RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
58 (3) 980-989, 2026
http://doi.org/10.54910/sabrao2026.58.3.3
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

MOLECULAR CHARACTERIZATION OF DIFFERENT PATHOGENIC VIRUSES INFECTING SWEET CHERRY (*PRUNUS AVIUM* L.)

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SUMMARY

This study employed total RNA extraction, cDNA synthesis, and PCR analysis for molecular characterization of pathogenic viruses infecting stone fruits. Leaf samples of sweet cherry (*Prunus avium* L.) showing symptoms of viral infection entailed collection from the orchards located in the districts of Bostanliq and Parkent of Tashkent Region, Uzbekistan, with the total RNA subsequently extracted. The quantity and quality of the extracted RNA reached their evaluation, performing cDNA synthesis using high-quality RNA samples. Polymerase chain reaction (PCR) proceeded with virus-specific primers targeting major pathogens of stone fruit crops. These include the Prunus dwarf virus (PDV), Plum pox virus (PPV), Prunus necrotic ringspot virus (PNRSV), Peach rosette mosaic virus (PRMV), Little cherry virus (LCV), Cherry leaf roll virus (CLRV), and Cherry green ring mottle virus (CGRMV). During the examination, the presence of CLRV was successful for validation by PCR in the analyzed stone fruit samples. The obtained results revealed a distinct distribution of CLRV infection and provide a practical basis for the selection of virus-resistant cultivars. The results demonstrate a significant practical value for the selection of virus-resistant stone fruit cultivars. The obtained results highlight the practical relevance of molecular diagnostics for selecting virus-resistant stone fruit cultivars.

Communicating Editor: Prof. Naqib Ullah Khan

Manuscript received: September 13, 2025; Accepted: February 22, 2026.

Citation: Bekmatova EE, Akhmadaliev BJ, Abduvaliev BA, Mukhamedova MS, Avazmetova IR, Akhmedova MSH, Norkobilova ZB, Fayziev VB (2026). Molecular characterization of different pathogenic viruses infecting sweet cherry (*Prunus avium* L.). *SABRAO J. Breed. Genet. 58 (3) 980-989. http://doi.org/10.54910/sabrao2026.58.3.3*.

Keywords: Sweet cherry (*P. avium* L.), Cherry leaf roll virus (CLRV)*, Prunus* spp., RNA extraction, cDNA synthesis, PCR, molecular diagnostics

Key findings: Molecular characterization confirmed the cherry leaf roll virus (CLRV) in the studied virus samples of the cherries and stone fruits in Uzbekistan.

INTRODUCTION

Sweet cherry (*Prunus avium* L.), a species native to Europe and Western Asia, is a commercially considerable fruit crop cultivated extensively across the temperate regions worldwide. The sweet cherry production is susceptible to various biotic stresses, among which viral infections pose substantial threats. In recent decades, several viruses have been notably infecting sweet cherry, leading to significant economic losses due to reduced fruit yield and quality. The notable viruses among these are the Prunus dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV), little cherry virus (LCV), Cherry leaf roll virus (CLRV), and Cherry green ring mottle virus (CGRMV) (Nemeth, 1986; Hadidi *et al*., 2011; Martelli and Jelkmann, 2018). These viruses, primarily transmitted through vegetative propagation and, in some cases, through pollen, have been the subject of extensive research, particularly concerning their impact on plant physiology, symptomatology, and management strategies (Hadidi *et al*., 2011; Jelkmann and Eastwell, 2011).

Prunus dwarf virus (PDV) is a member of the genus *Ilarvirus*, characterized by its ability to infect various *Prunus* species, including sweet cherry. The virus-inducing symptoms are stunting, leaf chlorosis, and reduced fruit size, eventually leading to significant yield losses. Past studies have shown PDV is transmittable through grafting and vegetative propagation, complicating efforts to control its spread within orchards (Fuchs *et al.,* 2019).

Prunus necrotic ringspot virus (PNRSV), belonging to the genus *Tobamovirus*, is one of the most prevalent viruses affecting *Prunus* species. The infected fruit trees exhibit symptoms like necrotic ring spots on leaves, reduced growth, premature leaf drop, and reduced fruit yield. The said virus' main transmission is through vegetative propagation, making infected nursery stock a significant source of new infections in orchards (Fuchs *et al.,* 2019).

