar, Merkez, Erbaa, Basciftlik, and Artova districts of Tokat province in 2018. Collected samples were stored at -20 °C until use.

Molecular studies

RNA isolation

Potato leaves samples were tested for the presence of PVY, PVS, PVX, and PLRV using virus-specific primers with RT-PCR methods. Total RNA was extracted from potato leaves according to the protocol described by Astruc et al. [27]. RT-PCR was done as a two-step method. Firstly, a total of 500 ng RNA was reverse transcribed to complementary DNA (cDNA) using random primers and Superscript III reverse transcriptase (Thermo Fisher Scientific, Vilnius, Lithuania). For 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 into a tube, and the tube was incubated for five minutes at 5 ºC and then transferred on ice for three minutes. After, 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. Tubes were incubated at 25 °C for 5 min and 42 °C for 60 min, followed by incubation at 85 °C for 5 min.

PCR studies

Obtained cDNA was used as a template for the PCR assays using specific primer pairs to four viruses (Table 1). PCR amplification was carried out in a 25 µl mixture containing 2.5 µl of cDNA, 0.2 µl of 25mM dNTPs, 2 µl of 25 mM $MgCl_2$, 5 µL of 5X green reaction buffer, and 0.5 µl of 10 µM of each specific primer (Table1), 1.25-units *Taq* DNA polymerase (Promega, Madison, USA), and sterile ultra-pure water. PCR products were electrophoresed in 1% agarose gel stained with ethidium bromide.

Table 1. The primer sequences were used in the study.

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

Phylogenetic analysis

The phylogenetic tree of data was constructed based on the nucleotide sequences of the CP region of isolates obtained in this study and references isolates available in the GenBank database using the Neighbour likely method in MEGAX software [28]. The bootstrap values were calculated on 1,000 replicates.

Results

Survey

In this study, a total of 418 leaf samples were collected to investigate the presence of viruses during the survey (Table 2). Potato plants exhibited a wide range of symptoms like yellowing, stunting, leaf deformation, necrotic spots on the leaves (Fig. 1).

Total RNA extractions and RT- PCR were done to determine the presence of potato viruses (PVY, PLRV, PVX, and PVS). According to RT-PCR results, a total of 220 leaf samples were determined to be infected with one or more viruses. The rates of viruses were 47.12%, 16.74%, 5.98%, 5.26% for PVY, PVS, PVX, PLRV, respectively (Table 2). The incidences of viruses according to the district are shown in Table 2. PVY and PVS were the most common potato viruses in the surveyed potato plantations in Tokat province.

Table 2
The occurrence rates of detected viruses.

District	Number of collected samples	Number of infected samples	Infected samples and rates (%)			
			PVY	PVS	PVX	PLRV
Center	116	64	63	1	-	-
Niksar	92	47	38	8	-	1
Erbaa	101	14	14	-	-	-
Başçiftlik	65	60	52	47	3	18
Artova	44	35	30	14	22	3
Toplam	**418**	**220**	**197**	**70**	**25**	**22**

Double infections with PVY + PVS, PVY + PVX, PVY + PLRV, PVS + PVX, and PVS + PLRV were detected as 6.9, 2.39, 2.15, 0.23, 0.47 and 1.19%, respectively (Table 3). Triple infections of PVY + PVS + PVX and PVY + PVS + PLRV were determined as 2.39% for both. The 3 samples were detected with mixed infection of four viruses (PVY + PVS + PVX + PLRV) tested.

Table 3
The number of mixed virus infections

District	Number of collected samples	Number of infected samples	Infected samples and rates (%)							
			PVY + PVS	PVY + PVX	PVY + PLRV	PVS + PVX	PVS + PLRV	PVY + PVS + PVX	PVY + PVS + PLRV	PVY + PVS + PVX + PLRV
Center	116	64	1(0.86)	-	-	-	-	-	-	-
Niksar	92	47	1(1.08)	-	-	-	-	-	-	-
Erbaa	101	14	-	-	-	-	-	-	-	-
Başçiftlik	65	60	25(38.46)	-	1(1.53)	-	5(7.69)	1(1.53)	10(15.38)	2(3.07)
Artova	44	35	2(4.54)	9(20.45)		2(4.54)	-	9(20.45)	-	1(2.27)
Total	**418**	**220**	**29(6.93)**	**9(2.15)**	**1(0.23)**	**2(0.47)**	**5(1.19)**	**10(2.39)**	**10(2.39)**	**3(0.71)**

