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Determination and partial molecular characterization of *Plum pox virus* in Bolu province

Bolu ilinde *Plum pox virus*'un belirlenmesi ve kısmi moleküler karakterizasyonu

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ABSTRACT

Plum pox virus (PPV), the causal agent of Sharka disease, causes yield, quality, and economic losses in stone fruits. PPV has been reported worldwide, especially in Europe. In studies to date, the presence of the virus has been identified as being restricted in different regions of Turkey. However, there is no record of PPV in Bolu province so far. Hence, surveys were carried out in Bolu province between 2016-2019, and a total of 306 samples were collected. To determine the presence of PPV, the samples were first tested by DAS-ELISA, and only three peach samples were found to be infected. DAS-ELISA results of infected samples were confirmed by RT-PCR using universal primers (P1/P2), then infected samples were identified at the strain level using strain-specific primers. The samples were found to be infected with the PPV-M (Marcus) strain and 243-bp long nucleotide sequences containing the partial coat protein gene of three isolates were deposited to NCBI. Phylogenetic analysis (Neighbor-Joining) generated by 38 representative PPV sequences indicated that Bolu isolates were clustered with PPV-M isolates and separated from other strains, as in BLAST analysis. To our knowledge, this is the first report of PPV in Bolu. This study reveals the necessity to carry out more extensive surveys to prevent the PPV dissemination in Bolu and to identify the complete genomes of the obtained isolates to determine their genetic variation. All the PPV-infected trees were destroyed as a consequence.

INTRODUCTION

Plum pox virus causing Sharka disease is one of the most devastating viruses in stone fruits, in wild and ornamental plant species in *Prunus* spp., and in many varieties of weeds as a secondary host. PPV is the species of *Potyvirus* belonging to *Potyviridae* family and possess a single-stranded positive-sense RNA (ssRNA) molecule of the size of 660-750 nm in length and 12-15 nm in width. This RNA molecule is about 9,786 bases in length and consists of one open reading frame that encodes a single polyprotein at a weight of 355.5 kDa (Cui and Wang 2016, Revers and Garcia 2015, White 2015). Originated polyprotein precursor is co- and post- translationally cleaved by three virus-encoded proteinases into 11 mature proteins P1, HC-Pro, P3, P3N-PIPO, 6K1, CI, 6K2, NIa (respectively VPg and NIa-Pro), NIb and CP (Sochor et al. 2012).

PPV is transmitted over short-distances by different species of aphids, such as *Aphis fabae* Scopoli, *Aphis gossypii* Glover, *Aphis spiraecola* Patch, *Brachycaudus persicae* Passerini, *Myzus persicae* Sulzer in a non-persistent manner (Fernández –Calvino et al. 2006). Long-distance spread by means of vegetative propagation materials causes the viruses to be introduced to different geographical areas (Brunt et al. 1996, James and Varga 2005, Myrta et al. 2006, Palkovics et al. 1993).

The symptom severity in infected plants can vary depending on the virus strain, infection time, age, and growth conditions of the host plant, environmental factors, and cultivar. In general, plum, apricot, and peach are very susceptible to PPV and the virus causes severe symptoms. Symptoms observed in these species are specific indications of the disease that can be used to identify Sharka macroscopically. Disease symptoms occur on leaves, fruits, and flowers. Leaf symptoms on infected trees include chlorotic (yellowing) spots, vein clearing, vein banding, rings or blotches. Flower symptoms may exhibit color-breaking and deformation. Symptoms for the fruit consist of lightly pigmented yellow rings, having less sugar content, severe premature dropping, and deformation, rings, and spots on the seed surface (Cambra et al. 2006).

