Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2022, Vol. 6 (2), 90-102
https://doi.org/10.29329/ijiaar.2022.451.3

Original article

Detection and the Prevalence of Prune Dwarf Virus (PDV) in Important Cherry Plantations of the Eastern Mediterranean Region by DAS-ELISA and RT-PCR Analyzes

Pelin Gündoğan [a, *], Mona Gazel [a] & Kadriye Çağlayan [a]

[a] Department of Plant Protection, Faculty of Agricultural, Hatay Mustafa Kemal University, Antakya-Hatay, Turkey

Abstract

In this study, the prevalence of PDV, which infects cherry (*Prunus avium* L.) trees and is among the important viral pathogens in Adana, Kahramanmaraş and Osmaniye provinces where cherry is extensively produced in the Eastern Mediterranean region of Turkey, was investigated. A total of 173 cherry leaf samples with and without symptoms collected from Adana, Kahramanmaraş and Osmaniye provinces were tested by DAS-ELISA and RT-PCR methods. PDV was not detected in all samples tested by the DAS-ELISA method. As a result of the tests performed with RT-PCR, 874 bp long nucleotide sequences belonging to the coat protein gene region of the 15 samples were obtained and found positive. After the sequences were obtained, they were compared with the sequences from Turkey and the sequences of PDV reference isolates registered in GenBank. As a result of BLASTn analysis, it was determined that the Turkish PDV cherry isolates showed similarity between 89.58-99.32% at the nucleotide level with the PDV reference sequence. Phylogenetic analysis of the nucleotide sequences of the obtained isolates was performed and their relationship with PDV cherry isolates in the world and Turkey was determined.

Keywords: *Prunus avium* L., cherry, DAS-ELISA, RT-PCR, PDV, Sequence analysis.

Received: 03 June 2022 * **Accepted:** 20 June 2022 * **DOI:** https://doi.org/10.29329/ijiaar.2022.451.3

* **Corresponding author:**

Pelin Gündoğan, Department of Plant Protection, Faculty of Agricultural, Hatay Mustafa Kemal University, Antakya-Hatay, Turkey.
Email: gundoganpelin@yahoo.com

INTRODUCTION

Turkey is a country with a very high cherry growing potential in the world. Cherry production in the world shows a regular increase over the years. Turkey; while it ranks first in the world in cherry production, it is a very important fruit in terms of the country's economy and nutrition of its people. Turkey has a very high potential in cherry production, and factors such as the long harvest period due to the different soil and ecological characteristics of the regions, and the suitability for organic cherry production, especially in the highlands where diseases and pests are low affects cherry production (Kara et al., 2012).

Cherry is one of the most important export products in Turkey which renders 26% of the world's cherry production with a production of 689,834 tons with the number of fruit-bearing trees of 22,154,903 according to 2021 TUIK (Turkish Statistical Institute) data (Anonymous, 2022).

In this fruit species, which contributes greatly to the country's income economically, virus diseases which cannot be prevented cause great problems (Dunez, 1988). There are many viral diseases negatively affecting cherry production in terms of quality and quantity. Among them, especially Prunus necrotic ringspot virus (PNRSV), Apple chlorotic leaf spot virus (ACLSV), Apple mosaic virus (ApMV), Prune dwarfing virus (PDV) and Plum pox virus (PPV) are the most important ones (Nemeth, 1986; Dunez, 1988).

The host range of PDV is very extensive and is common in all cultivated Prunus species (peach, cherry, plum, almond and cherry). It has been reported that the virus causes symptoms in the form of chlorotic rings and spots on the leaves of cherry trees in spring (Smith et al., 1998). It has also been reported that 70-80% of Prunus species are infected with PDV (Kunze, 1988). PDV detected in cherry trees has a devastating effect by causing 50-70% product loss (Fulton, 1985; Çağlayan et al., 2011). It has been determined that PNRSV and PDV in cherries generally cause co-infection (Nyland et al., 1976; Brunt et al., 1996).

