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BEAN COMMON MOSAIC VIRUS (BCMV) IDENTIFICATION USING PROTEIN COAT GENE AND PHYLOGENETIC ANALYSIS

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SUMMARY

The climatic conditions in Uzbekistan are highly favorable for cultivating leguminous crops, particularly the common bean (*Phaseolus vulgaris* L.). In the recent past, infections with the bean common mosaic virus (BCMV) have been evident in various crops across the country, leading to a decline in grain yield and quality. Phytoviruses infect bean plants and manifest through specific disease symptoms with considerable damage in leguminous crops, especially beans, mung beans, and peas. According to a monitoring conducted in various districts of the Tashkent Region, the PCR analysis revealed that BCMV was the most widespread virus. The collected BCMV-diseased plant samples underwent molecular-genetic identification based on the capsid protein (*CP*) gene. The PCR product succeeded in sequencing, with the resulting isolate being registered in the NCBI database under the name 'Bean common mosaic virus isolate UZ-1' with the accession number PQ442186.1. Phylogenetic analysis of this isolate revealed 97% similarity with the Chilean isolate (LI9539.1) and 99% similarity with isolates from phylogenetic lineages in Vietnam (LC775775.1), Nepal (MW620828.1), Russia (KF919300), and Africa (AF361337).

Keywords: Common bean (*P. vulgaris* L.), RT-PCR, *CP* gene, bean common mosaic virus isolate 'UZ-1,' primer, phylogenetic analysis

Key findings: Using RT-PCR**,** the bean common mosaic virus isolate 'UZ-1' attained identification in the common bean (*P. vulgaris* L.) plants. The nucleotide sequence of the *CP* gene**,** responsible for encoding the viral coat protein**,** sustained assessment. Bioinformatics analysis resulted in the phylogenetic tree for BCMV-'UZ-1,' which helped in determining the evolutionary origin of the virus.

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INTRODUCTION

In common bean (*Phaseolus vulgaris* L.) production, India is the leading country, as it produced 6,610,000 tons of dry beans**,** accounting for 23.32% of the total global production (Adams*,* 2019)*.* In the Republic of Uzbekistan during 2023, more than 330,500 ha of land was under legume crop sowing, with 35,800 ha (11%) allocated for bean cultivation. Beans were the primary crop planted on 5,100 ha and on 24,500 ha as a secondary crop (Abduvaliev *et al*., 2024). However, in previous years, various regions of the country faced the bean common mosaic virus (BCMV) infection, leading to a decline in crop yield and deterioration in product quality (Makhmudov *et al*., 2023; Kholmatova *et al*., 2024).

Among CIS (Country/Commonwealth of Independent States) countries, beans (*Phaseolus vulgaris* L.) are the primary cultivated crop in Ukraine, Moldova, and the Caucasus Region. From an economic perspective, the world's largest suppliers of dry beans are China (USD 573 million), Myanmar (USD 570 million), and the United States (USD 390 million), collectively accounting for 47% of global exports. Additionally, Canada, Argentina, Egypt, Brazil, Nicaragua, Ethiopia, Australia, and Kyrgyzstan together contribute 34% of global bean exports (Mihálik *et al*., 2023; Zhao *et al*., 2023).

Currently, numerous phytopathogenic viruses affecting various crops have gained distinction worldwide. These viruses negatively impact the quantity and quality of cultivated crops, causing considerable economic losses, estimated at USD 60 billion annually (Fayziev *et al*., 2020; Sobirova *et al*., 2020, 2023). Phytoviruses infect bean plants, causing specific disease symptoms and severely damaging leguminous crops, such as beans, mung beans, and peas. The primary reason for these diseases is the susceptibility of beans to various microorganisms, particularly phytopathogenic viruses. Phytopathogenic plant viruses are tiny infectious particles composed of a protein coat and nucleic acid. They lack independent movement mechanisms and typically rely on other organisms as vectors, mainly insects, to transfer the virus from infected plants to healthy ones. These viruses' transmission mainly proceeds from sap-sucking insects, such as aphids, thrips, and whiteflies (Jovlieva *et al*., 2024; Yusubakhmedov and Fayziev, 2024).

According to past research, more than 20 different viruses have succeeded in their detection as pathogens of bean plants worldwide. The most active and widespread major viruses influencing common beans include the Bean golden mosaic virus (BGMV)*,* Bean common mosaic virus (BCMV), Bean common mosaic necrosis virus (BCMNV), Cucumber mosaic virus (CMV), and Bean endornavirus-1 (PvEV-1) and -2 (PvEV-2). These viruses, individually and in combination, can infect bean plants, leading to the development of various disease symptoms and, eventually, considerable yield losses. The bean common mosaic virus (BCMV) is also one of these viruses, and the first recorded data on its spread was from Yu. I. Vlasov in 1960 in Uzbekistan (Kanwal *et al*., 2024). BCMV is a phytovirus with a non-persistent transmission of aphids and seeds, causing an infection rate of 80% in common bean plants, depending on the bean's genotypes and virus strains, confirmed by several studies (Han *et al*., 2018; Abdukadirov *et al.,* 2024).