Little cherry virus (LCV) is a complex of viruses that cause the little cherry disease, characterized by small and pale fruits with poor flavor and reduced fruit yield. The virus complex includes several distinct viruses, and their exact composition can vary. Transmission occurs through vegetative propagation and possibly through pollen, though the latter route requires further investigation for confirmation (Fuchs *et al.,* 2019).

Cherry leaf roll virus (CLRV), a member of the family Betaflexiviridae, infects the sweet cherry and other *Prunus* species. It causes leaf rolling, chlorosis, and reduced fruit yield and quality. The transmission of the virus occurs through vegetative propagation and possibly through pollen, though the latter route requires further probing (Fuchs *et al.,* 2019).

Cherry green ring mottle virus (CGRMV) is a member of the genus *Tobamovirus*, infecting sweet cherry and other *Prunus* species. The virus induces symptoms, such as green ring mottling on leaves, reduced growth, fruit deformation, and reduced fruit yield. Transmission occurs through vegetative propagation and possibly through pollen; however, the latter route requires further scrutiny (Fuchs *et al*., 2019).

The primary mode of transmission of these viruses is through vegetative propagation, particularly by using infected scion wood and rootstocks. This emphasizes the importance of using certified virus-free planting materials to prevent the introduction and spread of these viral pathogens. Additionally, the potential for pollen-mediated transmission has been a subject of recent studies, highlighting the need for comprehensive management strategies that

include monitoring and controlling vector populations, removing infected trees, and employing resistant rootstocks where available.

Cherries are one of the most valuable stone fruits, with global production exceeding 3.5 million tons annually (FAOSTAT, 2020). Viral infections, particularly those caused by cherry leaf roll virus (CLRV), incur significant yield losses (15%–20%) each year in affected regions (Fuchs and Gutiérrez, 2021). Beyond fruit yield reduction, fruit quality is also severely compromised, limiting marketability and export potential. Economic assessments have authenticated that in major cherry-producing regions, such as the United States, Turkey, and several European Union countries, virus-associated diseases cause significant losses, ranging from USD 100 to 300 million annually (Barba *et al.,* 2019; Hadidi *et al*., 2022).

The systematic surveys of cherry viruses remain scarce, and the existing research on potato, tomato, and cereal viruses revealed their substantial impact on crop productivity and nutritional quality in Uzbekistan (Fayziev *et al*., 2020; Kholmatova *et al.,* 2024; Akhmadaliev *et al., 2025;* Sultonnazirova *et al., 2026*). These past findings considerably suggested the presence of CLRV in cherry orchards may represent a hidden but critical threat to the local fruit production and export market.

At present, no direct chemical treatment is available for controlling CLRV and other viruses infecting cherries (Maliogka *et al.,* 2018; Fuchs, 2021). Therefore, the preventive and integrated management strategies remain the cornerstone of control. The most effective approach involves the use of virus-free certified planting material, obtained through rigorous propagation and testing programs (Hadidi *et al.,* 2022). Additionally, strict adherence to sanitation and quarantine measures**,** coupled with the elimination of infected trees and control of potential insect vectors, has been remarkable in mitigating large-scale viral spread.

In molecular diagnostic technologies, recent advances, including reverse transcription polymerase chain reaction (RT-PCR), quantitative PCR, and next-generation sequencing (NGS), have considerably improved the sensitivity and reliability of virus identification and authentication in perennial crops (Fuchs, 2021). In Uzbekistan, the adoption of such diagnostic frameworks is crucial to establish an early detection system and prevent the widespread dissemination of CLRV and other viruses affecting *Prunus* spp.

In this context, the presented research represents the first molecular-genetic identification of CLRV in cherry leaves in Uzbekistan**,** using RT-PCR with virus-specific primers. These results will not only fill a critical gap in the knowledge of viral diseases affecting cherries in this region but also underscore the importance of establishing comprehensive monitoring and certification programs to ensure sustainable cherry production.