In the studies carried out to detect the presence of PDV in stone fruit trees in Mediterranean countries, the infection rate was reported to be 67.2-80% (Myrta and Savino, 2008), and 37.6% in Serbia (Mandic et al., 2007). It was found that 16% of 751 cherry samples tested in Bulgaria between 2000 and 2012 were infected with PDV (Borisova et al., 2013). Vašková et al. (2000) reported that the coat protein gene region of 11 PDV isolates, which were isolated from many stone fruit species, is a highly conserved region, and as a result of phylogenetic studies, no specialization was determined between geographic origin, host and isolates. In the analyzes performed with 32 PDV isolates, it was determined that the coat protein region was highly similar (86-96%) with the reference isolates, and 4 different groups were observed in the phylogenetic tree created (Boulila, 2010). In the phylogenetic tree obtained by sequencing nine apricot and one cherry isolates of PDV in our country, the isolates were collected in 4

different groups (Ulubaş Serçe et al., 2009). As a result of the phylogenetic analysis of the PDV cherry isolates obtained from the Lakes region of Turkey, the existence of subgroups under 3 main groups was mentioned (Öztürk and Çevik, 2015).

Studies using the ELISA method have shown that the most common virus in cherries from stone fruits is PDV, and the infection rate varies between 5-55% (Azeri, 1994; Çağlayan Yıldızgördü and Hurigil, 1996; Fidan and Özdemir, 2000; Paylan, 2006).

In Turkey, it has been determined that 22.2% of stone fruit trees grown in the Eastern Mediterranean Region are infected with ApMV, 17.1% with PNRSV, 15.5% with PDV and 13.3% with ACLSV (Çağlayan and Gazel 1998). Between 2000 and 2003, Çağlayan et al. (2008) studied a total of 240 cherry and 73 cherry specimens exhibiting virus-like symptoms and samples were collected from both commercial orchards and cultivar collections. As a result of DAS-ELISA and RT-PCR tests, it was determined that PDV was very common (10.8%) in cherry trees, while PDV could not be detected in sour cherry (*Prunus cerasus*) trees.

A total of 488 samples from stone fruit trees (cherry, cherry, plum, nectarine, peach, apricot) were taken from 13 provinces (Adana, Afyon, Ankara, Amasya, Burdur, Bursa, Çanakkale, Hatay, Isparta, İzmir, Kahramanmaraş, Tokat and Yalova) and were tested by DAS-ELISA method. Upon testing, PNRSV was detected in 31 samples and PDV in 71 samples. While PDV infection was mostly detected in cherry with an infection rate of 27.4% (60 positive), it was detected in sour cherry with 22.2% (6 positive) (Ulubaş, 2003).

As a result of the ELISA test applied to 369 samples collected from stone fruit trees in Van Province and its vicinity, it was determined that PDV infected 0.6% in apricot, 7.1% in cherry and 31.2% in sour cherry (Sipahioğlu et al., 2004).

As a result of testing 521 cherry samples collected from 142 orchards in Isparta province in 2010 by DAS-ELISA method, it was determined that 316 of them were contaminated with PDV. From these samples, RNA was isolated from 27 samples representing the production regions, and 862 bp DNA fragment, including the coat protein gene region of PDV, was amplified by reverse-transcription polymerase chain reaction (RT-PCR) method and the amplified DNA was directly sequenced. The obtained PDV coat protein (CP) gene sequences were compared with each other and with the CP genes of the PDV isolates from the gene bank database and the other production regions of the world, and the similarity rates and phylogenetic relationships of the isolates were revealed (Öztürk et al., 2011).

A total of 400 samples collected as a result of a survey study conducted in Bilecik and Bursa provinces where Early Burlat and 0900 Ziraat cherry varieties were grown in 2010 and 2011 were tested against Prune dwarf virus (PDV) by DAS-ELISA method. Infection was detected at a rate of 45% in 0900 Ziraat cherry cultivar and 52% in Early Burlat cultivar from Bilecik province Gölpazarı district.

Infection rate was 36% in 0900 Ziraat cherry variety and 43% in Early Burlat cherry variety taken from Keles district of Bursa province (Uzunoğulları and Akçay, 2012).

In this study, the presence of PDV in Adana, Kahramanmaraş and Osmaniye provinces was investigated by DAS-ELISA and RT-PCR methods and molecular characterization of the detected virus isolates was performed.

MATERIALS AND METHOD

Field studies and collection of plant material

Within the scope of the study, survey studies were carried out in the cherry orchards of Andırın and Dulkadiroğlu districts of Kahramanmaraş province, Pozantı districts of Adana province and Bahçe disctricts of Osmaniye province between 2019-2021. A total of 173 plant samples were collected from the cherry trees showing the virus symptoms as well as the symptomless cherry trees. The samples were placed in polyethylene bags and brought to the laboratory in the cold chain and stored at +4°C until used in PCR tests.