Phylogenetic analyses

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

Sequence data belonging to 3 Turkish PVY isolates were obtained and submitted to GenBank under the accession numbers: MW699160 (PM1-6), MW699161 (PA1-1), MW699162 (PE2-6). According to nucleotide sequence analysis, 2 PVY isolates showed 99% nucleotide similarity with isolates from Poland, Germany, and the United States, and the remaining isolate showed 99% similarity with isolates from India, the United Kingdom (UK), and China isolates. The phylogenetic tree based on the partial sequences of the P1 gene of Turkish PVY isolates and 12 reference isolates derived from GenBank represented different groups (Figure. 2). Two PVY isolates were clustered with Group PVY^{N-Wi}, and one isolate clustered with $PVYN^{NTN}$ isolates.

The sequences analysis was performed based on the CP gene of eight Turkish PVS isolates. The nucleotide sequences of PVS isolates were compared with reference PVS isolates derived from GenBank. Eight Turkish PVS isolates were submitted to GenBank under the accession numbers: MW699152 (PA3-2), MW699153 (PB5-4), MW699154 (PB6-2), MW699155 (PB7-1), MW699156 (PN3-6), MW699157 (PA3-3), MW699158 (PN5-2), MW699159 (PN14-3). PVS isolates were grouped into two major clusters, one of which included PVS ordinary strains (PVS^{O}), and the other contained PVS Andean strains (PVS^{A}). All Turkish PVS isolates were grouped into the PVS^{O} cluster (Fig. 3). Four isolates (PA3-3, PN3-6, PN5-2, PN14-3) obtained in this study were clustered with Yunnan isolate from China and Sam-24 isolates from the UK. Scotland and other strains (PB71-1, PB5-4, PA3-2, PB6-2) were grouped with Irena, Valery, and Alex isolates from Ukraine and Sam-13-O7 isolate from the UK. In genetically defined PVS^{O}, the nucleotide identity ranged from 92.6 to 100%. Cox and Jones (2010) reported that all PVS^{O} isolates could be classified into seven subclusters and the PVS^{A} into three groups. In our study, PVS isolates were separated into three subclusters (Fig. 3).

The accession numbers of three Turkish PVX isolates were MW699163 (PA1-5), MW699164 (PA3-3), MW699165 (PB7-6). The PVX isolates are clustered into two groups as reported before by Santa Cruz and Baulcombe [29]. Group 1 consisted of isolates from the UK, Asia, and Europe, whereas Group 2 consisted of isolates from the USA. Turkish PVX isolates were grouped into Group I and type X (Fig. 4). Two PVX isolates showed 98% nucleotide similarity with isolates from the UK, and China, and one isolate (PA1-5) showed 98% similarity with the UK, Colombian isolates. Santa Cruz and Baulcombe [29] reported that the distinction between type B and type X coat proteins correlated with the ability or inability, respectively, of the different strains to overcome Nx-mediated resistance.

The accession numbers of 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 CP region sequences of PLRV isolates showed that Turkish PLRV isolates shared 97–100% nucleotide identity. The phylogenetic analysis was based upon the partial sequences the of CP gene of Turkish PLRV isolates and 15 reference isolates from GenBank represented different

groups (Fig. 5). The Turkish isolates were divided into two major groups in the phylogenetic tree. Six isolates obtained from this study were grouped into Group 1 and one isolate was clustered into Group 2. In Group 1, the isolates were divided into three subgroups. Six isolates showed high homology with the isolates from China, Bangladesh, Iran, India, and one isolate (PB5-1) was grouped with Ireland and Colombia isolates. PB2-1 isolate showed 100% similarity with the isolates from Saudi Arabia (KC875235) and China (KR051194). The four isolates (PA3-4, PA4-4, PB4-2, PB4-10) showed 99% similarity with Bangladesh and Chinese isolates and grouped (Fig. 5).

