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MOLECULAR CHARACTERIZATION BASED ON COAT PROTEIN SEQUENCES OF THE COTTON LEAFROLL DWARF VIRUS IN UZBEKISTAN

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SUMMARY

Cotton leafroll dwarf virus (CLRDV) is a significant aphid-transmitted *Polerovirus,* causing yield loss in cotton globally, leading to symptoms like leaf reddening/rolling, stunting, maroon stems, and bushy tops through asymptomatic infection. CLRDV, which belongs to the family Solemoviridae, is an economically important phytopathogen that causes considerable growth reduction, yield losses, and fiber quality deterioration in cotton. In this study, the CLRDV investigation ensued in cotton samples collected from fields in the Tashkent Region, Uzbekistan. Total RNA extraction came from symptomatic plants, with polymerase chain reaction (PCR) assays performed using specific primers targeting the coat protein (*CP*) gene of the virus. Several cotton samples reached positive identification for the CLRDV, with the resulting amplicons sequenced to determine the viral nucleotides' composition. Based on sequence data, the obtained isolate, as deposited in the GenBank database, had the name UZKIB-1. Phylogenetic analysis revealed the UZKIB-1 shared 96.58% nucleotide identity with CLRDV isolates Henan and CN-S5 (China) and Q3514 (Thailand). Eleven key mutations also succeeded in their detection, indicating ongoing evolutionary divergence within regional CLRDV populations. The results confirmed the presence of CLRDV in Uzbekistan, which will contribute to understanding its genetic diversity and epidemiological significance.

Keywords: Cotton leafroll dwarf virus, field surveys, RT-PCR, coat protein gene, phylogenetic analysis, Tashkent Region

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Key findings: Cotton leafroll dwarf virus (CLRDV) detection was successful in various cotton samples collected from the Tashkent Region, Uzbekistan, using molecular analysis. The nucleotide sequence of the UZKIB-1 isolate showed 96.58% identity with CLRDV isolates Henan and CN-S5 (China) and Q3514 (Thailand). Phylogenetic analysis and the identification of 11 mutations revealed ongoing genetic divergence within the CLRDV population in Uzbekistan.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most viable industrial crops in Uzbekistan's agricultural sector and plays a crucial role in the textile, oil-processing, livestock feed, and associated industries. In the present era, the incidence and effect of viral diseases in cotton-growing regions have considerably increased, posing a serious threat to sustainable cotton production. Among these emerging pathogens, the cotton leafroll dwarf virus (CLRDV) has gained more attention due to its rapid spread and severe effects on plant growth and development, seed cotton yield, and fiber quality (Doyle and Doyle*,* 1990; Smith *et al.,* 2018). Typical symptoms of the CLRDV infection include leaf rolling, plant dwarfism, substantial reductions in boll number and productivity, and reduced fiber quality (Rojas and Gilbertson*,* 2017; Edula *et al.,* 2023).

The first report on the CLRDV was in Turkmenistan in 1938, followed by its detection in Tajikistan in 1946 and Armenia in 1947. Since then, the CLRDV has expanded its geographical range, with reports of prevalence now in major cotton-growing regions of Africa, South America, Asia, and North America. In the United States, identifying CLRDV first occurred in Alabama in 2017, and subsequent reports emerged in several other cotton-growing states, where it caused considerable yield losses and fiber quality deterioration (Silva *et al.,* 2008; Aboughanem *et al.,* 2019). CLRDV is the major concern in the U.S. cotton belt, requiring monitoring for management, with research exploring genetic resistance and advanced detection tools like CRISPR (clustered regularly interspaced short palindromic repeats). The virus belongs to the family Solemoviridae, genus *Polerovirus*, and possesses a positive-sense single-stranded RNA genome of approximately 5.7 kb comprising seven open reading frames (ORFs). The virus has a spherical shape and consists of a single-stranded RNA genome. Its transmission is not mechanical but is primarily through biological vectors, which play a key role in its epidemiology and transmission dynamics. CLRDV's persistent transfer results from the cotton aphid (*Aphis gossypii*), which plays a key role in its epidemiology (Heilsnis *et al.,* 2022).