DAS-ELISA tests

Collected cherry samples were subjected to DAS-ELISA (Double Antibody Sandwich- Enzyme Linked Immuno Sorbent Assay) test according to Clark and Adams (1977). DAS-ELISA tests were performed with the diagnostic kit (BIOREBA AG, Switzerland) according to the company's instructions. Plates were evaluated spectrophotometrically at a wavelength of 405 nm using the SEAC SIRIO S Microplate Reader. Samples with twice or more absorbance values as a result of readings were considered positive, that is, virus-infected (Clark, 1981; Gazel et al., 2018).

Total Nucleic acid isolation

Total nucleic acid (TNA) isolation from cherry samples collected in the survey studies was carried out using the Morante-Carriel (2014) method. The quality of the isolated RNAs was measured with a Nanodrop device (Nanodrop 1000c, Thermo Sci., USA) and kept at -80°C until cDNA synthesis and PCR applifications.

Complementary DNA (cDNA) Synthesis

Complementary DNA (cDNA) synthesis was carried out from the TNA extracted samples. For each sample, 1 µl of random hexamer primer (Thermo Fisher Scientific), 6.5 µl of d_2H_2O and 5 µl of RNA mixture was used. After 5 minutes of incubation at 94^{o}C in the thermocycler, the tubes were placed on ice for 5 minutes. Then, 4 µl of 5X reaction buffer (Thermo Fisher Scientific), 2 µl of d_2H_2O, 0.5 µl of dNTP (10 mM) and 1 µl of M-MuLV reverse transcriptase (Thermo Fisher Scientific) were added to each tube to reach a total volume of 20 µl. The cDNA synthesis step was completed by keeping the tubes in the PCR device at 42°C for 1 hour and at 72°C for 10 minutes.

Polymerase Chain Reaction (PCR)

In PCR analysis, the primer pairs, amplifying 874 bp long coat protein gene region of PDV, was used (Vašková et al., 2000). In PCR reaction, each tube contained a final volume of 23 µl reaction mixture; 16.8 µl d_2H_2O, 2 µl cDNA, 0.5 µl virus-specific primers (at 10 pmol/µl concentaration of each primer (PDV/CP-F 5'-GTGTAGAAAGAAGAGAAGTCCGACAAG-3' and PDV/CP-R, 5'-ATCTAGAGCAGCATTTCCAACTACGA-3' (Vaskova et al., 2000), 0.5 µl of 10 mM dNTP, 2 µl of 25 mM $MgCl_2$, 2.5 µl of 10XB reaction buffer, 0.2 µl of *Taq* DNA polymerase (Thermo Scientific). The PCR cycling program was: 94°C for 2 min for initial denaturation, then 40 cycles of 94°C for 30 sec, 50°C for 30 sec, 72°C for 1.5 min and aditional cycle at 72°C for 10 min. After electrophoresis of PCR products on 1.2% agarose gel, DNA was stained with ethidium bromide and the results were observed in a UV transilluminator.

DNA Sequencing and Phylogenetic Analysis

The PCR amplicons of the expected size (874 bp) were randomly selected and sequenced. The raw sequence data obtained from each isolate were edited and processed with the MEGA 11 program, and consensus blocks were formed with the nucleotide sequences of PDV isolates from different countries of the world. The isolates obtained within the scope of the study were compared with other PDV isolates at the nucleotide level. Phylogenetic trees were created with the MEGA 11 program, using the 1000-repetition boostrap analysis method according to the Tamura 3-parameter algorithm (Tamura et al., 2021).

The nucleotide-level similarity ratios of the CP gene regions of PDV isolates obtained from Turkey and from different countries of the world were calculated using the Sequence Demarcation Tool (SDT) v.1.2 program, and their colored matrices were created (Muhire et al., 2014).

RESULTS and DISCUSSION

Field Surveys

A total of 173 cherry samples were collected, 62 from Andırın and Dulkadiroğlu districts of Kahramanmaraş, 67 from Bahçe district of Osmaniye province and 44 from Pozantı district of Adana province. The most common symptoms observed in survey areas are stunting, bare ends of branches, curling of leaves, vein banding and mosaic symptoms (Figures 1 and 2).

Figure 1. Symptoms of stunting and bare ends of branches observed in cherry trees in Kahramanmaraş province

Figure 2. Curling, vein banding and mosaic symptoms observed on the cherry leaves in Kahramanmaraş province

Results of DAS-ELISA tests

The all samples collected from Adana, Kahramanmaraş and Osmaniye provinces were tested for PDV by DAS-ELISA method, and PDV was not detected in any of the tested cherry samples. In the DAS-ELISA test, low absorbance values were obtained in the negative controls, while the absorbance value was found to be quite high in the positive control.