MATERIALS AND METHODS

Samples collection

The latest study collected leaf samples of sweet cherry (*P. avium* L.) showing symptoms of viral infection during April-May 2025 from intensive orchards located in the districts of Bostanliq and Parkent, Tashkent Region, Uzbekistan. Leaves from each sampled cherry tree entailed placement into sterile polyethylene bags, storage in a portable freezer at -20 °C, and subsequent transport to the laboratory for further analysis.

Isolation of total RNA

Isolation of total RNA from virus-infected cherry leaf samples proceeded using PureLink RNA mini kits from Invitrogen (ThermoFisher, USA) according to protocol. The sweet cherry leaf samples with PDV, PPV-D strains, PNRSV*,* PRMV, LCV, CLRV, and CGRMV disease symptoms sustained collection and extraction by placing 5 g in a porcelain mortar and adding liquid nitrogen. The 200 mg of the prepared extract, upon being taken, reached placement in a 2 ml Eppendorf test tube before thoroughly mixing in a vortex (Vortex MX-S, DLAB) after adding 1.5 ml of the lysis buffer (prepared in advance by adding 10 µl of 2-

mercaptoethanol to 1 ml of the lysis buffer). Then, it underwent incubation at room temperature for 3 min and spinning in a centrifuge for 5 min (2600 × g rpm). The separated supernatant succeeded its placement in a new test tube, adding the 96% ethanol at the ratio of 1:1.5 to the volume of the supernatant before being vortexed. Afterward, being poured into a filter tube included in the kit, it further incurred centrifugation for 15 s (12000 × g rpm), discarding the precipitate thereafter. The 700 µl of washing buffer (Wash buffer I), upon its addition, obtained centrifugation again for 15 s (12000 × g rpm).

Discarding the bottom of the column tube obtained a replacement with a new tube. Then, adding 500 µl of the second wash buffer (Wash buffer II), its centrifugation continued for 15 s (12000 × g rpm). After removing the filter part of the test tube, placing it in a new sterile test tube followed. Slowly adding the 100 µl of RNase-free sterile water (RNase-free water) to the central part of the test tube, incubation continued at room temperature for 2 min. After the specified time, its centrifugation for 2 min (12000 × g rpm) ensued, with the filter part of the tube removed. In this way, total RNA extraction succeeded in the lower tube.

Preparation of cDNA by reverse transcription

The use of the Invitrogen (Thermo Fisher, USA) reagent kit served for cDNA synthesis based on the isolated RNA matrix. For this, the preparation of a reaction mixture ensued by adding ddH_2O (2 µl) and 5 µl of isolated RNA and a reverse primer (Integrated DNA Technologies, Belgium) with a concentration of 10 pM/µl before incubation at 70 °C for 2 min. At the end of the incubation time, the mixture entailed a quick transfer to an ice-cold container. The reaction mixture for obtaining cDNA from RNA by the reverse transcription method includes reaction buffer (8 µl), dNTP (2 µl), reverse transcriptase (1 µl), and RNA (9 µl). The prepared reaction mixture succeeded in being placed in the amplifier at 37 °C for 60 min and 70 °C for 10 min.

RESULTS

The different viruses have wide distribution in stone fruits, causing severe infections in host plants (Sattorov *et al*., 2020). These mainly include Prunus dwarf virus (PDV), Plum pox virus (PPV), Prunus necrotic ringspot virus (PNRSV), Peach rosette mosaic virus (PRMV), Little cherry virus (LCV), Cherry leaf roll virus (CLRV), and Cherry green ring mottle virus (CGRMV). To date, no virus-tolerant commercial cultivars are available in stone fruits. For managing virus-induced diseases, the main integrating strategies include the use of virus-free planting material, the eradication of perennial plants that may serve as virus reservoirs, and the use of insecticides to control insect vectors responsible for virus transmission.

In surveyed areas, leaves of cherry, apricot, plum, and peach trees exhibited frequent physiological alterations and characteristics and virus-associated visual symptoms, including leaf curling, mosaic patterns, chlorosis, and necrosis (Figure 1). In the laboratory, successful design of virus-specific primers detected and authenticated the PDV and PPVD strains and PNRSV, PRMV, LCV, CLRV, and CGRMV, as well as viruses belonging to subgroup-C of the genus *Nepovirus*, using the RT-PCR method.