Recent studies have emphasized the importance of molecular characterization and phylogenetic analysis in understanding the evolution, genetic diversity, and geographical distribution of CLRDV isolates. Comparative sequence analyses revealed distinct phylogenetic clustering patterns associated with regional virus populations, revealing that geographical separation and vector dynamics notably influenced the CLRDV evolution (Edula *et al.,* 2023). Molecular diagnostic tools, including RT-PCR and nucleotide sequencing, appeared to be crucial for the accurate detection and differentiation of CLRDV isolates, particularly in arable regions where the virus has had no previous reports.

Effective management of CLRDV relies on integrated disease control strategies, including the use of virus-free planting materials, aphid vector management, optimized agronomic practices, and the development of resistant cotton cultivars. However, management of these strategies largely depends on the early detection and a clear understanding of the virus population structure at the regional levels. In Uzbekistan, several cotton-infecting viruses have also succeeded in their identification using molecular approaches, including the cotton leaf curl virus (CLCuV) (Kadirova *et al.,* 2024).

Current studies have reported the molecular identification of viruses infecting various vegetable and cereal-legume crops, indicating an increasing genetic diversity of economically important plant viruses in

Uzbekistan (Sobirova *et al.,* 2024; Abduvaliev *et al.,* 2025; Akhmadaliev *et al.,* 2025; Makhmudov *et al.,* 2024, 2026). However, molecular and genomic characterization of viruses affecting cotton, a crop of strategic economic importance, remains insufficiently explored, highlighting the urgent need for comprehensive and systematic research in this area. Therefore, the presented study aimed to determine the CLRDV occurrence and genetic diversity in cotton fields of the Tashkent Region, Uzbekistan, based on coat protein (CP) gene sequences.

MATERIALS AND METHODS

Field sampling and RNA extraction

In July-August 2024, the survey of cotton plants showing typical CLRDV symptoms transpired in cotton fields of the Tashkent Region, Uzbekistan. Symptomatic leaf samples entailed collection, transport on ice, and storage at −80 °C until analysis. Total RNA extraction was from 0.1–0.2 g of leaf tissue using the PureLink™ RNA Mini Kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions**.** The RNA concentration and purity assessment used a NanoDrop Eight spectrophotometer.

Synthesis of the cDNA

The cDNA synthesis employed the SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific, USA) and buffers in a final volume of 20 µl. The study took 5 ml from the total extracted RNA solution, mixing it with 1 µl of 10 µM reverse primer CLRDV-CP_R1 (Table 1), 4 µl of 2.5 mM dNTP, and 3 µl of nuclease-free water. Afterward, the samples' incubation commenced at 65 °C for 5 min before quenching in ice for 3 min. The adding of other reaction components had the following order: 4 µl of 5X PCR buffer, 1 µl of 0.1 M dithiothreitol and 0.5 µl (20 U) of SuperScript IV reverse transcriptase, and nuclease-free water to give a reaction volume of 20 µl. The tubes underwent incubation for 1 h 55 min at 41 °C on a T960 PCR Thermal Cycler, followed by 10 min of heating at 70 °C to denature the enzyme.

Amplification by PCR

Reverse primer Pol3982R and forward primer CLRDV3675F sought to amplify a 310 bp PCR product within the viral coat protein gene of BYDV (Sharman *et al.,* 2015). The PCR reactions proceeded using the Platinum hot start PCR 2x master mix (Thermo Fisher Scientific, USA) in a 25 µl volume, containing 4 µl of 2X Master Mix, 0.5 µl of each primer (10 µM), 0.9 µl of 25 mM MgCl, and 4 µl of the cDNA. The following thermocycling program continued for PCR: initial denaturation at 94 °C for 2 min, then 45 cycles: denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, elongation at 72 °C for 45 s, 1 cycle, and final elongation at 72 °C for 5 min. The names and sequences of the forward and reverse PCR primers served to detect the CLRDV (Table 1).