RT-PCR and Sequence Analysis

In the RT-PCR analyzes performed to detect the presence of PDV in samples collected from cherry trees in Adana, Kahramanmaraş and Osmaniye provinces, primers that amplify a part of the CP

region of PDV were used. As a result of the analysis of 173 cherry samples collected as a result of this study by RT-PCR method, 15 of them were found to be infected with PDV (Figure 3). The infection rate of PDV in the tested cherry samples was determined as 8.67%. While 7 of 62 cherry samples collected from Kahramanmaraş province were found to be PDV positive, 5 of 67 cherry samples collected from Osmaniye and 3 of 44 samples collected from Adana were found to be PDV positive. PDV infection rates in cherry trees in Kahramanmaraş, Osmaniye and Adana provinces were determined as 11.29, 7.46 and 6.81%, respectively. In previous studies conducted in our country, PDV was detected in cherry trees grown in Afyon, Adana, Ankara, Amasya, Burdur, Kahramanmaraş, Isparta and İzmir provinces (Ulubaş Ç., 2003, Paylan, I.C., 2006, Ulubaş et al., 2009a, Ulubaş et al., 2009b, Öztürk et al., 2011, Öztürk and Çevik, 2015). In this study, PDV was detected for the first time in cherry trees in Osmaniye province.

Biological, serological and molecular methods are generally used for the detection of viruses in stone fruit trees. Although ELISA is a reliable test as a routine diagnostic method, it has been reported that its sensitivity is low and pathogens with very low titers cannot be detected. RT-PCR is increasingly used for the detection and identification of viruses due to its higher level of sensitivity (Herranz et al., 2005). It has been demonstrated that the RT-PCR method is 100-1000 times more sensitive than ELISA (Sánchez-Navarro et al., 1998).

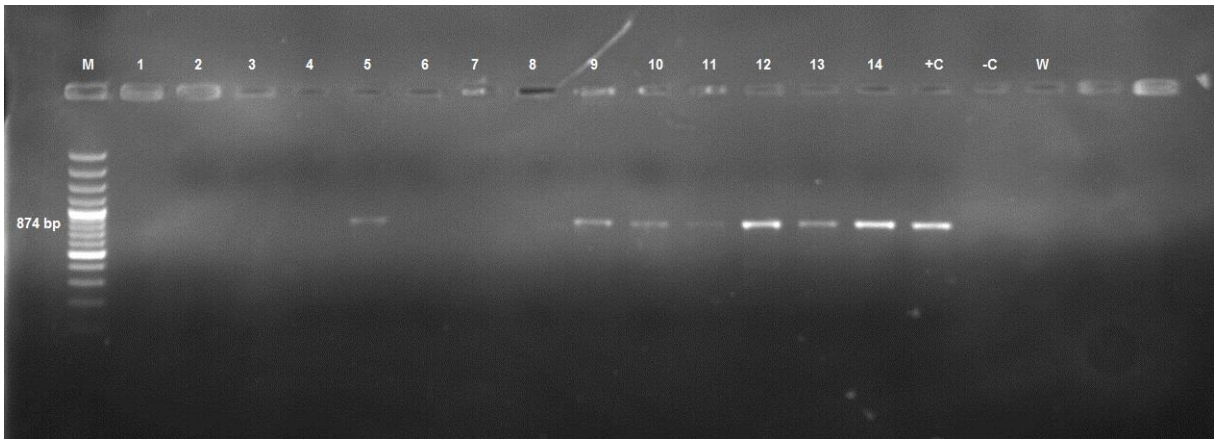

Figure 3. Agarose gel electrophoresis results of RT-PCR analysis using prune dwarf virus (PDV) specific primer pair in cherry samples collected from Kahramanmaraş and Osmaniye provinces. M: Marker (SMO#321 MBI Thermo Sci, USA); 1-10: Osmaniye cherry samples, 11-14: Kahramanmaraş cherry samples, +C: Positive control, -C: Negative control, W: Water control

