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INTEGRATED PEST MANAGEMENT SYSTEM FOR CUCUMBER (*CUCUMIS SATIVUS*) UNDER GREENHOUSE CONDITIONS

Abstract

The research was conducted in 2024 at the «Dzhantor» greenhouse complex of Kavunov, located in the Enbekshikazakh district of the Almaty region. The aim of the study was to develop a biologically based integrated protection system against major greenhouse whiteflies damaging cucumber crops grown under protected conditions. The entomophagous insect *Encarsia formosa* was used as a biological control agent, and its effect on pest population dynamics was evaluated. The biological efficiency of releasing *Encarsia formosa* at a rate of 2 individuals/m² was 61.3%. Due to unfavorable weather conditions, the biological preparation Aktarofit 1.8 was additionally applied against the pest, and its efficiency was studied. The biological efficiency of two applications of the preparation against the pest was 87,5%. After the full harvest, chemical treatments were carried out in the greenhouse to completely eliminate the remaining sucking pests, including individuals entering diapause. The use of a biologically based integrated protection system is of great importance in cucumber cultivation under greenhouse conditions.

Keywords: cucumber, greenhouse, greenhouse whitefly, Encarsia, integrated plant protection system, biocontrol agents, biopesticides

Авторлардың үлесі

К.А. және Б.Ж.: қияр дақылы өсірілетін жабық алаңда фитосанитарлық мониторингін жүргізу бойынша зерттеу әдістемесін әзірлеу, әдеби деректерді талдау, мақаланың негізгі бөлігін жазу. А.М. және Ә.С. энтомофагтар мен биопрепараттардың тиімділігін бағалау бойынша зертханалық тәжірибелерді ұйымдастыру, сондай-ақ мақаланы редакциялау және жариялауға дайындық жүргізу. Э.А. және Б.Б.: зертханалық тәжірибелер жүргізу, деректерді жинау және өңдеу, алынған нәтижелерді талдауға қатысу. Барлық авторлар жұмыста белсене қатысып, мақаланың соңғы нұсқасымен танысты.

IRSTI 34.25.21 **DOI** *https://doi.org/10.37884/3-2025/25*

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DETECTION OF HOP STUNT VIROID (HSVd) AND PEACH LATENT MOSAIC VIROID (PLMVd) IN STONE FRUIT CROPS USING DUPLEX RT-PCR METHOD

Abstract

Viroids are small plant pathogens that do not encode proteins. Despite their simple structure, viroids can cause serious metabolic and developmental disorders in plants, leading to a various of symptoms, as well as a decrease in yield and product quality. The diagnosis of viroids is especially relevant for fruit crops, since infections are often asymptomatic in the early stages.

This study presents the results of simultaneous detection of Hop stunt viroid (HSVd) and **Peach** latent mosaic viroid (PLMVd) in peach trees. Leaf samples from trees showing specific symptoms of viroid infection were collected from the Nauryzbai district of Almaty city. By reverse transcription followed by duplex polymerase chain reaction (duplex RT-PCR) using specific primers designed in this study, HSVd and PLMVd RNAs were detected in a number of samples, and the presence of RNAs of both viroids was shown in one sample. The purified amplicons were subjected to Sanger sequencing to determine the nucleotide sequences of two cDNA fragments. BLAST analysis confirmed that the plant was infected with both HSVd and PLMVd. These results indicate the spread of HSVd and PLMVd in the Almaty region. This highlights the need of introducing regular viroid diagnostics in stone fruit crops in Kazakhstan.

Keywords: *peach, viroid, PLMVd, HSVd, duplex RT-PCR, sequencing, nucleotide analysis*

Introduction

Viroids are a unique class of plant pathogens — small, circular, single-stranded RNA molecules that do not encode proteins but can replicate within the cells of higher plants [1–4]. Despite their very small size (240–440 nucleotides), viroids can cause serious symptoms in a wide range of cultivated plants, including fruit trees [4, 5]. The clinical features of viroid infections resemble those of many plant viruses and include epinasty and leaf chlorosis, deformation of flowers and fruits, stem cracking, stunted growth, and even plant death [5].