Electrophoresis in agarose gel

The analysis of PCR products continued on a 2% agarose gel prepared in 1× TBE buffer before staining with ethidium bromide. The samples' mixture with the DNA Gel Loading Dye (6X) runs alongside a 100 bp DNA ladder. Electrophoresis took place at 100 V using a horizontal system, with PCR products visualized under UV light and photographed using a gel documentation system.

Table 1. Primers used in this study.

Target region	Name of the primers	Sequence (5´-3´)	Product size (bp)	Source
CP	Pol3982R	CGAGGCCTCGGAGATGAACT	310	Sharman *et al.* (2015)
CP	CLRDV3675F	CCACGTAGRCGCAACAGGCGT		

Sequencing of PCR products

Excising one PCR band used a razor blade before being transferred to a 1.5 ml centrifuge tube and purified using the PureLink™ Quick Gel Extraction Kit (Invitrogen, USA), according to the manufacturer's instructions. The cycle sequencing reaction proceeded using the BigDye® Terminator v 3.1 kit (Applied Biosystems, USA). The cycle sequencing reaction comprised ddH_2O–3.5 µl, BigDye–1 µl, 5x Seq. Buffer–2 µl, sequencing primer–0.5 µl, and purified PCR product–2 µl. CLRDV-CP_R1 and CLRDV-CP_F1 primers served for sequencing. Using the following thermocycling program for cycle sequencing reaction included: initial denaturation stage at 96 °C for 1 min; denaturation at 96 °C for 10 s; annealing at 60 °C for 10 s; and elongation at 60 °C for 3 min, with repetitions for 45 consecutive cycles. The product of the sequence reaction entailed storage at 4 °C.

The sequencing reaction products received purification from fluorescently labeled terminator nucleotides using the Dynabeads Sequencing Clean-Up Kit (Applied Biosystems, USA). Separation and analysis of DNA sequence reaction products occurred by capillary gel electrophoresis, performing laser-induced fluorescence detection on the Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific). Sequence alignments and phylogenetic analyses comprised the use of the SnapGene 5.3.1 program to edit the raw data obtained from the DNA sequence analysis system. Nucleotide sequence examination used the BLASTN program (basic local alignment search tool nucleotide-nucleotide, http://www.ncbi.nlm.nih.gov/BLAST/). Performing multiple sequence alignments preceded by ClustalW. Phylogenetic tree construction was by the neighbor-joining (NJ) method using the Kimura 2-parameter model (Kimura*,* 1980), with statistical support performed by the bootstrap method of interior-branch test for phylogenetic trees. The conduct of the bootstrap test of phylogeny and visualization of the final phylogenetic tree engaged the MEGA11 software package and Molecular Evolutionary Genetics Analysis version 11 (Tamura *et al.,* 2021).

RESULTS

In July-August 2024, phytosanitary monitoring commenced in cotton fields planted in the Qibray District, Tashkent Region, to explore the CLRDV’s presence. The damaged wheat plant samples underwent collection from field areas with the following geographic coordinates: 41°22′53.4″ N 69°21′18.4″ E and 41°24′39.99″ N 69°29′01.8″ E. During the survey, the virus-infection-specific symptoms were visible in cotton plants, including leaf rolling and deformation, reddening of leaves, stunted growth, leaf abscission, and disruption of reproductive development (Figure 1). Amplifying a partial sequence of the CP gene had the RT-PCR performed on the collected cotton samples. Using CLRDV3675F and Pol3982R specific primers helped obtain the PCR products of approximately 310 bp (Figure 2). The BLAST analysis in the NCBI database confirmed that the sequenced fragment belonged to the CLRDV serotype.

The BLAST analysis of the nucleotide sequence database revealed the Uzbekistan isolate of cotton leafroll dwarf virus (CLRDV), UZKIB-1, showed nucleotide sequence similarity, ranging from 87.94% to 96.58%, with other CLRDV isolates. The highest genetic similarity (96.58%) was evident with two Chinese isolates, viz., CN-S5 (KX588248.1) and Henan (OK050525.1), and one Thai isolate, Q3514 (KP176644.1). Additionally, the UZKIB-1 shared similarity with three different isolates, i.e., the Argentine isolate M5 atypical (95.34%: KF359947.1), the USA isolate MS06 (95.03%: OQ107461.1), and the Brazilian isolate CLRDV (94.69%: HQ827780). However, the lowest nucleotide sequence similarity appeared with the Sudanese isolate SuCp31-15 (87.94%: MK411565.1).