Phylogenetic Analysis

Within the scope of this study, 6 of 15 samples were sequenced, which gave the same level of band (874 bp) as the positive control in PCR analyzes using primers which amplify part of the CP region of PDV. The nucleotide sequences of the PDV cherry isolates were uploaded to the NCBI GenBank and

the GenBank accession number was obtained for each one (ON568342-ON568347). The studied PDV cherry isolates showed 99.32-89.58% similarity at nucleotide level with PDV cherry isolates from different countries registered in Genbank. It was determined that PDV cherry isolates showed 97.61% similarity with KF718663 isolate from PDV cherry isolates reported from Turkey in previous studies (Figure 4). In the phylogenetic analysis, 6 cherry isolates obtained from previous studies in Turkey and 17 cherry isolates from different countries were used. It was determined that the nucleotide sequence of AK-17 Adana isolate was 99.32% similar to the AF208740 Czech Republic cherry isolate registered in GenBank. As a result of the phylogenetic analyzes, the PDV isolates were divided into 2 groups, and only one isolate (ON568347) was found in one of these groups. Our isolate, numbered ON568347, was obtained from Kahramanmaraş province. The nucleotide sequence of this isolate is different from our other isolates and it shows 98.13-89.58% similarity with GenBank isolates. The other 5 of our PDV cherry isolates were in the second group (Figure 4).

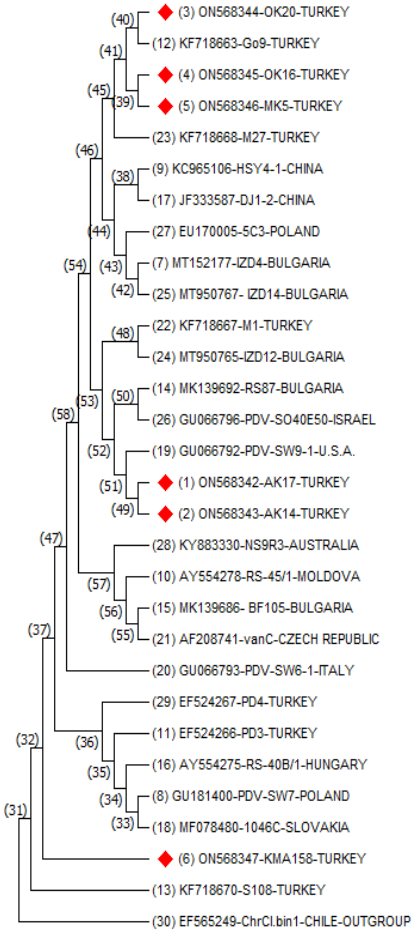

Figure 4. Phylogenetic tree created as a result of sequence analysis of PDV cherry isolates obtained from Eastern Mediterranean and from different geographical origins of the world. (In drawing the phylogenetic tree, the Tamura 3-parameter model and the 1000 boostrap parameter were selected, the sequence data obtained from the coat protein gene regions of Turkey and the world PDV isolates and the NJ algorithm of the MEGA 11 program were used).

The similarity rates of the nucleotide sequences obtained by sequencing the coat protein region of PDV cherry isolates show a high degree of similarity with the studies in the literature. Vaskova et al. (2000) reported that the coat protein region of the virus is a highly conserved and the similarity rate of isolates was 88% in studies. The similarity rate between nucleotide sequences obtained by sequencing the coat protein regions of PDV isolates in Bulgaria was found between 87-100% (Kamenova et al., 2019). PDV isolates obtained from cherry in Isparta province of Turkey and GenBank isolates were found to be 84-99% similar at the nucleotide level, and it was reported that the coat protein region of the virus did not show much polymorphism at the nucleotide level (Öztürk and Çevik, 2015). It has been found that 3 main groups and subgroups are formed in the phylogenetic tree formed with Turkey cherry PDV isolates. The majority of PDV Turkey cherry isolates clustered in the same main groups, and only 1 isolate was in a different group from the others (Öztürk and Çevik, 2015). The fact that Turkish isolates were included in the same main group in this study showed that this study highly collaborated with the study of Öztürk and Çevik (2015). The phylogenetic tree generated with the Turkish PDV cherry isolates in GenBank and the cherry isolates obtained within the scope of this study showed that Turkey PDV isolates were divided into 2 main groups.

The SDT program was used for the colored similarity matrix created at the nucleotide level with PDV isolates from Turkey and the world. The isolates sequenced on the colored similarity matrix created using the SDT program are shown in Figure 5. When the matrix was examined, the similarity rate between the world isolates sequenced and selected in this study varied between 89-100%. In this study, a high percentage of the isolates obtained with partial nucleotide sequences in the Czech Republic, Israel, China, and the USA was found to be similar to the isolates. These data seem to coincide with the distribution formed in the phylogenetic tree.