For this purpose, complete genome sequences and nucleotide sequences of the coat protein (CP) gene for CP synthesis of the aforementioned viruses preceded retrieval from the DNA database (GenBank, NCBI) and the Plant Virus Database through bioinformatic searches of strains reported worldwide. In FASTA format, the complete set of nucleotide sequences reached compilation, with multiple sequence alignment (MSA) of CP gene nucleotide sequences from different isolates also performed using bioinformatic tools. For PCR amplification of the CP gene, the design of primers used the Primer3Plus software (Figure 2). The primers correspond to the following nucleotide sequences (Table 1).

From infected leaves, the total RNA extraction utilized the Invitrogen™ PureLink™ RNA Mini Kit (Thermo Fisher, USA) as per the

Figure 1. Virus-specific disease symptoms observed in sweet cherry (*Prunus avium* L.) samples collected from District Bostanliq, Tashkent Region, Uzbekistan, including leaf curling, mosaic patterns, chlorosis, vein clearing, and necrotic lesions.

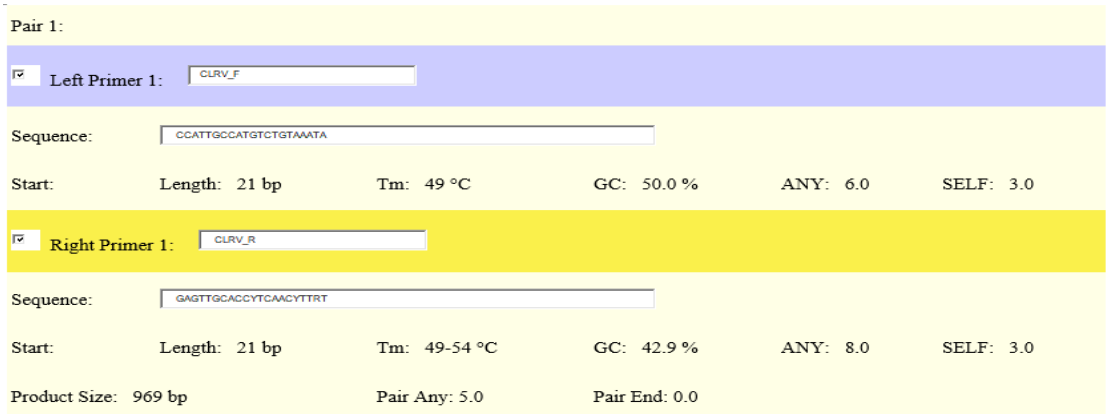

Figure 2. Primers designed using the Primer3Plus bioinformatics software.

manufacturer's instructions, with the same also described in the Materials and Methods section. The concentration and purity of the extracted RNA samples attained their determination using a NanoDrop Eight spectrophotometer (Figure 3). In determining RNA quantity and quality, the absorbance ratio (260/280) became the primary indicator (Table 2). The said ratio's consideration was because it is a reliable criterion for determining the presence of contaminants in RNA preparations, such as proteins, phenol, and other inhibitory substances. Calculated based on absorbance value and measured at the wavelengths of 260 nm and 280 nm, the ratio provides an estimate of the relative abundance of biomolecules within each sample. Therefore, the absorbance ratio (A260/A280) serves as an important diagnostic parameter for evaluating the quality of RNA extraction.

Table 1. Primers used in the analysis.

Primers	Primer sequence 5`----------3`	References
ChLRV-F	CCATTGCCATGTCTGTAAATA	This article
ChLRV-R	GAGTTGCACCYTCAACYTTRT	
PPV_F	ACCGGCAGCAACTAGCCCAA	This article
PPV_R	TCTTCTTGTGTTCCGACGTTTCCA	
PRMV_F	GACGCGTACGACAATGTTGA	This article
PRMV_R	CAATGCCATGTCATAGGAAAR	
PNSV_F	GTTCWGGRAGTGCTTRAGTGAC	This article
PNSV_R	TTCGCAATATCCACTAHCGGTGGT	
LCV_F	TTATTTGWTGAAACCCACATA	This article
LCV_R	CTATCCAAWAYTGMAAAATRAC	
PDV_F	GTGTAACGATTGGTTAACTCAC	This article
PDV_R	ATTCAGTGACAAAMTCTGAATG	
CGRMV_F	TTCAGCATYACTGCATTCATCAT	This article
CGRMV_R	ACTTTAGCTTCGCCCCGTGT	
Nepo_S_U_F	TTRKDYTGGYKAAMYYCCA	Michele Digiaro *et al*., 2007
Nepo_S_U_R	TMATCSWASCRHGTGSKKGCCA	

