# PERSIMMON (*DIOSPYROS KAKI* L.) AND JOHNSONGRASS [*SORGHUM HALEPENSE* (L.) PERS.] ARE NEW NATURAL HOSTS OF *PEACH LATENT MOSAIC VIROID*

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**Abstract.** *Peach latent mosaic viroid* (PLMVd) naturally infects stone fruits worldwide. Here, we report the first detection of PLMVd in persimmon (*Diospyros kaki* L.) and a weed Johnsongrass [*Sorghum halepense* (L.) Pers.]. Samples corresponding to 12 persimmon specimens and weeds nearby the persimmon trees were collected from a germplasm collection plot in Malatya (Turkey). Total RNAs were isolated using a silica-based method and the complete viroid genome was amplified by reverse transcriptase polymerase chain reaction (RT-PCR). From these samples, PLMVd was detected in 7 of the 12 persimmons and in Johnsongrass revealing 8 new sequence variants. Multiple alignment and phylogenetic analyses revealed that identified persimmon and Johnsongrass isolates clustered only with PLMVd-walnut isolates previously identified from same locality. The nucleotide sequences of PLMVd persimmon and Johnsongrass isolates showed 96.71-99.11% similarity with the PLMVd isolates detected in different fruit crops in the world. A single specific mutation identified in two PLMVd persimmon variants (-TH2 and -TH10) effectively changed the predicted secondary structure of the agent. The identification and the genetic analyses of PLMVd variants in persimmon and Johnsongrass confirm that the agent is a ubiquitous and genetically variable viroid that infects many cultivated fruit crops and weeds worldwide.
**Keywords:** *PLMVd, RT-PCR, Turkey, weed, persimmon, identification*

## Introduction

Viroids are small, circular RNAs replicating autonomously in many cultivated and wild plant species. They are noncoding plant pathogenic RNA molecules of 246 to 401 nucleotides in length, however, they induce visible symptoms in susceptible host plants that resemble those associated with many plant viruses. The disease symptoms greatly depend on the host they infect and the viroid strain (Adkar-Purushothama and Perreault, 2019).

PLMVd was first detected in peaches in France. For many years, PLMVd was believed to be restricted to peach (*P. persica*) and peach hybrids (Desvignes, 1976, 1999). Later on, the pathogen has been reported to infect members of the *Rosaceae* family including Japanese plum (*Prunus salicina Lindl.*), apricot (*Prunus armeniaca* L.), sweet cherry (*Prunus avium* L.), European *plum* (*Prunus domestica* L.) and cultivated and wild pears


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(*Pyrus communis* L. and *P. amygdaliformis*, respetively) (Shamloul et al., 1995; Faggioli et al., 1997; Hadidi et al., 1997; Giunchedi et al., 1998, 2011; Osaki et al., 1999; Kyriakopoulou et al., 2001). The agent was reported in many stone fruit growing countries including US, Italy, Spain, Austria, Turkey, Greece, Yugoslavia, Romania, Morocco, Algeria, China, Japan, Chile, and in Australia (Desvignes, 1986; Flores and Llácer, 1988; Flores et al., 1990, 1992; Albanese et al., 1992; Shamloul et al., 1995; Di Serio and Ragozzino, 1995; Skrzeczkowski et al., 1996; Di Serio et al., 1999; Pelchat et al., 2000; Fiore et al., 2003; Torres et al., 2004; Sipahioglu et al., 2006; Gumus et al., 2007; Gazel et al., 2008). Although it remains latent for many years following the first infection, PLMVd causes mosaic, bud necrosis, vain banding, blotch and death of branches in peach trees (Desvignes, 1999).

There are few studies dealing with the natural and experimental host ranges for PLMVd (Flores et al., 2000). With the advancements in plant pathogen diagnosis methods, new viroids and their hosts in different geographic areas of the world, are constantly being reported (Adkar-Purushothama and Perreault, 2019). Recently in Turkey, for instance, isolates of PLMVd have been fully characterized infecting walnut trees (Tuncel et al., 2020). There is no report of any persimmon disease associated with a viroid so far. Here, we studied and characterized PLMVd isolates infecting persimmon and Johnsongrass plants. The full-length genome of PLMVd isolates were amplified and sequenced from seven field-collected persimmon and a weed nearby the infected trees expressing no symptoms. The sequence analyses revealed the presence of PLMVd in persimmon and Johnsongrass. To our knowledge, there have been no previous reports of PLMVd infecting these two hosts in the world.

