

First report of *Peach Latent Mosaic Viroid* in symptomatic peach orchards in Southern Bulgaria

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Introduction

Viroids are plant infectious agents whose genome is represented by a small (246 to 401 bp), single-stranded, circular, non-coding RNA capable of autonomous replication in susceptible hosts[1]. *Peach latent mosaic viroid* (PLMVd) is a member of the family *Avsunviroidae*. Among the hosts of PLMVd are peach, nectarine, plum, apricot and cherry. The PLMVd causes a wide variety of peach symptoms, some of which are barely noticeable, while others such as mosaic or chlorotic spots on leaves or fruit tissue, even more severe like albino phenotype (calico peach) are well distinguishable [2]. Changes in the appearance of the fruit and the reduction in the lifespan of peach trees cause serious economic losses. In the present work, we study the occurrence of PLMVd in peach orchards in Bulgaria.

Materials and methods

Leaf samples were collected from 4-10-years-old symptomatic peach trees of cvs. ‘Royal Gem’ and ‘Gergana’, from peach orchards in Southern Bulgaria. Total RNA was isolated according to the protocol of Fekih et. al, 2006 [3]. cDNA was synthesized from 1 µg total RNA using the protoScript cDNA kit (NEB). RT-PCR was performed with degenerative (PLMVd Fw 5`-TCA GTG GGC TWW GCC CAG ACT T-3`; PLMVd Rev 5`-GCG ASG TNC TTA GCC TTT C-3`) and PLMVd-specific primers (PLMVd R1 5`-CCC GAT AGA AAG GCT AAG CAC CTC G-3`; PLMVd F1 5`- AAC TGC AGT GCT CCG AAT AGG GCA C-3`). PCR amplicons were extracted and gel purified with Qiaquick Qiagen protocol. A Geneious multiple alignment of PLMVd variants and isolates was performed. Buds of PLMVd positive peach samples were grafted on the indicator GF-305, peach cultivar ‘Summerset’ (*Prunus persica*) and myrobalan (*Prunus cerasifera*).

Results

Survey of peach orchards

The leaf samples were collected from six peach orchards trees. The selection of the analyzed trees was based on phenotypic changes such irregularly shaped colorless areas on fruits with cracked edges, bud necrosis and delay in leafing, flowering and fruit maturity. Samples were collected from the youngest upper leaves.



Figure 1. Symptomatic peach leaves and fruits of the cv. Royal Gem from peach orchards.

Sequence analysis

Amplified products were directly sequenced in GATC, Eurofins, Germany. BLAST analysis of the nucleotide sequences shared 90% identity with PLMVdMB-P2-17 isolate.