For phylogenetic analysis, the partial nucleotide sequence of the CP and MP genes of the Uzbekistan isolate, UZKIB-1 PV806584.1, sustained comparison with 11 strains of CLRDV available in the GenBank. Based on the nucleotide sequences of CLRDV isolates, the constructed phylogenetic tree revealed an apparent genetic clustering corresponding to their geographical origin. These isolates proceeded to group into distinct regional

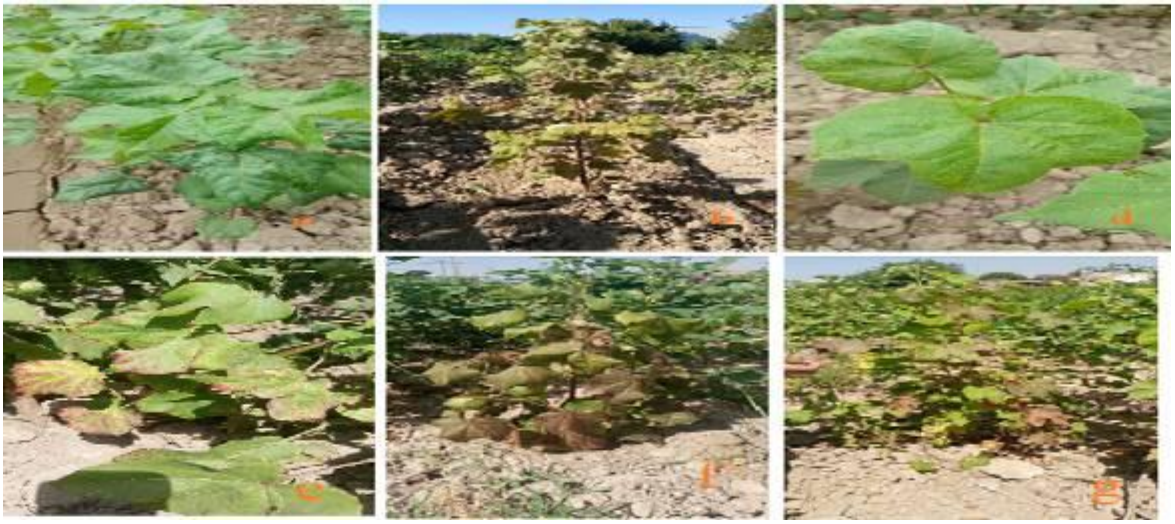


Figure 1. CLRDV disease symptoms in cotton (*Gossypium hirsutum* L.) fields of Tashkent Region, Uzbekistan.