## Materials and Methods

### *Collecting plant material and total RNA extraction*

In late summer of 2020, twelve asymptomatic leaf samples from persimmon trees and from the most common annual weeds around persimmon trees were collected from a fruit collection orchard in Malatya province (*Fig. 1*). The collected weeds were included: *Consolida regalis* Gray, *Sorghum halepense* (L.) Pers., *Carduus pycnocephalus* L., *Lactuca serriola* L., *Convovulus arvensis* L. and a weed from Apiaceae family. A sample from each weed species around the trees was collected and tested. No visual symptoms were observed either in persimmon or in weed samples except a persimmon tree which exhibited severe fruit deformation (*Fig. 2*). The all collected plant samples were shipped to laboratory in a cool chain for viroid testing. The leaf samples were rinsed in 70% ethanol for one minute and then rinsed twice with sterilized deionized water to eliminate the external microbial contaminants. Total RNA was extracted from fresh leaf tissues using a commercial genomic RNA purification kit (GeneJET Plant RNA Purification Kit). An isolate of PLMVd from a walnut tree identified positively from preliminary tests was used as positive control in diagnosis of the viroid. RNA from an asymptomatic persimmon tree was used as negative control.

### *Complementer DNA (cDNA) synthesis and amplification*

A total of 3 µl of purified RNA was added to cDNA reaction mixture containing 1 µl 20 pmol/ml genome specific reverse primer (PLMVd-R-5'-CCCGATAGAAAGGCTAAGCACCTCG-3') (Loreti et al., 1999), 1 µl of 10 mM


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dNTP, 8 µl of RNAase free water to a total volume of 12 µl. The mixture was heated for 5 min at 65 °C then chilled on ice. Then, four microliters of 5x first-strand cDNA buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, and 15 mM $MgCl_2$), 2 µl of DTT, and 1 µl of Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA) was mixed to reaction mixture and incubated at 42 °C for 50 min. For the inactivation of reverse transcriptase enzyme, the reaction mixture was incubated at 70 °C for 15 minutes and stored at -20 °C until use. PCR assay was performed in a 25 µl reaction volume in a sterile microfuge tube containing 5 µl of 5X Go*Taq* Green Buffer (Promega, Madison, WI, USA), 1 µl of dNTP (20 mM each), 1 µl of each forward and reverse primer (100 mM each) (PLMVd-F-5'-AAC TGC AGT GCT CCG AAT AGG GCA C-3' PLMVd-R-5'-CCC GAT AGA AAG GCT AAG CAC CTC G-3') (Loreti et al., 1999), 1.5 µl of $MgCl_2$, 3 µl of cDNA template and 12.3 µl nuclease-free water. PCR was performed in a Thermo Scientific Arktik Thermal Cycler (Waltham, MA, USA) and the cycling parameters were as follows: initial denaturation at 94 ºC for 2 min followed by 35 cycles of denaturation at 94 ºC for 1 min, annealing at 56 ºC for 2 min, extension at 72 ºC for 1 min, and final extension at 72 ºC for 10 min. Fifteen µl of RT-PCR product of each amplification was electrophoresed on 2% agarose gel in 1x TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) and stained with Pronasafe nucleic acid staining solution (CondaLab).


***Figure 1.*** *Map of Turkey showing the Malatya province where persimmon and the weed samples were surveyed for the presence of Peach latent mosaic viroid*

***Figure 2.*** *Severe fruit malformation in Peach latent mosaic viroid infected persimmon*


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### *Phylogenetic analyses and predicting the most stable secondary structure*

Reverse transcription polymerase chain reaction (RT-PCR) amplicons were sequenced bidirectionally by a commercial firm (BM Labosis, Ankara/Turkey) and compared with different PLMVd isolates from the NCBI database online. Multiple alignments were initially aligned by the neighbour joining method and analyzed with the CLC Main workbench program (CLC Bio, Qiagen, Aarhus, Denmark). The relationships were assessed phylogenetically using maximum likelihood algorithm of CLC Main Workbench Software by 1000 bootstrap replicates. Secondary structure analyses were obtained with the mfold structure prediction package of CLC Workbench program.

## Results

### *Symptoms in the field*

To investigate the presence of PLMVd in persimmon trees and weed plants we selected randomly and from those plants various viroid-like symptoms, including fruit malformation, stunting, and mosaics. During the field observation, almost no visual symptoms were seen in persimmon trees except one tree which exhibited severe fruit deformation (*Fig. 2*). The symptomatic persimmon tree was found infected by PLMVd in molecular tests (*Fig. 3*). However, we did not observe distinct mosaic and stunting symptoms in field plants. Likewise, no visual symptoms were observed in weed samples.