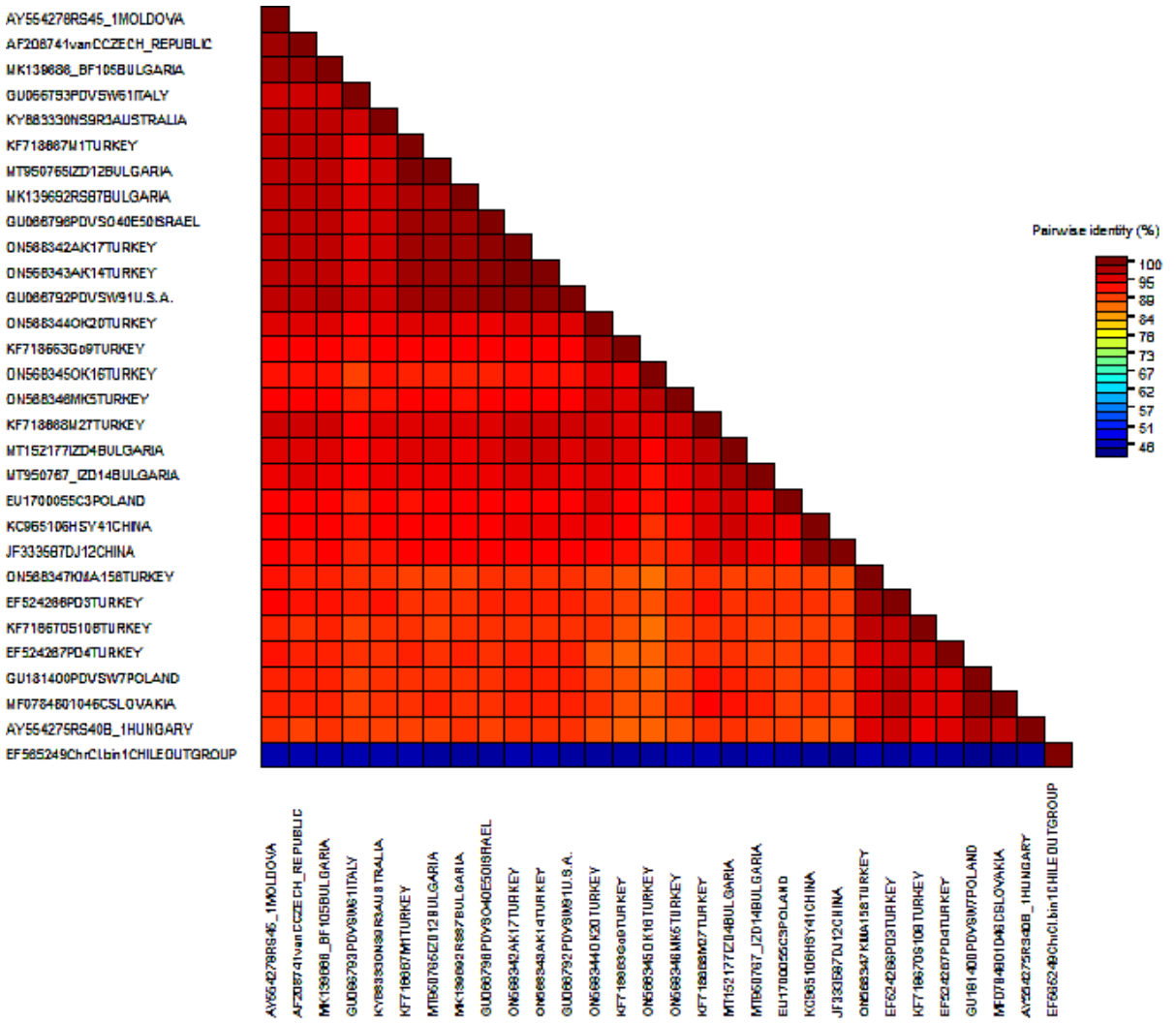

Figure 5. Colored similarity matrix created at nucleotide level of PDV Turkey and world isolates.

CONCLUSION

The presence of PDV in cherry trees grown in Adana, Kahramanmaraş and Osmaniye provinces in the Eastern Mediterranean Region was determined by DAS-ELISA and RT-PCR analyzes. It was determined that the tested cherry trees were infected with PDV at a rate of 8.671%. Sequences of 6 isolates were obtained and similarity ratios with reference PDV isolates were determined. Within the scope of the study, cherry PDV isolates whose nucleotide sequences were determined and isolates from different countries registered in Genbank were used in the phylogenetic analysis. The nucleotide sequences of the coat protein regions of cherry PDV isolates in Turkey were obtained. It is recommended to determine the whole genome sequences of the cherry PDV isolates obtained from the study. Identification of the whole genome sequence will contribute to the identification of different PDV variants in different hosts.