In 1961, the disease was identified for the first time on plum trees in Edirne, Turkey (Sahtiyanci 1969). In the following years, it was reported on apricot and plum trees in Ankara (Kurçman 1973), on apricot, plum, peach, and almond trees in Marmara Region (Bilecik, Bursa, İzmit, Sakarya, İstanbul, and Tekirdağ) between 1976 and 1982 (Yürektürk 1984). After these studies, more extensive surveys were carried out by many researchers to determine Sharka disease in Turkey. As a result of these studies, the presence of the disease was reported in following provinces: İzmir, Aydın, Balıkesir, Çanakkale, Manisa, Kahramanmaraş, Adana, Mersin, Isparta, Afyonkarahisar, Kütahya, Antakya-Hatay, Aksaray, Kırklareli, Kayseri, Konya, Antalya, Eskişehir, Samsun, Erzincan, Kırıkkale, Yozgat, and Sivas (Akbaş et al. 2011, Azeri 1994, Buzkan et al. 2006, Candresse et al. 2007, Çelik and Kütük 2013, Değirmenci et al. 2016, Deligöz et al. 2015, Dunez 1986, Gazel et al. 2010, Gumus et al. 2007, Gürcan et al. 2013a, 2013b, Koç and Baloglu 2006). As a result of the detection studies, the virus was detected locally in many provinces and most of the infected trees were destroyed.

PPV is comprised of ten following strains based on biological, serological, molecular and epidemiological properties: PPV-M (Marcus), PPV-D (Dideron), PPV-C (Cherry), PPV-EA (El Amar), PPV-T (Turkey), PPV-W (Winona), PPV-Rec (Recombinant), PPV-CR (Cherry Russia), PPV-An (Ancestor) and the most recently PPV-CV (Cherry Volga). PPV-M strain was first reported on peach in Greece (Myrta and Boscia 2001, Wang et al. 2006), PPV-D strain on apricot in France (Kerlan and Dunez 1979, Myrta et al. 2006), PPV-C strain on cherry in Moldova (Nemchinov et al. 1996, Wang et al. 2006), PPV-EA strain on apricot in Egypt (Myrta and Boscia 2001, Wetzel et al. 1991), PPV-T strain on plum in Turkey (Serçe et al. 2009), PPV-W strain on plum in Canada (James and Varga 2005), PPV-Rec strain on plum in Yugoslavia (Cervera et al. 1993), PPV-CR strain on sour cherry in Russia (Chirkov et al. 2013), PPV-An strain on plum trees in Albania (Palmisano et al. 2012), and PPV-CV strain on sour cherry in Russia (Chirkov et al. 2018), respectively. Among ten strains of PPV, only PPV-D (Elibuyuk 2004, Gürcan and Ceylan 2016, Gürcan et al. 2020), PPV-M (Elibuyuk 2004, Gürcan et al. 2019, Sertkaya et al. 2003), PPV-Rec (Candresse et al. 2007), and PPV-T (Serçe et al. 2009, Teber et al. 2019) have been reported in Turkey.

Over the past 100 years, Sharka disease epidemics severely affect prunus trees worldwide and cause enormous economic losses. Studies on the distribution of the disease in Turkey in the past years were generally limited to serological detection. Several studies conducted to determine the disease distribution in Turkey showed that PPV has a restricted distribution. However, the studies conducted in the last decade are more detailed at molecular level. Although there are few studies on the detection of PPV in the Black Sea Region (Akbaş et al. 2011, Deligöz et al. 2015), there are no studies conducted in Bolu province. Bolu is known to be virus-free province in terms of Sharka disease. In this study, the presence of PPV was determined serologically and molecularly in the home-garden in Seben district and the infected trees in question were destroyed. Seben district is a border to Ankara province which is reported to be infected with PPV. Thus, the objective of the present study was to determine the molecular similarities of isolates obtained from this study and other PPV isolates reported in Turkey and worldwide by phylogenetic analysis.