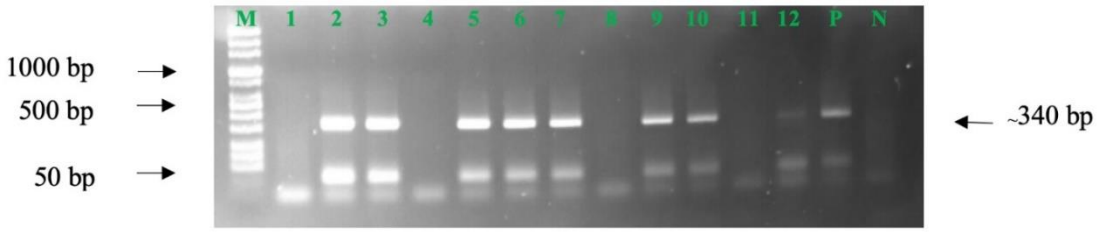


***Figure 3.*** *Agarose gel showing the reverse-transcription polymerase chain reaction assay of persimmon and weed samples using Peach latent mosaic viroid genome specific primer pairs, expected amplicon size ~340 bp. Lanes 1–12 are the tested persimmon samples, lane 2, lane 3, lane 5, lane 6 lane 7, lane 9 and lane 10 (0.34 kb) are positively reacted samples, P: positive control, N: negative control, M: 3.000 bp molecular markers*

### *RT-PCR detection*

In 2020, a total of 18 samples were collected from a persimmon orchard in Malatya province. Of these samples, 12 were from persimmon trees and remaining were various weed samples around the trees. PLMVd was detected in 7 persimmon samples and in one Johnsongrass plant (*Table 1*). Persimmon and Johnsongrass plant testing positive for PLMVd by RT-PCR were further sequenced. Among them, only the PLMVd-TH6 isolate was not registered in the gene bank because the poor sequence output. The results of RT-PCR assays and sequencing confirmed the presence of PLMVd infections in collected samples. PCR amplified products from viroid-infected samples were examined by agarose gel electrophoresis and typical bands specific to PLMVd from infected samples (approx. 0.34 kb) and the positive control were visualized in agarose gel (*Fig. 3*). No


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amplicon was observed when RNA from healthy plant was used as a template in the negative control.

***Table 1.*** *PLMVd infection of persimmon and weed samples*

| Sample short name | Sample | PLMVd Infection | Symptom observed | Accession No |
|---|---|---|---|---|
| TH1 | Persimmon | – | No visual symptoms | NA |
| TH2 | Persimmon | + | No visual symptoms | MZ289074 |
| TH3 | Persimmon | + | No visual symptoms | MZ289073 |
| TH4 | Persimmon | – | No visual symptoms | NA |
| TH5 | Persimmon | + | Fruit deformation | MZ289072 |
| TH6 | Persimmon | + | No visual symptoms | NA |
| TH7 | Persimmon | + | No visual symptoms | MZ289071 |
| TH8 | Persimmon | – | No visual symptoms | NA |
| TH9 | Persimmon | + | No visual symptoms | MZ289070 |
| TH10 | Persimmon | + | No visual symptoms | MZ289069 |
| TH11 | Persimmon | – | No visual symptoms | NA |
| TH12 | Persimmon | – | No visual symptoms | NA |
| G2 | *Sorghum halepense* (L.) Pers., Johnson grass | + | No visual symptoms | MZ289068 |
| T1 | *Consolida regalis* Gray, field larkspur | – | No visual symptoms | NA |
| E1 | *Carduus pycnocephalus* L., Italian thistle | – | No visual symptoms | NA |
| M1 | *Lactuca serriola* L., prickly lettuce | – | No visual symptoms | NA |
| S1 | *Convovulus arvensis* L., field bindweed | – | No visual symptoms | NA |
| A1 | a weed from Apiaceae | – | No visual symptoms | NA |

NA: Not applicable

The tested weeds were included: *C. regalis*, *S. halepense*, *C. pycnocephalus*, *L. serriola*, *C. arvensis* and an identified weed from Apiaceae family. Among these weeds, since PLMVd infection was detected only in Johnsongrass, it was thought that Johnsongrass may be the most important weed species to be a potential reservoir. However, no PLMVd was detected in other weed samples collected from persimmon orchard (*Table 1*).

The BLAST analyses of 7 complete PLMVd sequences did not reveal any hundred present identical hits to the currently available complete sequences of PLMVd in GenBank. Multiple alignment between the persimmon variants and all GenBank complete PLMVd sequences revealed that the 6 persimmon variants share 99.11–96.71% identity with available PLMVd sequences. The high level of nucleotide similarity with local isolates indicates that the agent might be spread from species to another by cross-infection due to use of contaminated pruning and grafting tools or by unknown insect vectors.

The full-length of the PLMVd variants identified in Malatya contains a few substitutions on their genomes. In the phylogenetic dendrogram, two major clusters (I and II) were observed. Majority of local PLMVd variants were clustered in branch-I,


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indicating a high level of genetic relatedness. With the exception of PLMVd-TH5, TH6 and TH7 variants, all local PLMVd variants were clustered into branch-I distantly from other Turkish and the world samples (*Fig. 4*). The PLMVd variant identified in *S. halepense* was clustered with local PLMVd walnut and the persimmon variants identified in this study. None of the PLMVd isolates, studied in this paper, were clustered with the similar sequences of the same species from other countries including Turkey.