Acknowledgements

This study was financially supported by Hatay Mustafa Kemal University Scientific Research Projects Commission within the scope of doctoral thesis (Project Number: MKU BAP-21D004).

REFERENCES

Anonymous (2022). Turkish Statistical Institute (TUIK). Crop Production Data. (www.tuik.gov.tr) (Access date: 24.03.2022)

Azeri, T. (1994). Detection of virus diseases of stone fruits in Aegean region of Turkey. Proceedings of 9th Congress of Mediterranean Phytopathological Union. p 511-513. Kuşadası, Aydın.

Borisova, A., Kamenova, I., Borovinova, M. (2013). Viral and fungal diseases of stone fruit species in Kyustendil region. Journal of Mountain Agriculture on the Balkans, vol. 16, 1, 164-182.

Boulila, M. (2010). Molecular characterization of an almond isolate of Prune dwarf virus in Tunisia: putative recombination breakpoints in the partial sequences of the coat proteinencoding gene in isolates from different geographic origin. Phytopathol. Mediterr. 48:411-421.

Brunt, H.A., Crabtree, K., Dallawitz, M.J., Gibbs, A.J., Watson, L. (1996). Viruses of plants. CAB Internation, Wallingford.

Clark, M.F., Adams, A.N. (1977). Characteristics of the microplate method of Enzyme Linked Immunosorbent Assay for the detection of plant viruses. Journal of General Virology, 34, 475-48.

Clark, M.F. (1981). Immunosorbent assay plant pathology. Annu. Rev. Phytopathol. 19: 83-106.

Gazel, M., Çağlayan, K. (1998). Diagnosis of virus diseases in Prunus species of Hatay region by ELISA and biological methods. Turkey 8th Phytopathology Congress. Ankara University, Faculty of Agriculture, Department of Plant Protection, Ankara, 322-325. *(In Turkish)*

Çağlayan Yıldızgördü, K., Hurigil, M. (1996). Virus Diseases of Peach Trees in Hatay Province. The Journal of Turkish Phytopathology, 25 (1-2), 65-69.

Çağlayan, K., Gazel, M., Ulubaş, Ç. (2008). Phytosanitary Status Of Cherry Trees In The East Mediterranean Region Of Turkey. V. International Cherry Symposium. ActaHorticulture,795,919-924.

Çağlayan, K., Ulubaş, Ç., Gazel, M., Kaya, K., Cengiz, F.C. (2011). Preliminary Findings on the Epidemiology of Plum Pox in Nutmeg Orchards. Turkey 4th Plant Protection Congress Proceedings, KSU, FP 106, 496s, Kahramanmaraş. *(In Turkish)*

Dunez, J. (1988). Situation of Virus and Virus Like Diseases of Stone Fruits in the Mediterranean and Near East Region, 227-276.

Fidan, Ü., Özdemir, S. (2000). Determination of viruses in cherry trees by DAS-ELISA. Plant Protection Research Institute, Bornova/İzmir. Final report Project Code No: BS/97/06/09/113. *(In Turkish)*

Fulton, R. W. (1985). Prune dwarf virus. In: ICTVdB- The Universal Virus Database, version 3. ICTVdB Management, Columbia University, New York.

Gazel, M., Tunç, B., Çağlayan, K. (2018). Detection and characterization of viruses that cause wood tissue deformation (Rugose Wood) in the vineyard areas of Hatay and Tekirdağ provinces by serological and

molecular methods. Journal of Mustafa Kemal University Faculty of Agriculture 23: 181-187. *(In Turkish)*

Herranz, M. C., Sanchez-Navarro, J. A., Aparicio, F. and Pallas, V. (2005). Simultaneous detection of six stone fruit viruses by non-isotopic molecular hybridization using a unique riboprobe or 'polyprobe'. J. Virol. Methods 124:49-55.

Kamenova, I., Borisova, A., & Popov, A. (2019). Incidence and genetic diversity of Prune dwarf virus in sweet and sour cherry in Bulgaria. Biotechnology & Biotechnological Equipment, 33(1), 980-987.
Kara, Z., Dağ, B., Yorgancılar, M. (2012). Determination of the Potentials of Agricultural Products Produced in Doğanhisar District, Especially Lupine. Mevlana Development Agency, Konya. *(In Turkish)*

Kunze, L. (1988). European Handbook of Plant Diseases, Blackwell Scientific Publications, Oxford. 15-17 pp.