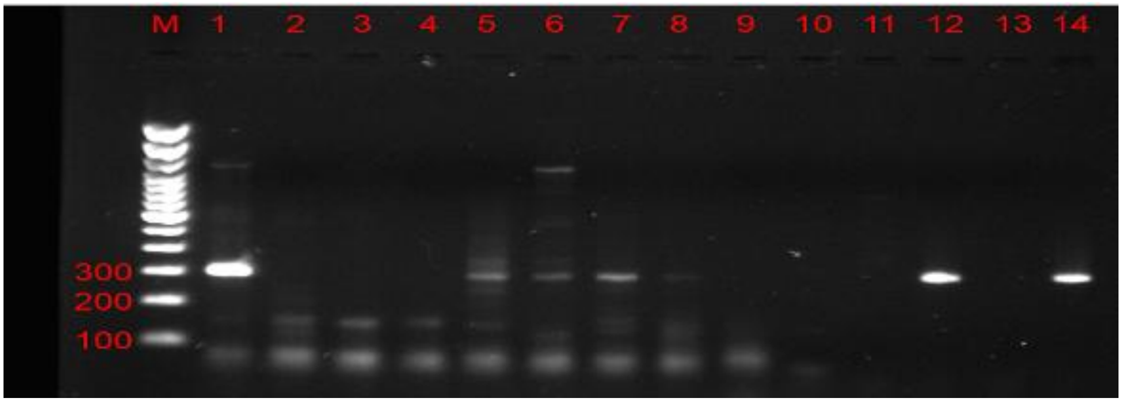


Figure 2. Electropherogram of RT-PCR performed with CLRDV-R1/CLRDV-F1 primers in cotton samples from Tashkent Region; M - DNA 100 bp DNA Ladder; 1,5,6,7,12, and 14 = cotton samples.

clades, confirming the evolutionary diversity and regional adaptation of the said virus. South American isolates from Brazil and Argentina formed a single clade supported by moderate to high bootstrap values (53%–69%), indicating this region plays a significant role in the virus's evolution.

In contrast, North American isolates from the United States clustered into a separate subclade, revealing a relatively stable CLRDV population in this region. The Uzbekistan isolate, UZKIB-1 (PV806584), clustered within the Asian clade and showed a close genetic relationship with isolates from China and Thailand, supported by the highest bootstrap values (88%–92%). These results suggested a shared evolutionary origin of CLRDV in Asia and the existence of regional dissemination pathways. The Sudan isolate formed a distinct branch, indicating the African CLRDV population represented an independent evolutionary lineage. Overall, the phylogenetic analysis confirmed the geographical separation emerged as a key factor shaping the genetic diversity of CLRDV and provided molecular evidence of the virus's presence in Central Asia (Figure 3).

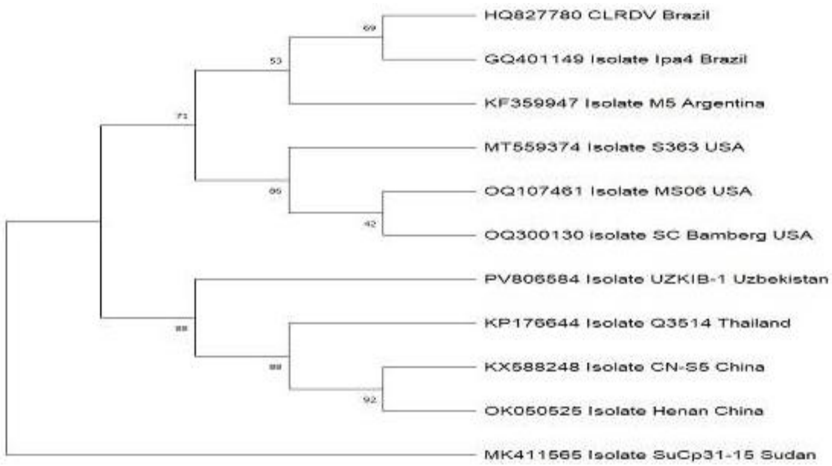
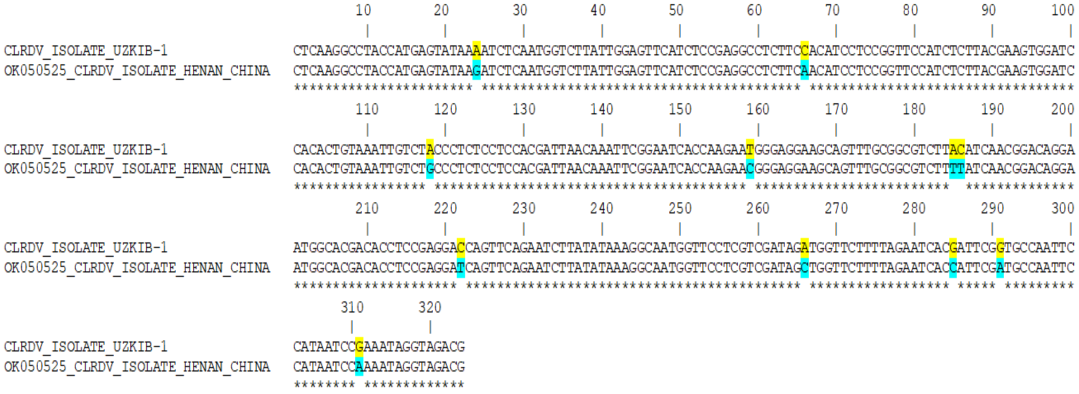


Figure 3. Neighbor-joining (NJ) tree for the CP gene of CLRDV UZKIB-1 isolates. Numbers above branches were the bootstrap support values based on 1000 replicates.