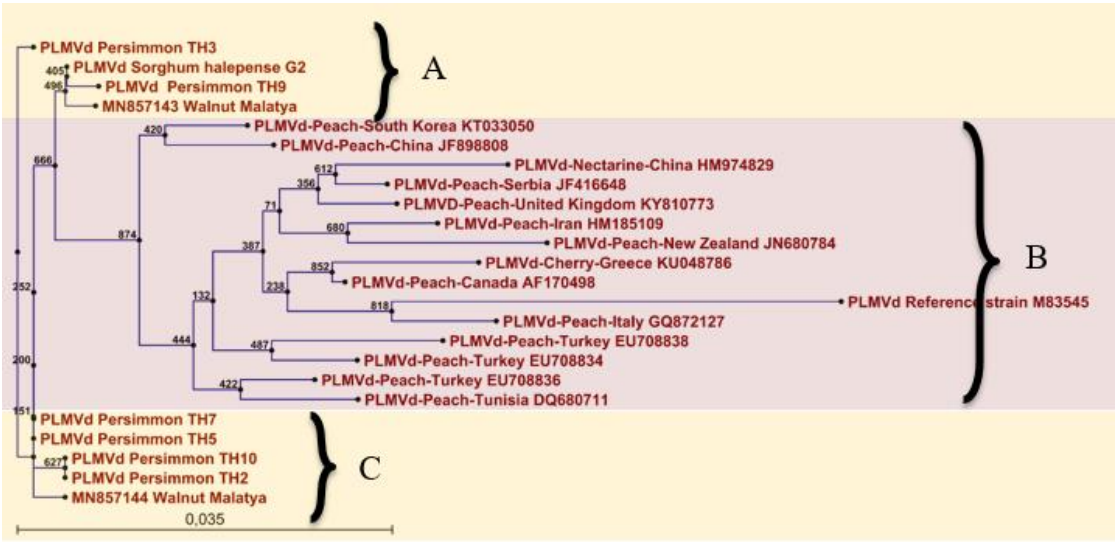


***Figure 4.*** *Phylogenetic relationships of Peach latent mosaic viroid (PLMVd) persimmon and Sorghum halepense L. variants along with PLMVd-walnut isolates compared to 20 genetically-related species reconstructed from the full-length viroid genome. The tree was generated by the neighbor joining algorithm and the bootstrap values of 1000 are indicated at key nodes*

The minimum free energy of optimal secondary structure was modelled for seven PLMVd variants using their consensus sequences (MZ289068, MZ289069, MZ289070, MZ289071, MZ289072, MZ289073 and MZ289074). The nucleotide sequence of PLMVd isolates studied in this paper had a nucleotide sequence identical to that determined by our group in walnut (Tuncel et al., 2020). All changes found in the new PLMVd variants did not affect the most stable secondary structure, except for PLMVd-persimmon TH2 and TH10 variants where serious changes observed. A specific mutation at the nucleotide 63 (G in position 63 instead of U) has changed the secondary structure drastically. However, the mutation did not interfere with the hypothesized hammerhead-like structure of PLMVd (*Fig. 5*). This nucleotide change was detected only in PLMVd variants of TH2 and TH10 (*Fig. 6*). Comparative analysis suggests that this single nucleotide change is solely responsible for the secondary structure difference between the reference PLMVd and the two persimmon PLMVd variants (TH2 and TH10), eliminating the P6 and P7 branches and creating one new branch and two spherical centers for branching of the viroid structure (*Fig. 5*). In order to prove the phenomenon, the nucleotide 63 was manually converted from Guanine to Uracil and the secondary structure analysis was reanalyzed using the same software. The results confirmed that the secondary structure of PLMVd-TH2 and TH10 variants were formed as a classical branched sphere.