Phylogenetically and biologically, viroids are divided into two families: **Pospiviroidae** and **Avsunviroidae,** which differ in their replication sites—nucleus and chloroplasts, respectively [6]. Some members of these families infect pome and stone fruit crops (apple, pear, plum, apricot, peach, cherry) and are widespread worldwide. Viroids are transmitted through various routes: mechanical injury to tissues, infected planting material, pollen, seeds, and, according to some reports, possibly by insect vectors [7].

Peach latent mosaic viroid (PLMVd) and Hop stunt viroid (HSVd) are the most studied and are economically important plant pathogens capable of causing significant yield losses in stone fruit crops [8]. Several international studies have shown that mixed infections can exacerbate symptoms and lead to overall plant decline [9, 10]. PLMVd is listed as a quarantine pathogen within the Eurasian Economic Union (https://adilet.zan.kz/rus/docs/H16EV000158) and has been detected in Kazakhstan for the first time by our research team [11, 12]. HSVd has a very broad host range and infects most stone fruit species [13], and to our knowledge, this viroid has never been screened for in Kazakhstan before.

Due to the absence of protein structures and antigenic properties, viroids cannot be detected using standard serological methods, making molecular diagnostics the primary detection approach. In practice, singleplex RT-PCR is used for individual viroid detection, multiplex RT-PCR for detection of multiple viroids simultaneously, as well as nucleic acid hybridization, and next-generation sequencing (NGS) [14].

The aim of this work was to use duplex RT-PCR method, developed by our research team, for molecular testing of peach samples from Almaty for the presence of viroids affecting stone fruit crops. This approach significantly reduces the time required for molecular screening of plant samples for viroids compared to single RT-PCR.

Materials and Methods

Samples. Leaf samples from peach trees (*Prunus persica*) exhibiting specific symptoms — including leaf curling, branch dieback, bark damage, and lack of fruit — were collected in the Nauryzbay district, Almaty region.

Extraction of total nucleic acids (TNA). The CTAB-based method was chosen for TNA extraction due to its effectiveness in removing PCR inhibitors like polysaccharides and polyphenols, which are abundant in fruit plants [15]. 100–200 mg of leaf tissue were homogenized in 1 mL CTAB buffer (cetyltrimethylammonium bromide). The quality of extracted nucleic acids was assessed by electrophoresis in a 1% native agarose gel.

Reverse Transcription and PCR. cDNA synthesis was performed using Maxima Reverse Transcriptase (Thermo Fisher Scientific) according to the manufacturer's protocol. The reaction mixture contained 1.5 µL RNA template (300–500 ng) and 0.5 µL random hexamer primers (100 µM), 3 µL H_2O. The mixture was incubated at 65 °C for 5 min and then chilled on ice. To the chilled mixture, 5 µL of RT mix were added (2 µL 5X RT buffer, 1 µL dNTP mix (10 mM), 0.25 µL Maxima RT, 0.25 µL RNase inhibitor). The RT was carried out at 25 °C for 10 min, 50 °C for 30 min, and 85 °C for 5 min.

The resulting cDNA was used for viroid PCR amplification employing Pfu DNA Polymerase (Thermo Fisher Scientific). The 25 µL PCR mix contained 2.5 µL cDNA, 2.5 µL $MgSO_4$ buffer, 0.5 µL dNTP mix (10 mM), 0.6 µL each of four primers (10 µM), 0.5 µL Pfu (1.25 U), and 16.6 µL sterile water. Amplification conditions: 95 °C for 2 min; 35 cycles of 95 °C 30 s, 58 °C 30 s, 72 °C 1 min; final extension at 72 °C for 5 min. PCR products were analyzed by electrophoresis in 1.8% agarose gel in TBE buffer, stained with ethidium bromide, and visualized under UV light.