MATERIALS AND METHODS

Survey

Surveys were carried out in Göynük and Seben districts (Figure 1) where stone fruits only grown in Bolu between May and June, 2016-2019 by Bolu Directorate of Provincial Agriculture and Forestry within the scope of "Sharka Survey Instructions". Not only the commercial fruit orchards, but also some of the residential gardens were also included in the survey area. Samples were collected considering the virus biology and climatic conditions of the location. For the sampling, in a total of 20 leaves were collected from each side of each trees and one tree was considered as one sample. Each collected sample was labeled with appropriate information (date, province, district, sample ID, owner, size of the field) to identify them at harvest. The labeled samples in polyethylene bags were shipped along with cold chain to the virology department of Directorate of Plant Protection

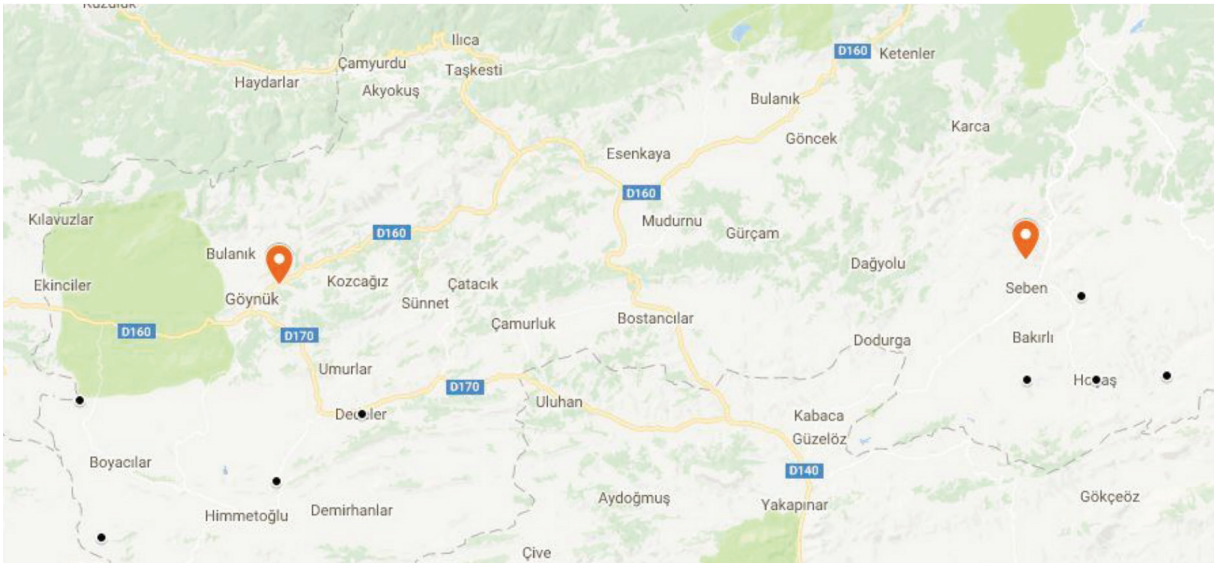

Figure 1. Map showing the survey areas of Göynük and Seben districts in Bolu province between 2016 and 2019 depicted in red, within the study region

Central Research Institue, Ankara. During 4 years, a total of 306 samples including 75 cherries and 166 peaches from Seben, and 29 cherries and 36 sour cherries from Göynük were analysed for the presence of PPV.

DAS-ELISA

Leaf samples were firstly tested by DAS-ELISA (Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay) using PPV-specific (PPV-C, PPV-D, PPV-EA, PPV-M, and PPV-W) polyclonal antisera (Agdia Inc. Elkhart, IN, USA) as stated in the manufacturer's instructions (Clark and Adams 1977). Absorbance at 405 nm was measured using a microplate reader (Tecan Sunrise Microplate Reader 16039400) at 30 to 60 min after the addition of the substrate (p-nitrophenyl phosphate). A threshold value for the samples was considered as positive when the mean value of healthy control of OD value is higher than twice the average.