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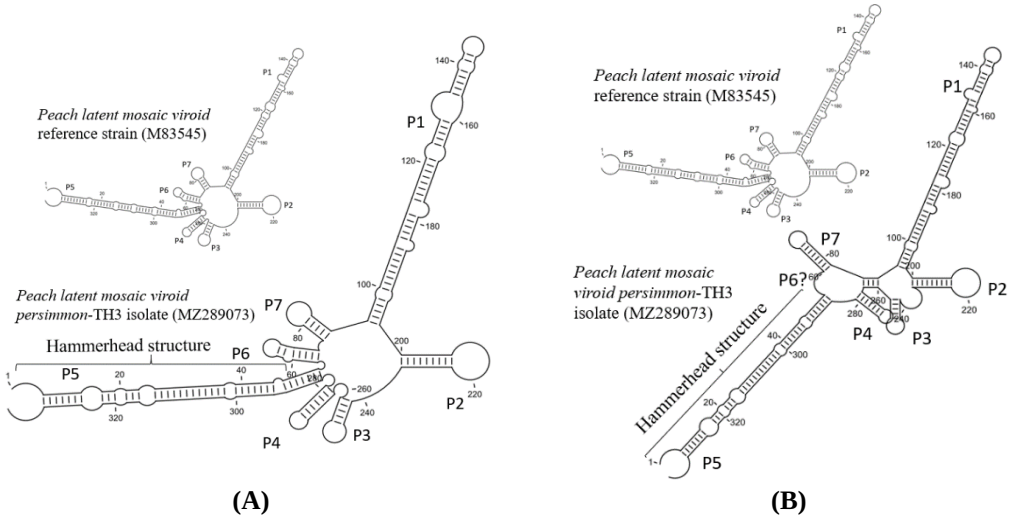


***Figure 5.*** *The most stable secondary structure model for Peach latent mosaic viroid (PLMVd) sequences: (A) PLMVd- TH3, TH5, TH6, TH7, TH9 and G2 variants (MZ289073, MZ289072, MZ289071, MZ289070, MZ289068) and GenBank sequence (M83545), (B) PLMVd-TH2 and TH10 variants (MZ289074 AND MZ289069)*

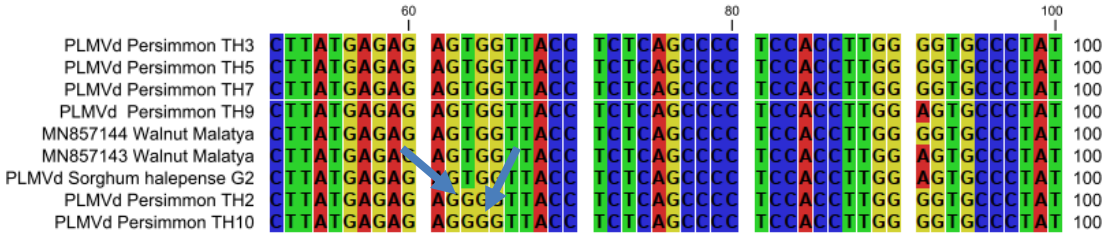


***Figure 6.*** *The multiple alignment of 10 complete Peach latent mosaic viroid (PLMVd) sequences from Malatya province. Six of these sequences identified in persimmon (this study), one in Sorghum halepense L. (this study) and two in walnut (Tuncel et al., 2020) are shown. The difference on the nucleotide sequences on PLMVd-TH2 and TH10 variants are indicated by arrows. The nucleotide changes at 63 T>G is exclusively responsible for the changed the most stable secondary structure of these two variants*

## Discussion

The present results indicate that a wide range of cultivated and weed plants may susceptible to PLMVd infections. PLMVd (genus *Pelamoviroid*, family *Avsunviroidae*) was first discovered in peach trees in France in 1976 and known to be latent in plum, apricot, cherry, apple, and pear varieties (Faggioli et al., 1997; El-Dougdoug, 1998; Fiore et al., 2000; Kyriakopoulou et al. 2001; Matic et al., 2005). PLMVd was recently reported for the first time on a crop outside of the *Prunus* genus as a widespread pathogen on walnut in Malatya province (Turkey) (Tuncel et al., 2020). In Turkey, this pathogen was reported previously in peach and nectarine (Sipahioglu et al., 1999; Gümüş et al., 2007; Gazel et al., 2008). Our work showed the existence of natural infections of PLMVd in persimmon trees and in Johnsongrass grown around the sampled trees. This is the first documented report of PLMVd infecting both species. Only one overt disease symptom of fruit malformation was observed in productive persimmon orchard, no other symptoms


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were recorded in the surveyed trees and weeds. However, it is still unknown whether the symptom of severe fruit deformation is caused by PLMVd. The identification of new natural hosts of PLMVd may help clarify the actual epidemiology of this pathogen in fruit crops. However, additional regional surveys both internationally and in Turkey are required to investigate the existence of PLMVd infections in other crops since it is occasionally latent in its hosts.