BCMV belongs to the Potyvirus family and causes economically significant diseases in leguminous crops globally. The said virus primarily affects the common bean and pea species, including *Phaseolus* L., *Vigna*, lupin (*Lupinus*), pea (*Pisum*), peanut (*Arachis*), and soybean (*Glycine*), particularly in the crop regions (Feng *et al*., 2019; Yang *et al*., 2019; Tang and Feng, 2023). The BCMV virion has a filamentous shape with dimensions of 750 nm × 12–15 nm and a sedimentation coefficient of 154–158 S. The purified virus preparation has an A260:A280 ratio of 1.12–1.27 (Su *et al*., 2022; Zhao *et al*., 2023). BCMV typically also spreads through seeds, aphids, and mechanical friction. The BCMV seed transmission rate varies between 3% and 95%, depending on the viral strains and host species (Aishwarya *et al*., 2020).

The seed transmission rate of the virus is a serious concern, as it serves as the primary source of viral epidemics. However,

the bean cultivars also influence the disease rate. The virus primarily remains in the seed coat; however, in susceptible cultivars, it can also spread through the endosperm (Kachroo *et al*., 2020; Mihálik *et al*., 2023; Tashpulatov *et al*., 2024). The BCMV infects the bean-susceptible cultivars and causes mosaic symptoms on leaves, appearing as light green-yellow patches, dark green areas, and both. Leaf discoloration is often in tandem with wrinkling, blistering, deformation, and downward curling. Overall, the virus intensity and manifestation of symptoms depend on the virus strains, bean cultivars, and the infected plants' life stage (Johary *et al*., 2016; Mbanzibwa, 2018; Worrall *et al*., 2019).

The bean common mosaic virus (BCMV) is one of the most widespread viral diseases affecting common beans *(Phaseolus vulgaris L.),* which are among the most crucial leguminous crops worldwide. BCMV is a phytovirus transmitted by aphids and can also be seed-transmitted, with infection rates reaching up to 80% in common bean plants, depending on bean variety and virus strain (Feng *et al*., 2014; Abdukadirov *et al.,* 2024; Nasirillayev *et al.,* 2025). Since its discovery in 1917, reports have stated BCMV has occurred in approximately 57 countries and regions globally.

The virus persists in various plant organs, altering their growth and development to different extents and ultimately reducing the seed yield. Therefore, research pertaining to the effects of various chemical compounds is crucial (Yusubakhmedov and Fayziev, 2024). In the recent past, effective research has succeeded in Uzbekistan on viruses affecting important crops (Fayziev *et al*., 2020; Sattorov *et al*., 2020; Jovlieva *et al*., 2024**)**. This research includes studies on determining the infection level of plants belonging to different genotypes **(**Sobirova *et al*., 2020**)**, molecular identification of the virus **(**Sattorov *et al*., 2020; Makhmudov *et al*., 2023; Sobirova *et al*., 2023), obtaining specific sera for virus immunodiagnostics and its practical application (Jovlieva *et al*., 2024), and research on the virus's effects on certain physiological traits of crop plants.

In implementing effective control measures against viruses, it is vital to study their biological characteristics and distribution levels using sensitive methods (Sherimbetov *et al*., 2020; Tamura *et al*., 2021). Additionally, examining the influences of various environmental and soil factors on crop plants and knowledge about their genetic makeup are highly significant (Yusubakhmedov and Fayziev, 2024). However, the most critical aspect is the rapid and sensitive detection of viruses in planting materials, including bean seeds, which can help control the virus spread and minimize yield losses (Mahsa *et al*., 2020). Therefore, the presented study primarily aimed at the molecular identification of the BCMV.

MATERIALS AND METHODS

Samples' collection

In May 2024, samples' collection of the common bean cultivar Ravot*,* showing visual symptoms of BCMV, came from bean fields of the Bo'ka, Piskent, Zangiota, and YangiYo'l Districts of the Tashkent Region, Republic of Uzbekistan.