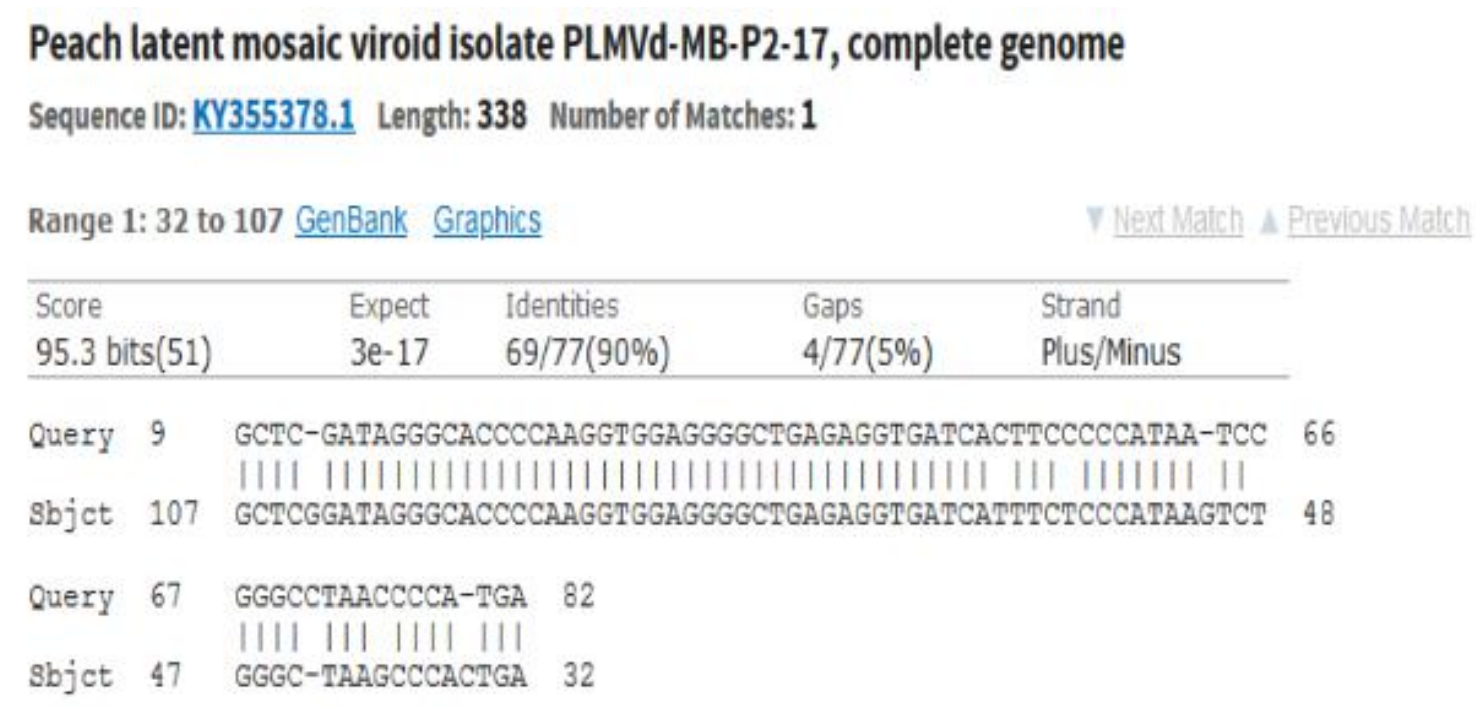


Figure 3. A graphical representation of the BLAST-generated alignment.

PLMVd detection

The multiple alignment of PLMVd variants and isolates was used to construct degenerate primers in the most conserved region of PLMVd. A specific amplification product with a size of 109 nt was obtained in peach sample 21/29, cv. Royal Gem by RT-PCR. Furthermore, using PLMVd- specific primers, a fragment of the expected size (348nt) was also amplified.

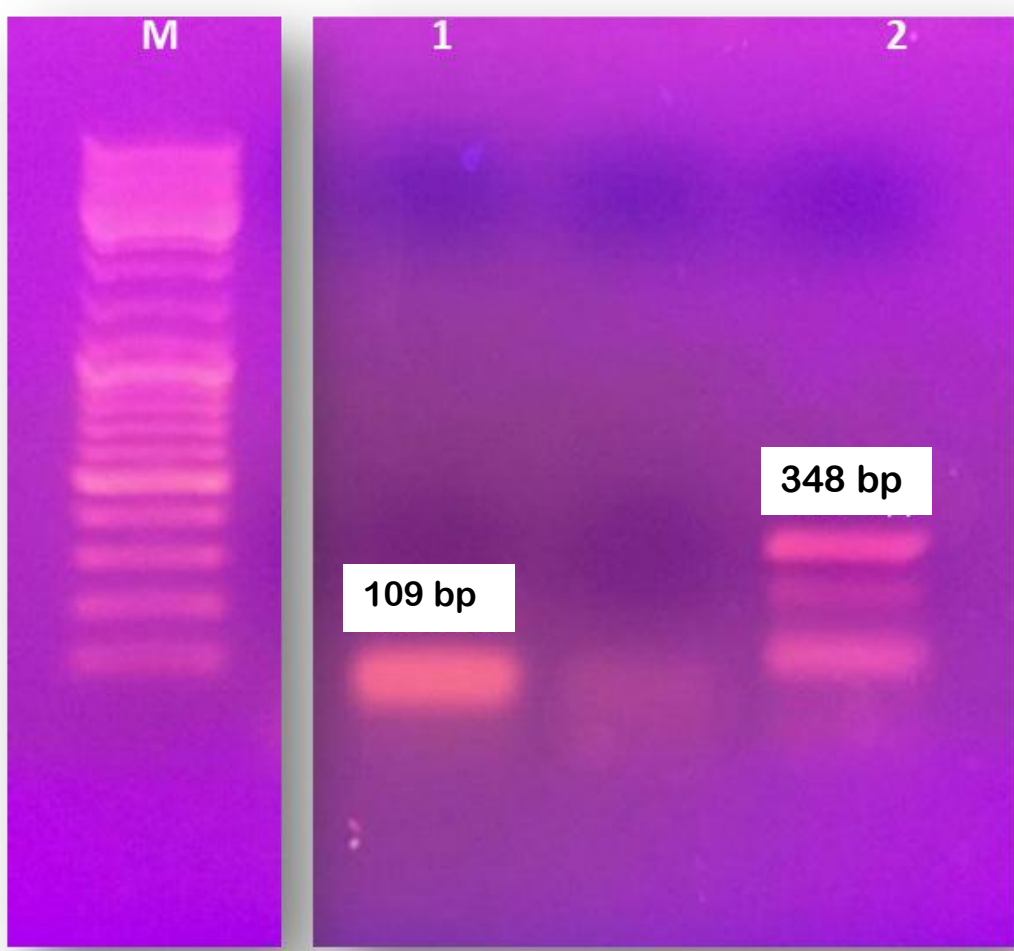


Figure 2. Detection of PLMVd by RT-PCR. M - Molecular marker (SM1331, Thermo Fisher Scientific); 1 - Amplicon obtained after RT-PCR with degenerate primers for detection of PLMVd in sample 21/29; 2 - Amplicon obtained after RT-PCR with PLMVd specific primers in sample 21/29;

Bioassay

Bioassay studies were conducted by budding from PLMVd infected sample 21/29 to GF-305 peach indicator plant, the late-maturing peach cv. Summerset and myrobalan.