As a preliminary attempt to characterize PLMVd, the nucleic acid sequences of 6 isolates were examined at nucleotide level and analyzed phylogenetically. For the phylogenetic analysis, 20 previous PLMVd sequences deposited in GenBank (NCBI) database were aligned with the sequences reported in this work from persimmon and Johnsongrass. Two isolates of PLMVd identified in the same location in previous study in walnut (Tuncel et al., 2020) were also included to phylogenetic study. The analysis showed that PLMVd persimmon and Johnsongrass isolates reported in this study clustered with only PLMVd isolates reported from the same region from walnut. None of the local PLMVd-persimmon variants were clustered either with other Turkish PLMVd isolates from elsewhere or world isolates indicating a high diversity of genetic relatedness. BLAST analysis of full genomes of PLMVd isolates showed a sequence similarity ranging from 96.71% to 99.11% for persimmon isolates and from 97.01% to 99.10% for Johnsongrass isolate with similar sequences belonging to different geographical origins of the world. All local PLMVd isolates were clustered into two major lineages (A and C) distantly from world isolates. Both clades consisted of PLMVd walnut isolate, previously identified at the same region indicating their close relationships. Sequence and phylogenetic analysis confirmed that the PLMVd sequences obtained from persimmon and Johnsongrass were highly similar to those from PLMVd-walnut isolates identified at the same region. Cross-infection due to pruning and grafting or vector transmission may be the main spreading mechanisms of the agent. Further studies of the origin and possible intermediate hosts of PLMVd are required to understand the nature of possible cross-infection particularly from walnut trees to persimmon and vice versa, where the persimmon samples were collected (Elleuch et al., 2013).

In previous studies, using the mfold software, the secondary structures of lowest free energy were predicted for several PLMVd isolates (Zuker, 1989; Bussiere et al., 2000; Rodio et al., 2006). In these variants, the main secondary structure, involving branched core and several stems, was always obvious in the resulting topologies. However, in our study the occurrence of a significant amount of structural diversity was observed only in PLMVd-TH2 and TH10 variants (*Fig. 5*). The reason for this variance is the mutation that caused the Uracil base at number 63 converting it to the guanine base. It has been observed that a single base change at a specific point can lead to a serious change in the secondary structure of PLMVd. For instance, stems P6 and P7 were absent in each predicted structure, whereas the central core was divided into two cores forming a tween center and e new stem instead P6 and P7.

*Sorghum halepense* (Pers.) L. (Johnsongrass), in the Poaceae family, is a perennial graminoid plant species (Holm et al., 1997) and distributed over one-third of the total global area, causing significant losses to agriculture (Chirita et al., 2007). It harbors many plant viruses including *Maize dwarf mosaic virus* (Achon and Sobrepere, 2001), *Wheat dwarf virus*, *Sugarbeet yellow virus*, *Maize chlorotic mottle virus* and *Wheat streak mosaic* (Warwick and Black, 1983; Ikley et al., 2015; Achon et al., 2016; Parizipour et al., 2016). Control of Johnsongrass throughout the season will probably prevent its seed production and possible transmissions by unknown vectors.


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Since PLMVd is graft transmissible pathogen in many host plants there has been an increased risk of spread via propagation plant material. To evaluate its natural spread and to determine the actual prevalence, further survey studies are needed in fruit crop orchards in Turkey and in the world. Using clean propagation material will be a basic approach for the fruit crop industry to prevent the spread of this newly discovered viroid agent in fruit orchards. This will also provide the means for the long-term elimination of disease from productive orchards. By identifying persimmon and Johnsongrass as new natural hosts, here we report that PLMVd is a ubiquitous viroid that infects many different fruit trees cultivated worldwide and the weeds.

## Conclusions

Very little is known about the possible origin of the viroid infections in crop plants. Here we present the natural infections, nucleotide sequences and several new features of PLMVd isolated from persimmon and Johnsongrass. This is the first description of PLMVd from its natural hosts. By identifying these two new natural hosts, it has been confirmed that PLMVd is a genetically variable viroid that infects numerous different cultivated fruit crops and weeds around the globe. Additional survey studies are needed on the occurrence and geographical distribution of PLMVd in other fruit crops and weed species.

## REFERENCES

[1] Achon, M. A., Sobrepere, M. (2001): Incidence of potyvirus in commercial maize fields and their seasonal cycles in Spain. – Journal of Plant Diseases and Protection 108: 399-406.
[2] Achon, M. A., Serrano, L., Clemente, O. G., Sossai, S. (2016): First Report of Maize chlorotic mottle virus on a Perennial Host, Sorghum halepense, and Maize in Spain. – Plant Disease 101(2). 10.1094/PDIS-09-16-1261-PDN.
[3] Adkar-Purushothama, C. R., Perreault, J. P. (2019): Current overview on viroid–host interactions. – WIREs RNA.
[4] Albanese, G., Giunchedi, L., La Rosa, R., Poggi-Pollini, C. (1992): *Peach latent mosaic viroid* in Italy. – Acta Horticulturae 309: 331-338.
[5] Bussière, F., Ouellet, J., Coteou, E., Perreault, J. P. (2000): Mapping in solution shows the *Peach latent mosaic viroid* to possess a new pseudoknot in a complex, branched secondary structure. – Journal of Virology 74(6): 2647-54.
[6] Chirita, R., Grozea, I., Sarpe, N., Lauer, K. F. (2007): Control of *Sorghum halepense* (L.) species in western part of Romania Communications. – Agricultural and Applied Biological Sciences 73: 959-964.
[7] Desvignes, J. C. (1976): The virus diseases detected in greenhouse and in field by the peach seedling GF 305 indicator. – Acta Horticulturae 67: 315-323.
[8] Desvignes, J. C. (1986): *Peach latent mosaic* and its relation to peach mosaic and peach yellow mosaic virus diseases. – Acta Horticulturae 193: 51-58.
[9] Desvignes, J. C. (1999): Virus diseases of fruit trees. – Edition CTIFL Publication, Paris.
[10] Di Serio, F., Ragozzino, A. (1995): Indagini sulla presenza del viroide del mosaico latente del pesco (PLMVd) in Campania. – Informatore Fitopatologico 9: 57-61.
[11] Di Serio, F., Malfitano, M., Flores, R., Randles, J. W. (1999): Detection of PLMVd in Australia. – Australasian Plant Pathology 28: 80-81.
[12] El-Dougdoug, Kh. A. (1998): Occurrence of *Peach latent mosaic viroid* in apple (*Malus domestica*). – Annals of Agricultural Science (Cairo) 43(1): 21-30.