Sequencing. PCR products were gel-purified using QIAquick PCR Purification Kit (Qiagen) and bidirectionally sequenced by Sanger method using Big Dye Terminator v.3.1 (Applied Biosystems). Capillary electrophoresis was performed on an ABI 3500XL Genetic Analyzer. For viroid identification and homology assessment, NCBI BLAST (https://www.ncbi.nlm.nih.gov/) was used to analyze the resulting nucleotide sequences.

Results and Discussion

Primer design. To test peach samples for the presence of HSVd viroid, a pair of oligonucleotides was designed to amplify the full genome sequence based on the consensus sequence shown in figure 1. This sequence was assembled across multiple HSVd genome sequences available in the GenBank database (https://www.ncbi.nlm.nih.gov) using NCBI Multiple Sequence Alignment Viewer 1.26.0. The HSVd isolate found in China (GenBank: EF076820) was used as a comparison sequence. The high variability and circular nature of viroid genomes was taken into account when designing PCR primers. The constructed primer pair hsvf (5'- CGGATCCTCTCTTGAGCC-3') and hsvr (5'- CGGCAGAGGCGCAGATAGAACA -3') covers positions from 255 to 294 of the genome of the reference isolate. For detection of PLMVd, the previously developed primer pair plmf (5'-GGATTACGACGTCTACCCGG-3') and plmr (5'-CCAGTTTCTACGGCGGTACCTG-3') was used [10]. Online analysis for self- and mutual complementarity between the 3'-ends showed the absence of primer-dimer formation (https://www.thermofisher.com/kz/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/multiple-primer-analyzer.html). PLMVd isolates range in size from 337 to 339 nucleotides, HSVd isolates range from 297 to 301 nucleotides, and the size difference between two viroids is approximately 40 nucleotides, which allows them to be separated in an agarose gel after duplex RT-PCR.