Note: According to the IUPAC nomenclature, S = C or G; V = A, C, or G; Y = C or T; R = A or G; W = A or T; M = A or C; D = A, G, or T; K = G or T; and H = A, C, or T nucleotides.

Table 2. Concentration of RNA samples.

Sample ID	Concentration (ng/µL)	A260/A280 ratio
1	113.7	1.74
2	94.6	1.76
3	45.2	1.55
4	39.1	1.60

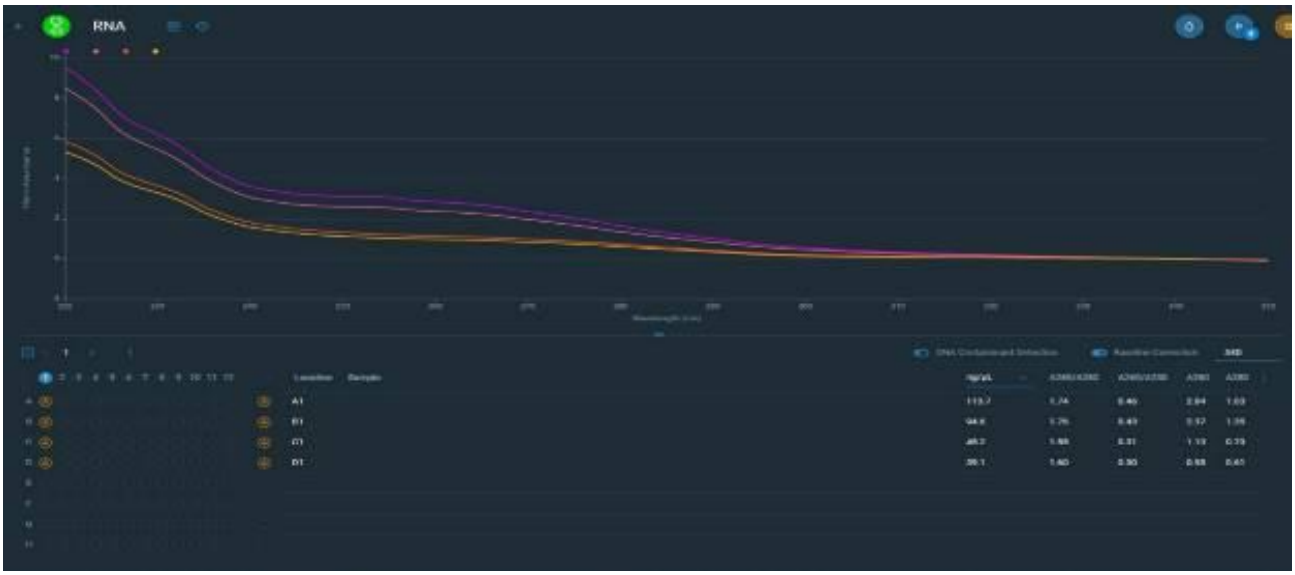

Figure 3. Spectrophotometric analysis of extracted RNA samples.