Four weeks after grafting, we observed irregularly shaped colorless areas and curling on newly emerged leaves. RT-PCR analysis to detect PLMVd in these leaves, verified the presence of an amplicon of the expected size in both PLMVd primer pairs.

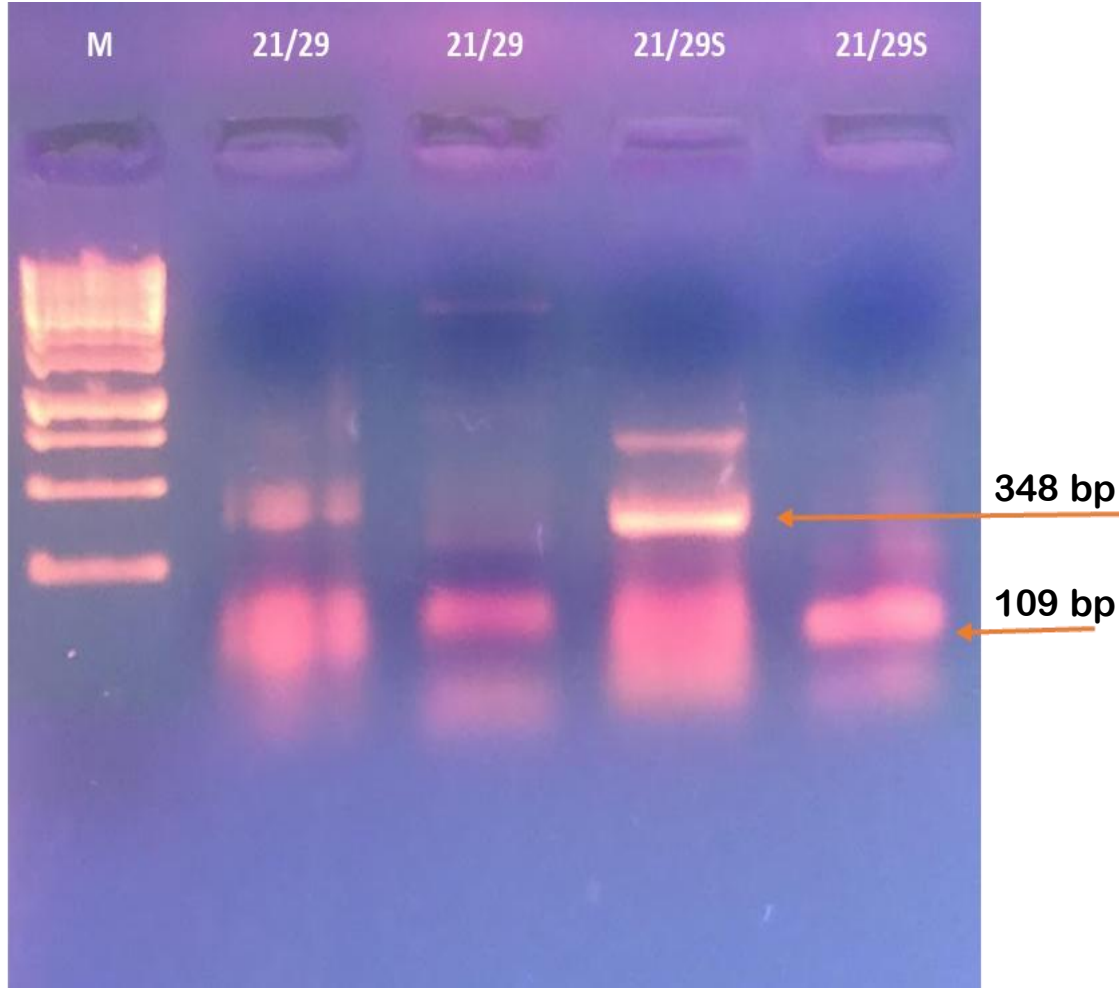


Figure 4. RT-PCR for detection of PLMVd in peach cultivar Summerset (S).

Conclusions

- A peach tree cv. Royal Gem from orchards near the city of Plovdiv tested positive for PLMVd. This is the first report of PLMVd infected peach in Bulgaria.
- We provide a set of degenerate PCR primers for PLMVd detection
- Bioassay on peach cv. ‘Summerset’ (*Prunus persica*) revealed PLMVd- specific symptoms in emerging leaves.

Acknowledgments

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Literature

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