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CTGGG-G-A-ATTCTCGAGTTGCCGCAAAAGGCATGCAAAG-AAAAAAACTAGGCAGGGA
GGYGCTTA-CCTGAGAAAGGAGCCCCGGGGCAACTCTTCTCAGAA-TCCAGCRAGAGGCG
T-GGAGAGAGGGCCGCGGTGCTCTGGAGTA--G--AGGCT-CTGCCTTCGAAACACCATC
GATCGTCCCTTCTTC-TTTACCTT-CT-TCTGGCTCTTCTTGGA-----GACGCGACCGG
TGGC--ACCCCTGCTCGGTTCGCTCCAACCTGCTTTTGTTCTATCTGCGCCTCTGCCGCG
GATCCTCTCTTGAGCCCCT
```

The annealing region of the hsvf and hsvr primers is underlined.

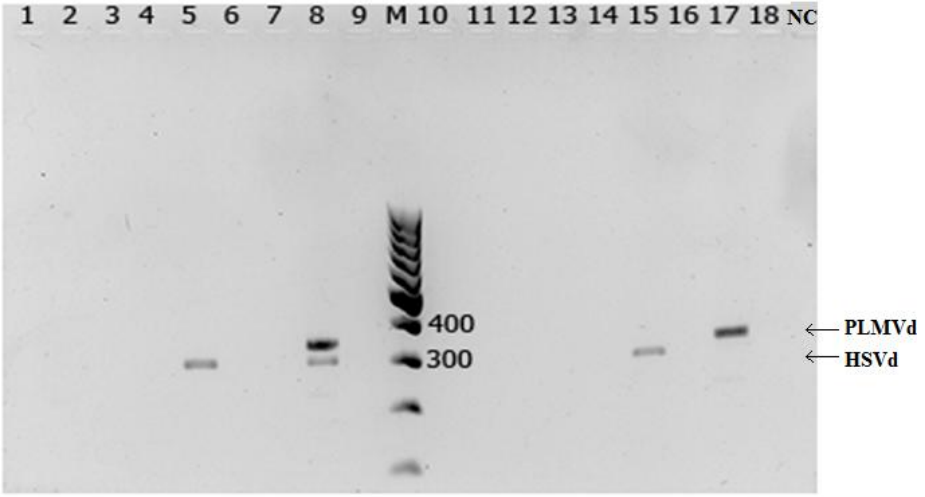
Figure 1 – HSVd consensus sequence

Sample collection. Various plant tissues and organs are used to detect viroids, including fruits, shoots, bark, and leaves. Eighteen peach leave samples were collected in the Nauryzbai district of Almaty in the fall of 2024. Some trees looked quite healthy, with no signs of infection, while others

had dried skeletal branches or curled leaves (fig. 2). The absence of peach calico symptoms such as mosaic and extensive chlorosis of leaves on the tested trees is noted.

Duplex RT-PCR. For the molecular detection of viroids, TNAs from the collected leaves were extracted using the cationic detergent CTAB. Finally, the TNA precipitate obtained from 100 mg of plant material was dissolved in 100 µl of sterile double distilled water. TNA samples were visual inspected after electrophoresis under native conditions in 1% agarose gel and subsequent staining with ethidium bromide. The presence of distinct 28S and 18S ribosomal RNA bands indicated a high-quality RNA suitable for further analysis. All 18 samples were tested with duplex RT-PCR with primers specific to PLMVd and HSVd. Figure 2 shows the test results alongside a negative control. As can be seen from the figure, a band of the expected size for PLMVd, approximately 340 bp, was observed in 2 out of the 18 samples, and a fragment around 300 bp in size, which is expected for HSVd, was detected in 3 samples. In sample No.8, the amplification of both fragments suggests a potential mixed infection, where both viroids are present in one sample. It is worth noting that the sample was taken from a peach tree, with specific symptoms, namely, curled leaves and dry skeletal branches. Of the 18 samples tested, 4 were infected. Overall, the infection rate was 22%, which is really quite high.

Figure 2 – Tested peach leaf samples showing signs of curling



Lanes 1–18 correspond to tested samples; NC – negative control; M – 100 bp DNA marker.

Figure 3 – Gel electrophoresis of duplex RT-PCR products of peach samples amplified with primer pairs plmf/plmr and hsvf/hsvr

Sequencing and comparative nucleotide analysis. Two cDNA-fragments (300 bp and 340 bp), amplified from sample No.8, were gel-purified and bidirectionally Sanger sequenced using

viroid-specific primers. The size of HSVd was found to be 298 nucleotides, while PLMVd was 338 nucleotides. Align sequences using BLAST confirmed their viroid nature and showed 95–99% homology for HSVd and 94–96% for PLMVd with published isolates.

Thus, we documented the simultaneous infection of a single plant by two viroids, which was confirmed via RT-PCR, sequencing, and alignment of the obtained sequences with known viroid sequences.

Such natural cases of co-infection by multiple viroids have also been reported by international researchers. One of the earliest observations, conducted in the Czech Republic in 2004, demonstrated the simultaneous presence of HSVd and PLMVd in *Prunus persica*. Among 66 tested trees, 46 were co-infected with both viroids, five were infected only with PLMVd, and 15 were free of both viroids [16].

Another example of natural dual infection was reported by Fekih Hassen and colleagues (2006), where PLMVd + HSVd and PBCVd + HSVd combinations were found in pear trees, underscoring the prevalence of mixed viroid infections under field conditions [17].