Nucleic acid extraction and RT-PCR

Based on DAS-ELISA results, all the positive samples and one negative sample (as a negative control) were selected, and the total RNAs of the selected samples were extracted to use in RT-PCR. The total RNA extraction protocol was similar to Foissac et al. (2001) with minor modifications. A hundred mg of plant tissue was ground using liquid nitrogen and homogenized with 1 ml of extraction buffer containing 6 M guanidine thiocyanate, 0.2 M sodium acetate, 25 mM EDTA, 1 M potassium acetate, 2.5% PVP-40, and 1% mercaptoethanol. Then, 500 µl of the extract was mixed with 100 µl of 10% sodium lauryl sulfate into the new tubes. The tubes containing sodium lauryl sulfate and extract were incubated at 70 °C for 10 min, placed in ice for 5 min, and centrifuged at 14000 rpm for 10 min respectively. Subsequently, 300 µl of supernatant was transferred to the new microcentrifuge tubes. Then, 150 µl of 99.9% ethanol, 35 µl silica gel (1 g silicon dioxide +1 ml H_2O pH: 2), and 300 µl of 6 M sodium iodide were added to the microcentrifuge containing 300 µl of supernatant. The mixtures were incubated at room temperature for 10 min in a shaker, then the tubes were centrifuged at 6000 rpm for 1.30 min. After discarding the supernatants, pellets in the tubes were washed two times by adding 500 µl washing buffer (10 mM Tris-HCl containing 0.05 mM EDTA, 50 mM NaCl and 50% ethanol) each time, then the pellets were dissolved in 75 µl of RNase-free water. The mixtures were incubated at 70 °C for 4 min, and centrifuged at 14000 rpm for 3 min, after that the supernatants were transferred into new tubes. The total RNAs were quantified by spectrophotometry using a Nanodrop (Thermo Scientific, Wilmington, ME). After all the total RNAs for each sample were adjusted to 50 ng/µl and were stored at -80 °C.

For the detection of PPV isolates, one-step RT-PCR was conducted according to Wetzel et al. (1991) using PPV-universal primers (P1/P2) that are specific to all strains. To identify the strain differentiation of PPV, one-step RT-PCR was carried out using additional strain specific primer pairs: P1/PD and P1/PM for PPV-D and PPV-M, respectively (Olmos et al. 1997).

Samples were amplified by PCR, using a thermal cycler (Applied Biosystems Veriti™ 96-Well Thermal Cycler), and the reaction was prepared as follows: 25 µl of a mastermix containing an initial concentration of 8 µl of 5X Go Taq Flexi Green buffer, 1.25 µl of 25 mM $MgCl_2$, 0.625 µl of 10 mM dNTPs, 1 µl of 10 µM P1 primer, 1 µl of 10 µM P2 primer, 0.2 µl of Taq DNA Polymerase (GoTaq® G2 Flexi

DNA Polymerase 5 U/µl), 0.15 µl of Reverse Transcriptase (ProtoScript® II Reverse Transcriptase-M-MuLV), 0.2 µl of RNase (RNase Inhibitor, Murine 1 U/µl) and 2 µl of 50 ng/µl total RNA. Mastermix applied in universal primers was also used for strain-specific primers (P1/PM-P1/PD).

The common thermal cycler program for universal and strain-specific primers was carried out under the following conditions: 45 min at 42 °C, 2 min at 94 °C, 40 cycles of 30 s at 94 °C, 30 s at 60 °C and 1 min at 72 °C, followed by a final extension for 10 min at 72 °C. A total of 8 µl PCR products were loaded into 2% 1X TAE agarose gel stained with Pronasafe nucleic acid staining solution (Conda, Madrid, Spain) for 1 hour at 80 V and visualized by UV transillumination. The remaining PCR products were stored at -20 °C until used for sequence analysis.

Bioinformatic analyses

The remaining 17 µl of RT-PCR products, by a commercial company (Macrogol The-BM Laboratory Systems, Turkey), were sequenced in both orientations using P1/P2 primer pairs. Raw sequence data were aligned through the Clustral W technique (Thompson et al. 1994) implemented in MEGA 7 software. Consensus sequences of 243 nucleotides were generated by using forward and reverse sequences. In total, three PPV sequences corresponding to the partial coat protein region of isolates were deposited in GenBank (National Center for Biotechnology Information-NCBI) and compared to reference sequences of the corresponding genomic region of PPV isolates available in the GenBank database using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The list of reference isolates obtained from the GenBank database is provided in Table 1. All sequences were aligned using ClustalW and the sequences were trimmed to 241 bp to form clear consensus sequences. Aligned nucleotide sequences were used to construct a phylogenetic tree using the MEGA 7 software. For the phylogenetic tree, sequences for other strains of PPV from NCBI were selected to fully represent the genetic variation. In addition, *Potato virus Y* (PVY) represents the type species of the Potyviruses, which were also included in the phylogenetic tree as an out-group. Thirty-five reference isolates (14 were belonging to Turkey and the remaining 21 were belonging to other countries) and three Bolu (Turkey) isolate was used. Phylogenetic trees were constructed based on the Neighbour-joining method (NJ) (Saitou and Nei 1987) and the Maximum Likelihood method (ML) (Kimura 1980, Kumar et al. 2016) with a bootstrap value of 1000. NJ tree was selected for this study because similar results were observed in both trees. The intragroup genetic variability analyses were conducted using the maximum composite likelihood model (Tamura et al. 2004) implemented in MEGA 7.