Discussion

Viral diseases are the most common problem in plants. Potyviruses are primary threats to potato production all over the world. There are a lot of studies about potato viruses in Turkey. However, there are limited studies on molecular detection and the presence of potato viruses in Tokat province. In this study, we studied the prevalence of potato viruses in potato-growing areas in Tokat province. RT-PCR assays and sequencing analyses were performed to determine and reveal the molecular relationship of four potato viruses. The results showed that 220 out of 418 samples were infected with one or more viruses. The rates of PVY, PVS, PVX, PLRV were 47.12%, 16.74%, 5.98%, 5.26%, respectively. The presence of these viruses was previously reported serologically and biologically in Turkey. The current study was the first study for the determination of the viruses via molecular tools in Tokat. In the previous serological studies, PVX were determined as prevalent in Tokat province by Çitir et al. [30]. Guner and Yorganci [31] noted that PVY, PVS, PVX, PVA, and PLRV were found as single or mixed infections in both potato leaves and tubers by using DAS-ELISA in Niğde and Nevşehir provinces. In Hatay province, the most common viruses were PVY (49.5%), followed by PLRV (5.4%) and AMV (5.4%), and PVY and PLRV were also determined as mixed infections in 2013–2014 [32]. In Isparta province, 44.23% rate of the prevalence of PVY in potato-growing areas was reported [33]. Topkaya [34] examined the infection of PVY, PLRV, and AMV serologically and molecularly in leaf samples of potatoes in Tokat and reported the prevalence of 50%, 5.5%, and 1.38%, respectively. The single infections of PVA or PVS are symptomless and difficult to characterize; however, the mixed infections of PVY, PVS, PLRV, and PVX may cause more severe symptoms such as rugosity and mottling of leaves, stunting [35]. In this study, double infections with PVY + PVS, PVY + PVX, PVY + PLRV, PVS + PVX and PVS + PLRV were detected as 6.90, 2.39, 2.15, 0.23, 0.47, and 1.19%, respectively. Similar results were reported by different researchers [17, 36]. PVY and PLRV were molecularly reported by a previous study in Tokat [34]. However, in this study, PVX and PVS were also determined first time by RT-PCR methods in this region. We showed that viruses are widespread in potato fields in Tokat province as mixed infections result in greater losses.

In the study, sequences of comparison of the viruses with the references sequences derived from the GenBank database showed that PVY isolates from this study shared a maximum 99% nucleotide similarity with PVY^{N-WI} and PVY^{NTN} from Germany, Poland, India, and the UK. PLRV shared a maximum 99–100% nucleotide identity with the isolates of China and Saudi Arabia, Bangladesh, Ireland; nucleotide identity of PVS isolates ranged from 92 to 99% and PVX shared 98–99% nucleotide identity with Chinese and British isolates. PVY isolates can be identified serologically but serological tests do not effectively discriminate among the different PVY strains [37, 38]. Molecularly PVY strains were separated as nonrecombinant isolates of PVY as PVY^{O}, PVY^{N}, and PVY^{C} [39, 40, 41], and recombinant isolates built of PVY^{O} and PVY^{N} parental sequences that were classified as either PVY^{NTN} or PVY^{NTN-NW}.

Phylogenetic analysis provided important information about the origin and the evaluation of viruses and showed that Turkish PLRV isolates were closely related to Canadian and Chinese isolates; PVX isolates to Chinese isolates; PVY isolates to the isolates from the USA, UK, and Germany; PVS isolates to Chinese and Iranian isolates, respectively. These results would be a foundation for further molecular evolutionary study on potato viruses in Turkey.

Conclusion

Viruses are major threats to potato fields worldwide as well as in Turkey. The viruses reduce potato yield significantly and are the most important limiting factor in potato planting areas. In this study, we searched the prevalence and genetic diversity of PVY, PVX, PVS, and PLRV potato growing areas in Tokat province. As far as we know, this is the first molecular report of PVX and PVS

in Turkey. These reports will provide a base for further research on the phylogenetic features of the above-mentioned viruses, including the effect.

Declarations

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Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors

Consent to participate is not applicable.