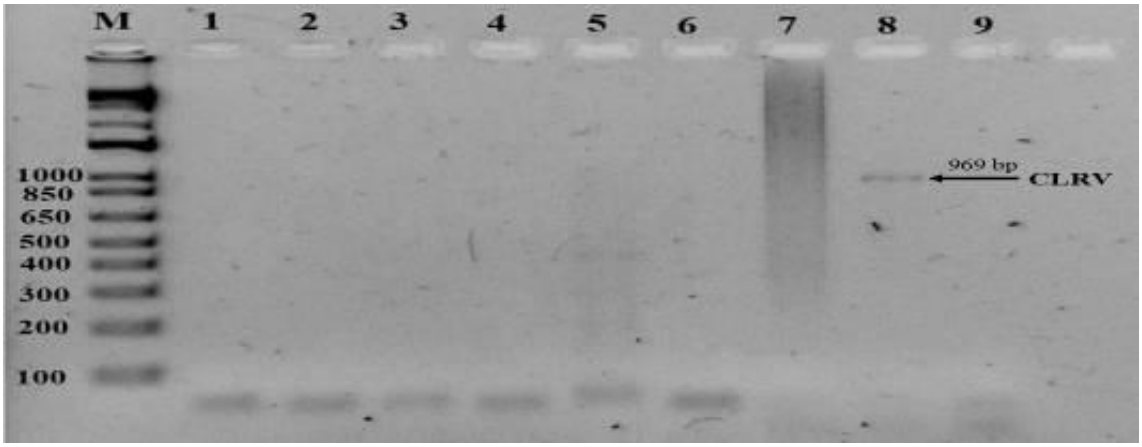

Figure 4. Electropherogram of amplification products obtained by two-step RT-PCR. *Note:* M – 1 kb DNA Ladder Plus; 1 – Plum pox virus (PPV-D strain); 2 – Peach rosette mosaic virus (PRMV); 3 – Prunus necrotic ringspot virus (PNRSV); 4 – Little cherry virus (LCV); 5 – Prunus dwarf virus (PDV); 6 – Cherry green ring mottle virus (CGRMV); 7 – Nepovirus subgroup C; and 8 – Cherry leaf roll virus (CLRV).

The RNA sample with the highest purity was the option for subsequent analyses. From the isolated RNA, the complementary DNA (cDNA) synthesis followed the established protocol, performing the two-step RT-PCR. Using specific primers individually, the conducted PCR assays detected PDV and PPV-D strains and PNRSV, PRMV, LCV, CLRV, and CGRMV, as well as viruses belonging to subgroup C of the genus *Nepovirus*. The resulting PCR amplicons incurred analysis and visualization using a gel transilluminator (BK AG-100 Gel Imaging Analysis System, Biobase, China) (Figure 4).

The electropherogram analysis confirmed that amplification using the primer pair CLRV_F/CLRV_R in two-step RT-PCR generated the expected amplicons, thereby providing considerable and reliable evidence for the presence of CLRV infections in the tested leaf samples. The results highlighted the effectiveness of the applied molecular diagnostic approach for accurate CLRV detection and authentication. Importantly, no amplification products corresponding to other target viruses were evident. This indicated the Cherry green ring mottle virus (CGRMV), Prunus dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV), Plum pox virus D strain (PPV-D), little cherry virus (LCV), and Peach rosette mosaic virus (PRMV) did not occur in the analyzed cherry leaf samples.

This result suggested that CLRV represents the predominant viral pathogen affecting the studied *Prunus* spp. under the existing field conditions. To date, numerous studies have taken place in Uzbekistan on the detection and molecular diagnostics of potato viruses (Fayziyev *et al*., 2020; Jovlieva *et al*., 2024; Kholmatova *et al*., 2024), tomato viruses (Akhmadaliev *et al*., 2024), and cereal and legume viruses (Makhmudov *et al*., 2025; Sobirova *et al*., 2025; Abduvaliev *et al*., 2025). However, not a single study has transpired to date on viruses infecting sweet cherry (*P. avium* L.) in Uzbekistan. The relevant research results demonstrated, for the first time, the molecular-genetic diagnosis of Cherry leaf roll virus (CLRV) in sweet cherry leaves using PCR.

However, at present, the management of viral diseases in stone fruits remains a major challenge worldwide, as no effective chemical treatments are available for directly targeting and controlling these viruses. Therefore, the early identification of infected plants is of crucial importance as a primary preventive measure to limit the spread of viral diseases in fruit trees.