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                                             10        20        30        40        50        60        70        80        90       100
                                              |         |         |         |         |         |         |         |         |         |
CLRDV_ISOLATE_UZKIB-1                CTCAAGGCCTACCATGAGTATAAAATCTCAATGGTCTTATTGGAGTTCATCTCCGAGGCCTCTTCCACATCCTCCGGTTCCATCTCTTACGAAGTGGATC
OK050525_CLRDV_ISOLATE_HENAN_CHINA   CTCAAGGCCTACCATGAGTATAAGATCTCAATGGTCTTATTGGAGTTCATCTCCGAGGCCTCTTCAACATCCTCCGGTTCCATCTCTTACGAAGTGGATC
                                     *********************** ***************************************** **********************************
                                            110       120       130       140       150       160       170       180       190       200
                                              |         |         |         |         |         |         |         |         |         |
CLRDV_ISOLATE_UZKIB-1                CACACTGTAAATTGTCTACCCTCTCCTCCACGATTAACAAATTCGGAATCACCAAGAATGGGAGGAAGCAGTTTGCGGCGTCTTACATCAACGGACAGGA
OK050525_CLRDV_ISOLATE_HENAN_CHINA   CACACTGTAAATTGTCTGCCCTCTCCTCCACGATTAACAAATTCGGAATCACCAAGAACGGGAGGAAGCAGTTTGCGGCGTCTTTTATCAACGGACAGGA
                                     ***************** **************************************** *************************  **************
                                            210       220       230       240       250       260       270       280       290       300
                                              |         |         |         |         |         |         |         |         |         |
CLRDV_ISOLATE_UZKIB-1                ATGGCACGACACCTCCGAGGACCAGTTCAGAATCTTATATAAAGGCAATGGTTCCTCGTCGATAGATGGTTCTTTTAGAATCACGATTCGGTGCCAATTC
OK050525_CLRDV_ISOLATE_HENAN_CHINA   ATGGCACGACACCTCCGAGGATCAGTTCAGAATCTTATATAAAGGCAATGGTTCCTCGTCGATAGCTGGTTCTTTTAGAATCACCATTCGATGCCAATTC
                                     ********************* ******************************************* ****************** ***** *********
                                            310       320
                                              |         |
CLRDV_ISOLATE_UZKIB-1                CATAATCCGAAATAGGTAGACG
OK050525_CLRDV_ISOLATE_HENAN_CHINA   CATAATCCAAAATAGGTAGACG
                                     ******** *************
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Figure 4. Alignment of partial sequence of coat protein and movement protein genes of CLRDV isolate UZKIB-1 (Uzbekistan) and CLRDV isolate Henan (China).

Multiple sequence alignment (using ClustalW) considerably suggested that 11 mutations, viz., 24G>A, 66A>C, 118G>A, 161C>T, 185T>A, 186T>C, 222T>C, 266C>A, 285C>G, 291A>G, and 311A>G, have considerably contributed to the exploration of the evolutionary history of the cotton leafroll dwarf virus isolate, UZKIB-1 Uzbekistan haplotype (Figure 4).

DISCUSSION

In this study, the coat protein (CP) gene of the cotton leafroll dwarf virus (CLRDV) isolate, UZKIB-1, succeeded in its analysis for the first time under the environmental conditions of Uzbekistan. Within plant tissues and systemic spread, the CP gene plays a crucial role in viral movement; therefore, its molecular characteristics proved crucial for understanding the virus's adaptation, dissemination mechanisms, and evolutionary trajectories. Recent studies have also emphasized the extensive evolutionary diversity of the CLRDV genome, showing that virus isolates from different regions, such as those from the USA, were genetically distinct from South American isolates (Olmedo *et al.,* 2025).