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

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Figures

Figure 1

Symptoms of viruses on potato samples. a: PLRV; b, d: PVY; c: AMV

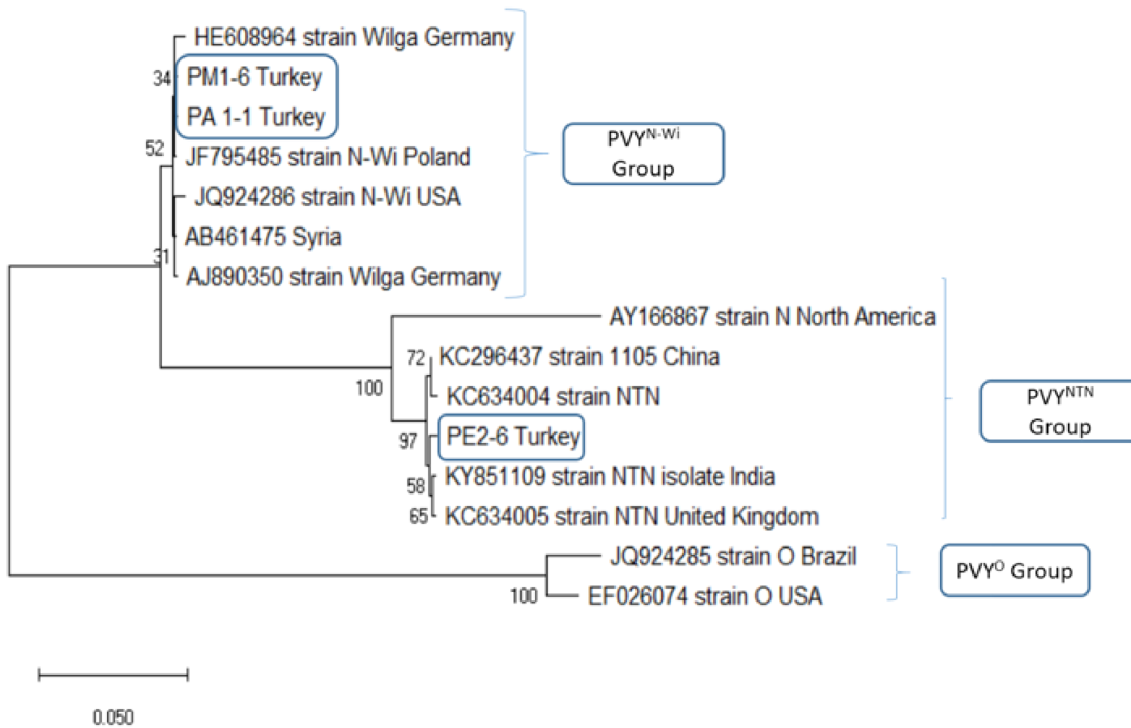

Figure 2

Phylogenetic analysis of PVY based on the P1 gene sequences of different selected isolates from the NCBI database.

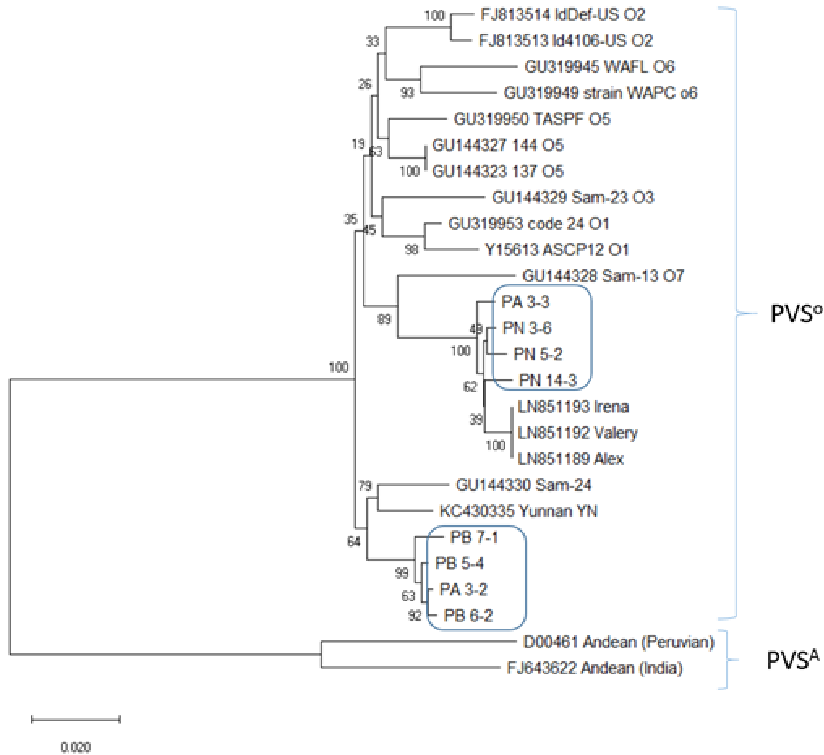

Figure 3

Phylogenetic analysis of PVS based on the CP gene sequences of different selected isolates from the NCBI database.