Furthermore, in Taiwan (2015), a study revealed the simultaneous presence of Citrus exocortis viroid (CEVd) and HSVd in citrus species. The investigation detected a titer-enhancing relationship, along with overlapping tissue localization patterns [18].

Therefore, dual viroid infections are not uncommon in either natural ecosystems or agroecosystems. This fact underscores the necessity of regular monitoring and certification of plants, particularly in fruit crop production. While infection by multiple viroids may be asymptomatic in itself, the interaction of two or more viroids can exacerbate symptom development, impair host defenses, increase susceptibility to viruses, reduce yield, and even contribute to plant mortality [9, 10].

*Scientific and practical significance***.** The duplex RT-PCR primer set developed by our research group enables simultaneous detection of PLMVd and HSVd in one reaction, saving resources and time compared to singleplex RT-PCR. The identified isolates from sample No.8 can serve as positive controls in viroid detection screens not only for stone fruits, but also for pome fruits like apples and pears. Whole-genome sequencing of local PLMVd and HSVd isolates provides new data on regional strain distribution.

Conclusions

In Almaty city, a study using duplex RT-PCR identified in peach trees two serious viroid pathogens, PLMVd and HSVd, also it is the first documented case of co-infection in Kazakhstan. Identification was confirmed by direct sequencing of amplicons, showing high homology to known published isolates, suggesting potential introduction via imported planting material. These findings carry significant epidemiological implications, revealing a previously undetected spread of viroids in the country. The observed mixed infection in peaches—manifested by leaf curling and branch dieback—highlights the importance of such studies. Considering the fact that viroid infections may remain asymptomatic for an extended period, regular monitoring in nurseries and stricter control on imported seedlings are crucial. Moreover, the developed duplex RT-PCR method and the isolated strains can serve as positive controls for new viroid detection systems in phytosanitary monitoring of stone fruit crops.

Acknowledgments. This work was supported by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan under scientific-technical program BR21881942 “Development of biotechnological approaches for phytopathogen control to enhance agricultural crop productivity” for 2023–2025.

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СҮЙЕКТІ ЖЕМІС ДАҚЫЛДАРЫНДА HOP STUNT VIROID (HSVd) ЖӘНЕ PEACH LATENT MOSAIC VIROID (PLMVd) ВИРОИДТАРЫН ДУПЛЕКСТІ КТ-ПТР ӘДІСІМЕН АНЫҚТАУ

Аңдатпа

Вироидтар – бұл ақуыз кодтамайтын өсімдіктердің шағын патогендері. Қарапайым құрылымына қарамастан, вироидтар өсімдіктерде метаболизм мен даму үдерістерінің айтарлықтай бұзылуын тудыруы мүмкін, бұл симптомдардың кешеніне, сондай-ақ өнімділік пен сапаның төмендеуіне алып келеді. Вироидтарды анықтау, әсіресе, ерте кезеңдерінде симптомсыз өтетін инфекцияларға байланысты, жеміс-жидек дақылдары үшін өзекті мәселе болып табылады.

Бұл зерттеуде шабдалы ағаштарында Hop stunt viroid (HSVd) және Peach latent mosaic viroid (PLMVd) бір мезгілде анықтаудың нәтижелері ұсынылады. Алматы қаласы, Наурызбай ауданында вироидтық инфекцияның клиникалық белгілерін көрсеткен шабдалы жапырақтарының үлгілері зерттелді. Арнайы әзірленген праймерлерді пайдалана отырып, кері транскрипция әдісімен және кейінгі дуплексті полимеразалық тізбекті реакция (дуплекс КТ-ПТР) арқылы жүргізілген талдау нәтижесінде кейбір үлгілерде HSVd және PLMVd вироидтарының РНҚ анықталды, ал бір үлгіде екі вироидтың РНҚ-сы қатар анықталды. Кейін ампликондарды тазартып, олардың нуклеотидтік ретін анықтау үшін Сэнгер әдісімен секвенирлеу жүргізілді. BLAST талдауы HSVd және PLMVd вироидтарының сәйкестігін растады. Алынған деректер HSVd және PLMVd вироидтарының Алматы қаласы аумағында айналымда екенін көрсетеді. Бұл Қазақстанда сүйекті жеміс дақылдарының вироидтық инфекцияларын жүйелі түрде диагностикалаудың қажеттілігін дәлелдейді.