Mandic, B., Matic, S., Rwahnih, M. A. L., Jelkman, W., & Myrta, A. (2007). Viruses of sweet and sour cherry in Serbia. Journal of Plant Pathology, 89, 103-108.

Morante-Carriel J., Selles-Marchart S., Martínez-Márquez A., JoséMartínez-Esteso M., Luque I., Bru-Martínez R. (2014). RNA isolation from loquat and other recalcitrant woody plants with high quality and yield. Analytical Biochemistry, Volume 452, Pages 46-53.

Muhire, B. M., Varsani, A. ve Martin, D. P. (2014). SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS ONE, 9(9), e108277.

Myrta, A., Savino, V. (2008). Virus And Virus-Lıke Diseases Of Cherry In The Mediterranean Region. Acta Horticulturae V. International Cherry Symposium, 795, 891-896.

Nemeth, M. (1986). Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees. Martinus Nijhoff Publishers, Dordrecht, The Netherlands, 47 p.

Nyland, G., Gilmer, R.M., Moore, J.D. (1976). Prunus Ringspot Group, In: Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. U.S. Dep. Agric. Handb, pp 104-132 .

Öztürk Y., Çevik B. (2015). Genetic Diversity in the Coat Protein Genes of *Prune dwarf virus* Isolates from Sweet Cherry Growing in Turkey. Plant Pathol J. 2015 Mar; 31(1): 41–49.

Öztürk, Y., Çevik, B., Kaymak, S. (2011). Detection of Plum dwarf virus (Prune dwarf virus, PDV) by Serological and Molecular Methods and Determination of Coat Protein Gene Sequences in Cherry Production Areas in Isparta Province. Proceedings of the 4th Plant Protection Congress of Turkey, 28-30June 2011, Kahramanmaraş, p.399. *(In Turkish)*

Paylan, I. C. (2006). Research on the Determination of Virus Diseases in Cherry Production Areas in Izmir Province. Ege University Institute of Science and Technology, Master Thesis, 73s, İzmir. *(In Turkish)*

Sánchez-Navarro, J. A., Aparicio, F., Rowhani, A., Pallás, V. (1998). Comparative analysis of ELISA, nonradioactive molecular hybridization and PCR for the detection of prunus necrotic ringspot virus in herbaceous and Prunus hosts. Plant Pathology, 47(6), 780-786.

Sipahioğlu, H.M., Demir, S., Polat, B., Akköprü, A., Usta, M. (2004). Viral and Fungal Disease Factors Detected in Fruit Trees Cultivated in Van and its Surroundings. Yuzuncu Yıl University Journal of Agricultural Sciences, 14(2),133-139. *(In Turkish)*

Smith, I. M., Dunez J., Phillips, D. H., Lelliot, R. A., Archer, S. A. (1998). European Handbook of Plant Diseases. Blackwell Scientific Pupl., 583 p.

Tamura, K., Stecher, G., Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, Volume 38, Issue 7, July 2021, Pages 3022–3027.

Ulubaş Serçe, Ç., Candresse, T., Svanella-Dumas, L., Krizbai, L., Gazel, M., Çağlayan, K. (2009b). Further characterization of a new recombinant group of Plum pox virus isolates, PPV-T, found in orchards in the Ankara province of Turkey. Virus Research, 142(1-2), 121-126.

Ulubaş Serçe, Ç., Ertunç, F., Oztürk, A. (2009a). Identification and genomic variability of Prune dwarf virus variants infecting stone fruit trees in Turkey. Journal of phytopathology, 157(5), 298-305.

Ulubaş, Ç. (2003). Diagnosis and characterization of Ilarviruses infecting stone fruit trees by RT-PCR. Ankara University Institute of Science and Technology, PhD thesis, 95s, Ankara. *(In Turkish)*

Uzunoğulları, N., Akçay, M. (2012). Investigation of Prune dwarf virus (Prune dwarf ilarvirus, PDV) that causes infection in cherry orchards in Bilecik and Bursa provinces. Plant Protection Bulletin, 52 (4), 367-374. *(In Turkish)*

Vaskova, D., Petrzik, K., Karesova, R. (2000). Variability and molecular typing of the woody-tree infecting PNRSV. Archives Virology, 145, 699-709.