Table 1. The list of isolates subjected to sequence analysis in this study

No	GenBank access. No	Country-Province	Strain
1	MG941018.1	Turkey-Bolu*	M
2	MG941019.1	Turkey-Bolu*	M
3	MG941020.1	Turkey-Bolu*	M
4	MF371001.1	Turkey-Istanbul	M
5	MF370999.1	Turkey-Istanbul	M
6	MF370998.1	Turkey-Istanbul	M
7	MF370995.1	Turkey-Istanbul	M
8	KX423957.1	Turkey-Bursa	M
9	KX423940.1	Turkey-Bursa	M
10	KX423931.1	Turkey-Bursa	M
11	KX423897.1	Turkey-Bursa	M
12	HF585103.1	Slovakia	M
13	LC228949.1	Japan	M
14	FJ361234	Greece	M
15	HF585104.1	Slovakia	M
16	HF585102.1	Slovakia	M
17	KX423952.1	Turkey-Bursa	D
18	KX423946.1	Turkey-Bursa	D
19	KT827161.1	Turkey-Istanbul	D
20	KT827117.1	Turkey-Istanbul	D
21	LT158756.1	Slovakia	D
22	AB576049.1	Japan	D
23	EF640933.1	USA	D
24	FN179154.1	Hungary	D
25	AY953266.1	Canada	D
26	HG964685.1	Canada	Rec
27	EU117116.1	Poland	Rec
28	KT827131.1	Turkey	T
29	KT827147.1	Turkey	T
30	AM157175.1	-	EA
31	DQ431465.1	Egypt	EA
32	KC347608.1	Russia	W
33	AY912055.1	Canada	W
34	KJ787006.1	Russia	C
35	AY184478.1	-	C
36	KC020124.1	Russia	Cr
37	KC020126.1	Russia	Cr
38	HF674399.1	Albania	An
39	KR528584.1	Uruguay**	PVY

*Novel isolates identified in this study ** Selected isolate as an outer group

RESULTS AND DISCUSSION

From 2016 to 2019, a total of 306 samples were collected from two districts (Göynük and Seben) and eight villages (neighborhoods) of those districts by Bolu Directorate of Provincial Agriculture and Forestry. Sweet and sour cherry samples were collected from Göynük district, peach and sweet cherry samples were collected from Seben district during the surveys. Based on molecular and serological results, in total, only three peach samples belonging to Çeltikdere neighborhood of Seben district in 2017 were shown to be PPV-infected.

Characteristic symptoms of PPV were not seen on the infected peach trees, but different sizes of white spots on the fruits and fruit deformation were observed. The occurrence of the PPV symptoms on peach leaves and fruits can vary depending on virus strain, climate factors, plant age, and cultivar (Desvignes et al. 1999, Elibüyük 2005). Some papers also reported that PPV may sometimes fail to produce any characteristic symptoms on peach trees (Desvignes et al. 1999, Elibüyük 2005, Polak et al. 2003, Varn et al. 2004). Therefore, this could be the reason why we did not observe PPV symptoms on the infected trees in Bolu province. In a total of 306 samples, examined macroscopically in both field and laboratory, were first analyzed by DAS-ELISA and 3 samples were determined to be infected with PPV. Furthermore, 3 km perimeter of the infected garden is fully examined and analyzed in terms of PPV infection in the later years (2018-2019). After the analysis, the region was found PPV negative.