Кілт сөздері: шабдалы, вироидтар, PLMVd, HSVd, дуплексті КТ-ПТР, секвенирлеу, нуклеотидтік талдау.

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ДИАГНОСТИКА ВИРОИДОВ КАРЛИКОВОСТИ ХМЕЛЯ (HSVd) И ЛАТЕНТНОЙ МОЗАИКИ ПЕРСИКА (PLMVd) КОСТОЧКОВЫХ КУЛЬТУР МЕТОДОМ ДУПЛЕКСНОЙ ОТ-ПЦР

Аннотация

Вироиды — это небольшие патогены растений, не кодирующие белки. Несмотря на простую структуру, вироиды могут вызывать серьёзные нарушения метаболизма и развития у растений, приводя к ряду симптомов, а также к снижению урожайности и качества продукции. Диагностика вироидов особенно актуальна для плодовых культур, поскольку инфекции часто протекают бессимптомно на ранних стадиях.

В работе представлены результаты одновременного выявления вироидов карликовости хмеля (HSVd) и латентной мозаики персика (PLMVd) в персиках**.** Тестированы образцы листьев персика, собранных на территории Наурызбайского района г. Алматы, проявившие клинические признаки вироидного заражения. Методом обратной транскрипции с последующей дуплексной полимеразной цепной реакцией (дуплекс ОТ-ПЦР) с использованием разработанных нами специфичных праймеров, в ряде образцов были

выявлены РНК HSVd и PLMVd, а в одном образце было показано присутствие РНК обоих вироидов. В последующем была проведена очистка ампликонов, которые далее были секвенированы по Сэнгеру для определения нуклеотидных последовательностей обоих вироидов. BLAST-анализ показал выявление HSVd и PLMVd**.** Полученные данные указывают на циркуляцию HSVd и PLMVd в районе города Алматы. Это обосновывает необходимость внедрения регулярной диагностики вироидных инфекций косточковых плодовых культур в Казахстане.

Ключевые слова: персик, вироиды, PLMVd, HSVd, дуплекс ОТ-ПЦР, секвенирование, нуклеотидный анализ

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МРНТИ 68:68.35:68.35.03 **DOI** *https://doi.org/10.37884/3-2025/26*

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КОМПЛЕКСНАЯ ОЦЕНКА ПЕРСПЕКТИВНЫХ СОРТООБРАЗЦОВ ТОМАТА ОТКРЫТОГО ГРУНТА В ПИТОМНИКЕ КОНКУРСНОГО ИСПЫТАНИЯ

Аннотация

В статье представлены результаты оценки перспективных сортообразцов томата открытого грунта отечественной селекции за 2023-2024гг. Все изученные сортообразцы были описаны по морфологическим параметрам, всем была дана комплексная оценка по хозяйственно-ценным признакам. Образцы имели детерминантный тип куста, характеризуются нештамбовыми растениями, отличаются по габитусу, величине листьев, длительности периодов вегетации, по величине срока созревания, общему и товарному урожаю. Также отличаются друг от друга по комплексу признаков плода: по окраске, размеру (крупные и средние), форме (округлые, сливовидные, грушевидные), с большим и небольшим углублением рубца в месте отрыва плода от плодоножки, по камерности - 2-3 камерные и многокамерные. Изученные образцы характеризовались хорошей продуктивностью, относительной устойчивостью к основным видам болезней и высокими биохимическими показателями. Все эти признаки имеют как теоретическое, так и практическое значение. Созданные сортообразцы превосходят или находятся на уровне стандарта по общему урожаю и качеству плодов, имеют разные сроки созревания, некоторые находятся в госсортоиспытании, а остальные сортообразцы в дальнейшем будут рекомендованы для передачи, а также могут служить в качестве исходного материала для дальнейшей селекции томатов.

Ключевые слова: *томат, сортообразец, габитус, лист, форма плода, окраска плода, урожайность, качество.*