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1906_46294639

[13] Elleuch, A., Hamdi, I., Ellouze, O., Ghrab, M., Fkahfakh, H., Drira, N. (2013): Pistachio (*Pistacia vera* L.) is a new natural host of *Hop stunt viroid*. – Virus Genes 47(2): 330-337.
[14] Faggioli, F., Loreti, S., Barba, M. (1997): Occurrence of *Peach latent mosaic viroid* (PLMVd) on plum in Italy. – Plant Disease 81(4): 423.
[15] Fiore, N., Aboughanem, N., Infante, R., Myrta, A., Pallás, V. (2000): Detection of Peach Latent Mosaic Viroid in Stone Fruits from Chile. – In: Myrta, A., Di Terlizzi, B., Savino V. (eds.) Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region. Bari: CIHEAM, pp. 143-145.
[16] Flores, R., Llácer, G. (1988): Isolation of a viroid-like RNA associated with peach latent mosaic disease. – Acta Horticulturae 235: 325-332.
[17] Flores, R., Hernández, C., Desvignes, J. C., Llácer, G. (1990): Some properties of the viroid inducing the peach latent mosaic disease. – Research in Virology 141: 109-118.
[18] Flores, R., Hernández, C., Avinent, L., Hermoso, A., Llácer, G., Juárez, J., Arregui, J. M., Navarro, L., Desvignes, J. C. (1992): Studies on the detection, transmission and distribution of *Peach latent mosaic viroid* in peach trees. – Acta Horticulturae 309: 325-330.
[19] Flores, R., Daro`s, J. A., Hernández, C. (2000): The *Avsunviroidae* family: viroids with hammerhead ribozymes. – Advances in Virus Reseach 55: 271-323.
[20] Gazel, M., Ulubas Serce, C., Caglayan, K., Luigi, M., Faggioli, F. (2008): Incidence and genetic diversity of *Peach latent mosaic viroid* isolates in Turkey. – Journal of Plant Pathology 90: 495-503.
[21] Giunchedi, L., Gentit, P., Nemchinov, L., Poggi-Pollini, C., Hadidi, A. (1998): Plum spotted fruit: a disease associated with *peach latent mosaic viroid*. – Acta Horticulturae 472: 571-579.
[22] Giunchedi, L., Kyriakopoulou, P., Barba, M., Hadidi, A. (2011): *Peach latent mosaic viroid* in naturally infected temperate fruit trees. – In: Hadidi, A., Barba, M., Candresse, T., Jelkmann, W. (eds.) Virus and Virus-Like Diseases of Pome and Stone Fruits. St. Paul, MN, USA, The American Phytopathological Society Press, pp. 225-227.
[23] Gumus, M., Paylan, I. C., Matic, S., Myrta, A., Sipahioglu, H. M., Erkan, S. (2007): Occurrence and distribution of stone fruit viruses and viroids in commercial plantings of *Prunus* species in Western Anatolia, Turkey. – Journal of Plant Pathology 89(2): 255-258.
[24] Hadidi, A., Giunchedi, L., Shamloul, A. M., Poggi-Pollini, C., Amer, M. A. (1997): Occurrence of *Peach latent mosaic viroid* in stone fruits and its transmission with contaminated blades. – Plant Disease 81: 154-158.
[25] Holm, L. G., Plucknett, D. L., Pancho, J. V., Herberger, J. P. (1997): Sorghum halepense L. Pers. – In: Holm, L. G. (ed.) The world's worst weeds, distribution and biology. The University Press of Hawaii, Honolulu, pp. 54-61.
[26] Ikley, J. T., Wise, K. A., Johnson, W. G. (2015): Annual Ryegrass (*Lolium multiflorum*), Johnsongrass (*Sorghum halepense*), and Large Crabgrass (*Digitaria sanguinalis*) are alternative hosts for *Clavibacter michiganensis* subsp. *nebraskensis*, causal agent of Goss's wilt of corn. – Weed Science 63: 901-909.
[27] Kyriakopoulou, P., Giunchedi, L., Hadidi, A. (2001): Peach latent mosaic and pome fruit viroids in naturally infected cultivated pear *Pyrus communis* and wild pear *P. amygdaliformis*: implications on possible origin of these viroids in the Mediterranean region. – Journal of Plant Pathology 83: 51-62.
[28] Loreti, S., Faggioli, F., Cardini, M., Mordent, G., Bambini, A. R., Poggi-Pollini, C., Barba, M. (1999): Comparison of different diagnostic methods for detection of *Peach latent mosaic viroid*. – EPPO Bulletin 29: 433.
[29] Matic, S., Al Rwahnih, M., Arben, M. (2005): Occurrence of Stone Fruit Viroids in Bosnia and Herzegovina. – Phytopathologia Mediterranea 44: 285-290.
[30] Osaki, H., Yamamuchi, Y., Sato, Y., Tomita, Y., Kawai, Y., Miyamoto, Y., Ohtsu, Y. (1999): Peach latent mosaic isolated from stone fruits in Japan. – Annuals of Phytopathology Society of Japan 65: 3-8.