The BLAST analysis revealed the UZKIB-1 isolate exhibited the highest level of nucleotide similarity with CLRDV isolates from the Asian region, particularly those from China and Thailand, suggesting potential regional transmission pathways and possible mechanisms of virus introduction into Uzbekistan. The recent phylogenetic studies enunciated the geographic structuring of CLRDV isolates as a critical factor influencing the virus's dissemination history, with the whole-genome analysis confirming the clustering of the virus population into distinct clades (Ramos *et al.,* 2021).

Phylogenetic analysis disclosed the UZKIB-1 isolate belongs to the Asian clade, supporting either a common evolutionary origin in Asia along with the existence of regional dissemination pathways. However, the Sudanese isolate formed a separate branch, highlighting that the African CLRDV population may represent an independent evolutionary lineage, indicating regional genetic diversity of the virus.

The multiple sequence alignment revealed the 11 identified mutations crucially contributed to the evolutionary history of the UZKIB-1 isolate. The RNA viruses typically exhibited the highest mutation rates, leading to the emergence of new isolates and variants, which may result in diverse symptom expression and virulence levels (Akinyuwa *et al.,* 2024). Vectors, particularly the aphid (*Aphis gossypii*), play a pivotal role in understanding CLRDV phenotypes and transmission mechanisms. Past studies have shown that these aphids efficiently transmit the CLRDV virus and determine its epidemiological sensitivity, which requires consideration for developing regional monitoring and management strategies (Heilsnis *et al.,* 2024).

The *A. gossypii*'s role as a vector is primary in understanding CLRDV epidemiology. Several studies have authenticated that CLRDV transmission exclusively happens via *A. gossypii* in a persistent and non-propagative manner; therefore, the virus does not replicate within the vector but can be carried and transmitted over extended periods (Heilsnis *et al.,* 2022). The vector aphid quickly acquires the virus, transmitting it to new plants shortly thereafter—a late (winged) morph proved capable of transmitting CLRDV within 40 seconds, thereby increasing its epidemiological potential (Michelotto *et al.,* 2007). The *A. gossypii* with the highest efficiency revealed the number of vectors, their acquisition and transmission rates, and its population dynamics directly affect the CLRDV spread (Heilsnis *et al.,* 2022).

Furthermore, due to the long-lasting nature of virus retention in aphids and the short transmission times, chemical insecticide-based control of *A. gossypii* populations does not significantly reduce CLRDV spread. This is because the aphids often transmit the virus to new plants before being controlled through different measures (Mahas et *al.,* 2022). The results emphasized the need for improved regional monitoring systems, in-depth studies of vector population dynamics, and integrated management strategies that explore the vector-virus relationship.

Overall, the pioneering study provides the first molecular evidence of CLRDV presence in cotton fields of Uzbekistan and offers valuable insights into the virus's genetic characteristics and regional evolutionary relationships. These findings can establish a robust scientific base for virus surveillance, enhanced quarantine measures, cotton cultivar screening for resistance, and the development of improved diagnostic strategies.

CONCLUSIONS

The timely study provides the first molecular evidence of cotton leafroll dwarf virus (CLRDV) in cotton fields of Kibray District, Tashkent Region, Uzbekistan. Virus-specific symptoms observed during field surveys reached confirmation by the RT-PCR and nucleotide sequencing. The isolate, UZKIB-1 (PV806584.1), exhibited the highest nucleotide sequence similarity with other CLRDV isolates, showing the close genetic relationship with isolates from China and Thailand. Phylogenetic analysis revealed that CLRDV isolates clustered according to their geographical origin, firmly placing the isolate UZKIB-1 within the Asian clade. The multiple sequence alignment identified 11 key mutations that likely contributed to the evolutionary history of the UZKIB-1 haplotype. Overall, the results confirmed the CLRDV presence in Central Asia. These highlighted the importance of geographical separation in shaping viral diversity, providing a molecular basis for regional surveillance, vector management, and the development of disease control strategies in cotton cultivation.

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