RT-PCR was performed for three peach samples, which were all positive according to DAS-ELISA results. Based on DAS-ELISA results, one PPV-free sample from the peach tree used as a negative control, one PPV-infected sample from the apricot tree used as a positive control and one water control were also included into RT-PCR reactions. Universal primers (P1/P2) were used first to amplify the partial coat protein region of PPV, then strain-specific primer pairs were used to determine the strains of PPV (P1/PM-P1/PD). The samples of P157, P179, P214 and positive control amplified the bands of the expected size of 243 bp for P1/P2 primer pairs. Thus, the results of RT-PCR analyses using universal primers were in complete agreement with DAS-ELISA results. A 198 bp product was amplified with P1/PM primers using positive samples, which was designated as PPV-M (Figure 2).

Figure 2. Agarose gel electrophoresis of the RT-PCR amplification products using universal and strain-specific primer pairs. The first and last lanes represents DNA 100 bp Ladder; lane 1-2-3, Bolu isolates; lane 4, positive control; lane 5, negative control; lane 6, water control

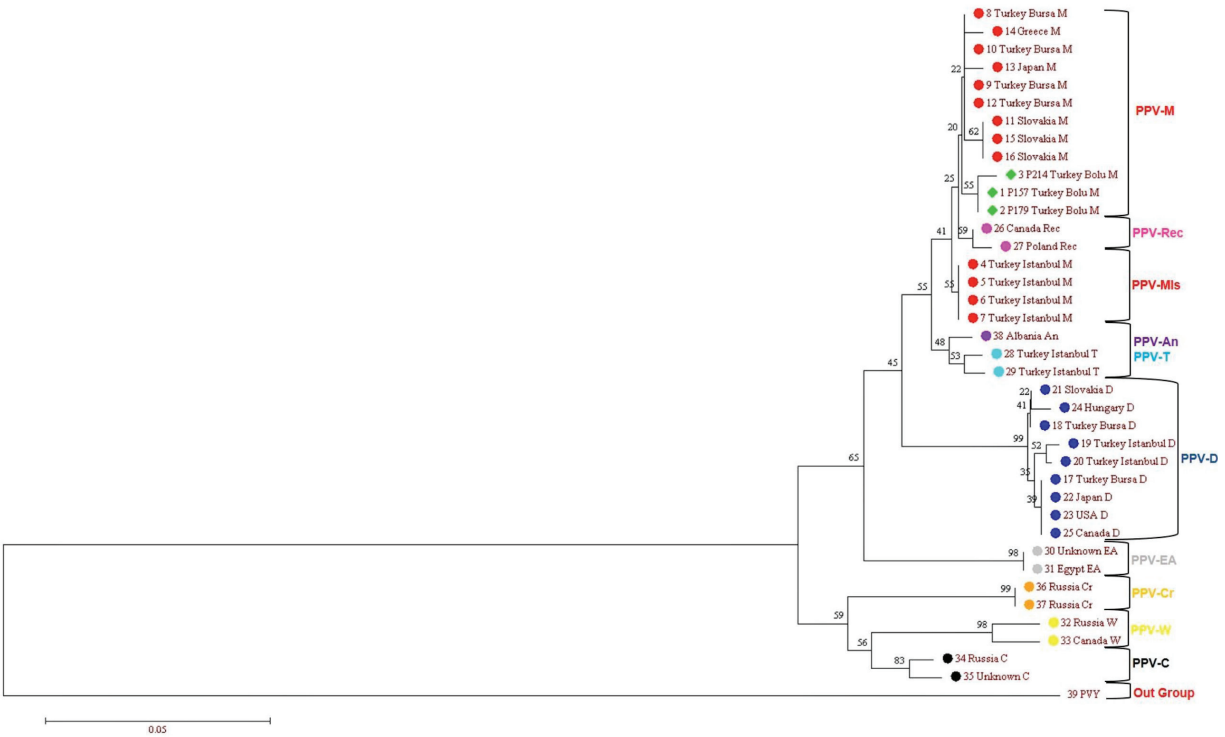

Figure 3. Phylogenetic tree constructed based on nucleotide sequence of the partial coat-protein gene (241 bp) showing the genetic relationships between Bolu and representative PPV isolates. The sequence obtained in this study is marked as diamond (♦)

PCR amplicons of P1/P2 primer pairs were sequenced and then compared with the corresponding worldwide PPV sequences available in GenBank to reveal the similarities. Three Bolu isolates (P157, P179, and P214) showed 99-100% similarity to GenBank accessions from Turkey (Edirne-Bursa) and Macedonia.