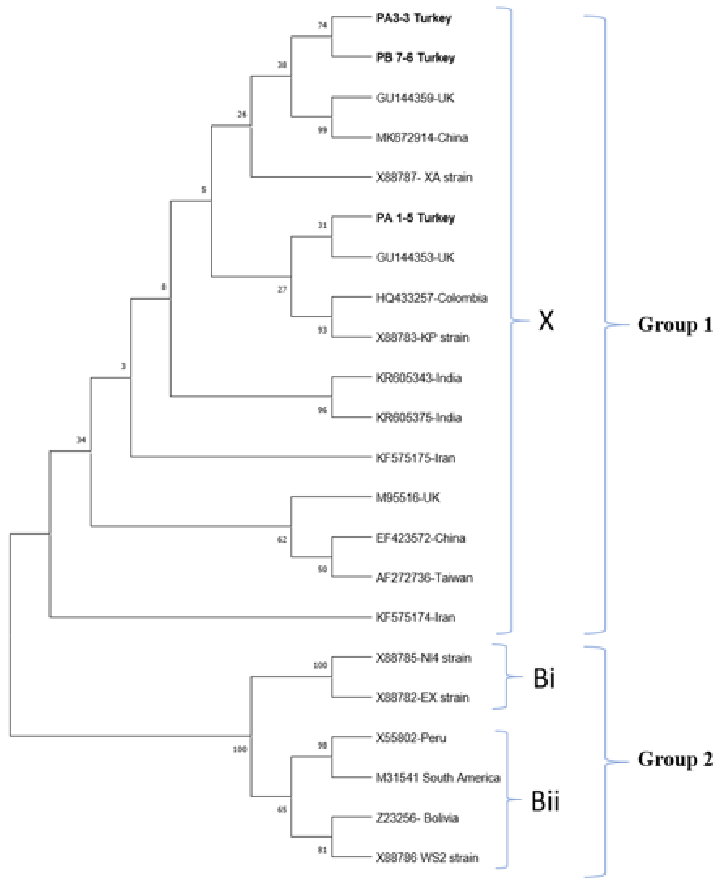

Figure 4

Phylogenetic analysis of PVX based on the CP gene sequences of different selected isolates from the NCBI database.

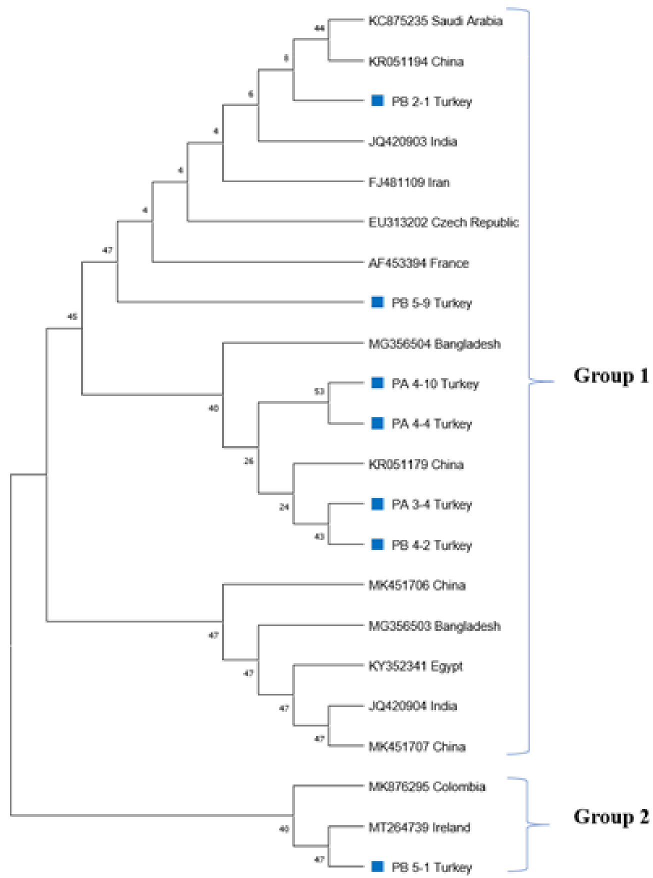

Figure 5

Phylogenetic analysis of PLRV based on the CP gene sequences of different selected isolates from the NCBI database.