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DOI: http://dx.doi.org/10.15666/aeer/1906_46294639

[31] Parizipour, M. G., Behjatnia, S. A. A., Afsharifar, A., Izadpanah, K. (2016): Natural hosts and efficiency of leafhopper vector in transmission of *Wheat dwarf virus*. – Journal of Plant Pathology 98: 483-492.
[32] Pelchat, M., Levesque, D., Ouellet, J., Laurendeau, S., Levesque, S., Lehoux, J., Thompson, D. A., Eastwell, K. C., Skrzeczkowski, L. J., Perreault, J. P. (2000): Sequencing of *Peach latent mosaic viroid* variants from nine North American peach cultivars shows that this RNA folds into a complex secondary structure. – Virology 271: 37-45.
[33] Rodio, M. E., Delgado, S., Flores, R., Di Serio, F. (2006): Variants of *Peach latent mosaic viroid* inducing peach calico: uneven distribution in infected plants and requirements of the insertion containing the pathogenicity determinant. – Journal of General Virology 87: 231-240.
[34] Shamloul, A. M., Minafra, A., Hadidi, A., Giunchedi, L., Waterworth, H. E., Allam, E. K. (1995): *Peach latent mosaic viroid*: nucleotide sequence of an Italian isolate, sensitive detection using RT-PCR and geographic distribution. – Acta Horticulturae 386: 522-530.
[35] Sipahioglu, H. M., Myrta, A., Abou-Ghanem, N., Di Terlizzi, B., Savino, V. (1999): Sanitary Status of Stone Fruit Trees in East Anatolia (Turkey) with Particular Reference to Apricot. – Bulletin OEPP/EPPO Bulletin 29: 439-442.
[36] Sipahioğlu, H. M., Demir, S., Myrta, A., Al Rwahnih, M., Polat, B., Schena, L., Usta, M., Akkopru, A., Selcuk, M., Ippolito, A., Minafra, A. (2006): Viroid, phytoplasma and fungal diseases of stone fruits in Eastern Anatolia, Turkey. – New Zealand Journal of Crop and Horticultural Science 34: 1-6.
[37] Skrzeczkowski, L. J., Howell, W. E., Mink, G. I. (1996): Occurrence of *Peach latent mosaic viroid* in commercial peach and nectarine cultivars in the U.S. – Plant Disease 80: 823.
[38] Torres, H., Gómez, G., Pallás, V., Stamo, B., Shalaby, A., Aouane, B., Gavriel, I., Kominek, P., Caglayan, K., Sipahioglu, H. M. (2004): Detection by tissue printing of stone fruit viroids, from Europe, the Mediterranean and north and south America. – Acta Horticulturae 657: 379-383.
[39] Tuncel, F., Tekkaş, N., Türk, G., Oksal, H. D., Sipahioğlu, H. M. (2020): First Detection and Molecular Characterization of *Peach latent mosaic viroid* in a New Natural Host: Walnut (*Juglans regia* L.). – Alinteri Journal of Agriculture Sciences 35(2): 37-44.
[40] Warwick, S. I., Black, L. D. (1983): The biology of Canadian weeds. *Sorghum halepense* (L.) Pers. – Canadian Journal of Plant Sciences 63: 997-1014.
[41] Zuker, M. (1989): On finding all suboptimal foldings of an RNA molecule. – Science 244: 48-52.


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