PPV-M was first reported in infecting peach in Greece and known to be widely distributed in European countries (Myrta and Boscia 2001, Wang et al. 2006). They are often associated with rapidly spreading epidemics in peach but are less frequently found in plums. Usually, PPV-M isolates are transmitted efficiently by aphids (Glasa and Candresse 2008). By their serological properties, Dallot et al. (2011) separates the M-strain into two following subgroups: PPV-Ma (mostly from Mediterranean countries) and PPV-Mb (mainly from Central–Eastern Europe isolates). In phylogenetic analysis for Bolu isolates (Figure 3), PPV-M isolates were clustered in two different groups (M and MIs). M (Ma and Mb) isolates consist of isolates belonging to Turkey, European, and Mediterranean countries; MIs isolates, reported by Gürcan and Ceylan 2016, comprised of Istanbul (Turkey) isolates. In the phylogenetic tree, Bolu isolates were clustered on the branch called M.

The sequences of Bolu isolates were compared to the other PPV isolates in NCBI. The first ten isolates were presented in Table 2. The high similarities among the isolates of Bursa and Edirne were observed. The same results were reported by other researchers. Bolu isolates were derived from Europe isolates due to the similarities in sequences of Europe originated Turkish isolates and its place in the group on phylogenetic tree M. One of them is European PPV-M which is considered as spreading by arboriculture from Europe to Turkey. The latest one, PPV-MIs is a subgroup of the M strain discovered only in Istanbul. The complete genome of 10 isolates from this group were sequenced and reported to be genetically different from the European M group (Gürcan 2017, Teber and Gürcan 2016). The phylogenetic tree in this study shows that Istanbul PPV-MIs isolates are clustered by different groups from PPV-M isolates.

Apart from these data, it is seen that PPV-An isolate is closely related to Istanbul PPV-T isolates in the phylogenetic tree. PPV-An was detected in Albania in 2012. It has been reported that PPV-An isolate is most closely related to PPV T with 93.5% similarity and is the most distant to the strains PPV-W and PPV-C, with identities of 78.8% and 78.4% respectively (Palmisano et al. 2012). The other PPV strains were clustered among themselves as expected. The phylogenetic tree supported the results obtained in other studies (James et al. 2013).

In summary, a total of 306 stone fruit samples representing eight villages (neighborhoods) of 2 districts of Bolu province were examined for the presence of PPV. The results of molecular and serological analysis revealed that three peach trees were infected with PPV-M.

PPV-M strain was previously reported in Aydın, Çanakkale, Denizli, İstanbul and Isparta (Gürcan and Ceylan 2016, Gürcan et al. 2019), Adana (Koç and Baloğlu 2006), Çanakkale, Mersin, Hatay (Ulubaş-Serçe et al. 2011), Kayseri (Ceylan et al. 2014), and Antalya (Çelik and Kütük 2013). In all these studies, the detection of PPV in newly established apricot or peach orchards (usually 4-7 years old) was reported (Gürcan and Ceylan 2016). In this study, PPV-M strain on peach was also detected in Bolu.

However, more detailed surveys in Bolu province are necessary to prevent PPV dissemination. In addition, the complete genome sequence of the isolates obtained from those surveys should be carried out to find an answer for the strain level of PPV isolates. PPV is considered as EPPO A2 quarantine pest in many countries as well as in Turkey and has a strict eradication program regulated by the Republic of Turkey Ministry of Agriculture and Forestry. If the infection is detected early, then disease eradication may be reached by destroying infected-trees rapidly.