DISCUSSION

The molecular diagnostic approach employed in this study-conventional two-step RT-PCR targeting the coat protein gene-provided reliable detection of CLRV in symptomatic tissues. However, the landscape of plant virus diagnostics has evolved substantially, with recent advances highlighting both opportunities and limitations of different methodological platforms. Real-time quantitative PCR (RT-qPCR) and reverse transcription loop-mediated isothermal amplification (RT-LAMP) have emerged as valuable complementary tools, offering enhanced sensitivity for detection in asymptomatic tissues and reduced dependency on sophisticated thermocycling equipment (Babu *et al*., 2024). These methodologies are particularly relevant for CLRV, which can establish persistent infections in cherry germplasm without inducing overt symptoms, thereby serving as cryptic inoculum reservoirs within propagation networks (Digiaro *et al*., 2024). The integration of such high-throughput compatible assays into routine screening programs would significantly strengthen early warning capabilities in Uzbekistan's emerging cherry industry.

The molecular diagnostic techniques' implementation, particularly reverse transcription polymerase chain reaction (RT-PCR) and next-generation sequencing (NGS), has significantly enhanced our ability to detect and authenticate the viruses infecting sweet cherry (*P. avium* L.). In this study, the presence of Cherry leaf roll virus (CLRV) attained successful identification in sweet cherry leaf samples collected from the districts of Bostanliq and Parkent, Tashkent Region, Uzbekistan, utilizing RT-PCR assays targeting the coat protein gene. The results were greatly analogous to previous research findings, indicating the widespread occurrence of CLRV in various *Prunus* species. For instance, a study by Fuchs *et al*. (2019) reported the detection of CLRV in sweet cherry orchards in the United States, highlighting the global distribution of this pathogen. Similarly, Milusheva *et al*. (2019) identified a novel virus, tentatively named Cherry Virus Trakiya (CVT), in sweet cherry trees in Bulgaria, underscoring the diversity of viral pathogens affecting cherry fruit orchards.

In asymptomatic trees, the detection of CLRV suggested the presence of latent infections, which could serve as reservoirs for further spread of the virus. This observation was also consistent with past findings by Zhang *et al*. (2014), who also reported that certain viruses can persist in asymptomatic hosts, complicating the effective strategies planned for disease management.

This study's use of RT-PCR revealed the highest sensitivity and specificity, confirming its efficacy as a diagnostic tool for viral infections in sweet cherries. These results agreed with the previous work of Simkovich *et al*. (2021), who also highlighted the reliability of RT-PCR in detecting Prune dwarf virus (PDV) in sweet cherries. Moreover, the development of full-length infectious cDNA clones offers valuable resources for functional studies and the development of resistant cultivars through breeding and intensive selection in sweet cherry (Simkovich *et al*., 2021).

The presented study focused on CLRV; however, it is noteworthy to mention that sweet cherry fruit trees are susceptible to a wide range of other viruses, including PDV, PNRSV, and the little cherry virus 2 (LChV2). The simultaneous detection of multiple viruses using multiplex RT-PCR assays can provide a comprehensive understanding of the viral landscape in cherry fruit orchards (Zong *et al*., 2014). The development of a multiplex reverse transcription-polymerase chain reaction (mRT-PCR) was successful, which was standardized for the simultaneous detection of four cherry viruses. These are Cherry virus A (CVA, genus: *Capillovirus*), Cherry necrotic rusty mottle virus (CNRMV, unassigned species of the Betaflexiviridae), little cherry virus 1 (LChV-1, genus: *Closterovirus*), and Prunus necrotic ringspot virus (PNRSV, genus: *Ilarvirus)* with nad5 as plant internal control (Noorani *et al.,* 2013).

The detection of CLRV in sweet cherry orchards highlights its potential epidemiological significance, as latent infections may serve as reservoirs for virus spread through propagation materials, posing a risk to regional cherry production.

CONCLUSIONS

In the pioneering study, the employed molecular diagnostic approaches have proven to be effective in identifying viral infections in sweet cherry (*P. avium* L.). The detection of CLRV in asymptomatic trees emphasized the need for regular monitoring and using certified virus-free planting materials to prevent the spread of viral pathogens. Further research is essential to explore the full spectrum of viral infections in sweet cherries and develop integrated disease management strategies that incorporate molecular diagnostic tools. These findings emphasize the importance of using certified virus-free planting materials and implementing regular molecular monitoring programs to prevent the spread of CLRV.

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