In Bolu, infected trees determined through this study were destroyed quickly. Within the scope of International Standards for Phytosanitary Measures (ISPMs) adopted

Table 2. The similarity rates of the novel isolates identified in this study to the isolates deposited into GenBank

Isolate	Strain	Country	GenBank access. No	Similarity (%)		
				P157	P179	P214
EdMrPc292	M	Turkey	MG686904	100.0	100.0	99.59
EdMrPc287	M	Turkey	MG686901	100.0	100.0	99.59
EdMrPI286	M	Turkey	MG686900	100.0	100.0	99.59
MK175	M	Macedonia	MK562732	99.59	99.59	99.17
MK41	M	Macedonia	MK562730	99.59	99.59	99.17
BrPc110	M	Turkey	KX423957	99.59	99.59	99.17
BrPc70	M	Turkey	KX423940	99.59	99.59	99.17
BrPc56	M	Turkey	KX423934	99.59	99.59	99.17
BrPc54	M	Turkey	KX423932	99.59	99.59	99.17
BrPc53	M	Turkey	KX423931	99.59	99.59	99.17

by International Plant Protection Convention (IPPC), Republic of Turkey Ministry of Agriculture and Forestry General Directorate of Food and Control have implemented regional eradication programs and buffer zones of at least 1000 meters in diameter and established to prevent the further spread of the disease. As a result of this study, PPV quarantine regulations were established to regulate any movement of *Prunus* spp. into and out of the area. Planting of pome fruits and field crops (non-Prunus species) was allowed within the buffer zone. Replanting *Prunus* species was also banned until the achievement of a 3-year virus-free period within the affected area.

ÖZET

Plum pox virus (PPV), sert çekirdekli meyvelerde verim, kalite ve ekonomik kayıplara sebep olan Şarka hastalığının etmenidir. PPV, Avrupa başta olmak üzere dünya çapında tespit edilmiştir. Türkiye'de bugüne kadar yapılan çalışmalarda etmenin varlığı farklı bölgelerde sınırlı olarak tespit edilmiştir. Bolu ilinde şu ana kadar PPV'nin tespitine ilişkin herhangi bir kayıt bulunmamaktadır. Bolu ilinde 2016-2019 yılları arasında yapılan sürveyler neticesinde 306 örnek toplanmıştır. PPV'nin varlığını tespit etmek amacıyla ilk olarak DAS-ELISA ile örnekler analiz edilmiş, sadece üç adet şeftali örneğinin PPV ile enfekteli olduğu belirlenmiştir. Enfekteli örnekler daha sonra üniversal primerler (P1/P2) kullanılarak RT-PCR ile test edilmiş ve DAS-ELISA sonuçları doğrulanmıştır. Ayrıca PPV'nin ırka spesifik primerleri ile ırk düzeyinde de teşhisleri yapılmıştır. Irk düzeyinde yapılan bu teşhislerde örneklerin PPV-M (Marcus) ırkı ile enfekteli olduğu belirlenmiştir. Moleküler çalışmalar sonucunda üç izolatın kısmi kılıf protein bölgesine ait 243 nükleotidlik dizileri elde edilerek NCBI'a kaydedilmiştir. Ayrıca 38 temsili PPV sekans dizisi ile yapılan filogenetik analiz (Neighbour-Joining) sonucunda, BLAST analizinde olduğu gibi Bolu izolatlarının PPV-M izolatları ile kümelenerek diğer PPV ırklarından ayrıldığı görülmüştür. Yapılan bu çalışma ile elde edilen PPV izolatları Bolu ili için ilk kayıt niteliğindedir. Sonuç olarak, Bolu ilinde daha kapsamlı sürveylerin yapılması ve elde edilen izolatlarının tam genom dizileri elde edilerek genetik varyasyonlarının belirlenmesinin gerekliliği ortaya çıkmıştır. Enfekteli bulunan ağaçların ise tamamı imha edilmiştir.

Anahtar kelimeler: PPV, Şarka, şeftali, M ırkı, sekans, filogenetik ağaç

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