Extraction of total RNA

The RNA extraction from infected plant leaves proceeded following the instructions of the Invitrogen™ PureLink™ RNA Mini Kit (Thermo Fisher, USA). With the instability of the RNA, it required storage at -20 °C.

cDNA synthesis from the extracted RNA

The reverse transcription reaction, as carried out, had a total volume of 20 µL in two stages: Stage 1—for each sample, the reaction mixture (12 µL) comprised 5 µL RNA, 1 µL BCMV-CP reverse primer, 2 µL dNTP (10 mM), and 5 µL ddH_2O. The mixture's incubation continued in a thermocycler at 65 °C for 5 min for one cycle; Stage 2—Reverse Transcription Reaction. For each sample, the second-stage reaction mixture (19 µL) comprised 12 µL first-stage reaction product, 4 µL 5× buffer, 2 µL DTT,

and 5 µL ddH_2O. This process used 0.01 M pH 7.2 phosphate buffer.

The mixture underwent incubation at 37 °C, then proceeded to the third stage. Stage 3—Enzyme addition and cDNA synthesis. In the 19 µL reaction mixture, the addition of 1 µL MMLV reverse transcriptase enzyme ensued (Thermo Fisher, USA). The reaction, as performed in a thermocycler (HEAL FORCE-T960, China), had the following conditions: 25 °C for 10 min, 40 °C for 115 min, and 70 °C for 10 min. Afterward, the synthesized cDNA remained stored at **-**20 °C until further use.

Reverse transcription-polymerase chain reaction (RT-PCR)

For the detection of the BCMV-CP gene, designing the primers used the NCBI data, employing the following sequences: forward 5-GGAGAATCTGTRCAYCTACA-3 and reverse primer 5-ACGCGARATGCTAACTGTG-3. These primers entailed utilization to identify the coat protein (*CP*) gene found in BCMV. The PCR reaction preparation had a total volume of 25 µL per sample, consisting of 6.6 µL ddH_2O, 0.9 µL $MgCl_2$ (50 mM), 12.5 µL 2.5x Hot Start Platinum Master Mix (Invitrogen, USA), 0.5 µL SMV-CP primers (forward/reverse, 10 pmol/µL), and 4 µL cDNA.

The PCR amplifier (HEAL FORCE-T960, China) programming was as follows: initial denaturation—1 cycle at 94 °C for 2 min; amplification—45 cycles of denaturation including 94 °C for 30 s, annealing at 60 °C for 45 s, elongation at 72 °C for 1 min, and final elongation at 72 °C for 10 min. After completion, mixing 10 µL of the PCR product proceeded with 3 µL of Gel Loading Buffer (Invitrogen, USA), and the separation used 1% agarose gel electrophoresis in 1x TBE buffer. The gel staining with 3 µL of ethidium bromide solution took place before electrophoresis at 80 V for 120 min in 1x TBE buffer. The DNA fragment lengths, when determined, used a DNA marker, with the gel analyzed using a transilluminator, and the results recorded accordingly (BK AG-100 Gel Imaging Analysis System, Biobase, China).

RESULTS

The study carried out disease monitoring activities in the districts of Bo'ka, Piskent, Zangiota, and YangiYo'l, Tashkent Region, Uzbekistan, to determine the virus spread. As a result of the virological monitoring, diverse disease symptoms identified in bean plants included a mixture of yellow and green mosaic patterns, green mosaic, leaf veins' epidermal thickening, chlorotic spot patterns, leaf mosaic roughening, and mosaic leaf curling. Plant samples showing virus symptoms succeeded in their individual collection in polyethylene bags for virological research. The assessment of their infection levels had the infectious sap initially prepared and mechanically inoculated onto virus-sensitive test-indicator plants. The collected samples from the bean cultivar Ravot (Figures 1a, b, and c) and other cultivars exhibited green mosaic, vein epidermal thickening, chlorotic spot patterns, mosaic roughening of leaves, and mixed yellow and green mosaic symptoms. They also showed the characteristics similar to those associated with BCMV.

The molecular identification of the virus progressed using a PCR kit (Invitrogen, USA) to confirm these symptom characteristics of BCMV. For this purpose and according to the protocol provided in the reagent kit and test manual, the viral RNA extraction from plant leaf samples used the Invitrogen™ PureLink™ RNA Mini Kit (Thermo Fisher, USA). The extracted viral RNA required RT-PCR utilizing the "Common Bean Mosaic Virus Genetic Material Detection Test Kit" from Invitrogen (USA). The results, as analyzed and documented, involved a gel transilluminator, with the PCR results presented in Figure 2 (BK AG-100 Gel Imaging Analysis System, Biobase, China).

The PCR analysis confirmed that all the tested bean cultivars, QoraKo'z and Ravot, identified during monitoring, acquired severe infection with BCMV. As mentioned earlier, the collected bean plant samples exhibited infectious symptoms and also showed infections of BCMV using the RT-PCR method.

Figure 1. BCMV disease symptoms on the common bean (*Phaseolus vulgaris L.*) plant. The image shows: a) the overall appearance of a virus-infected plant and b and c) symptoms on the leaves.

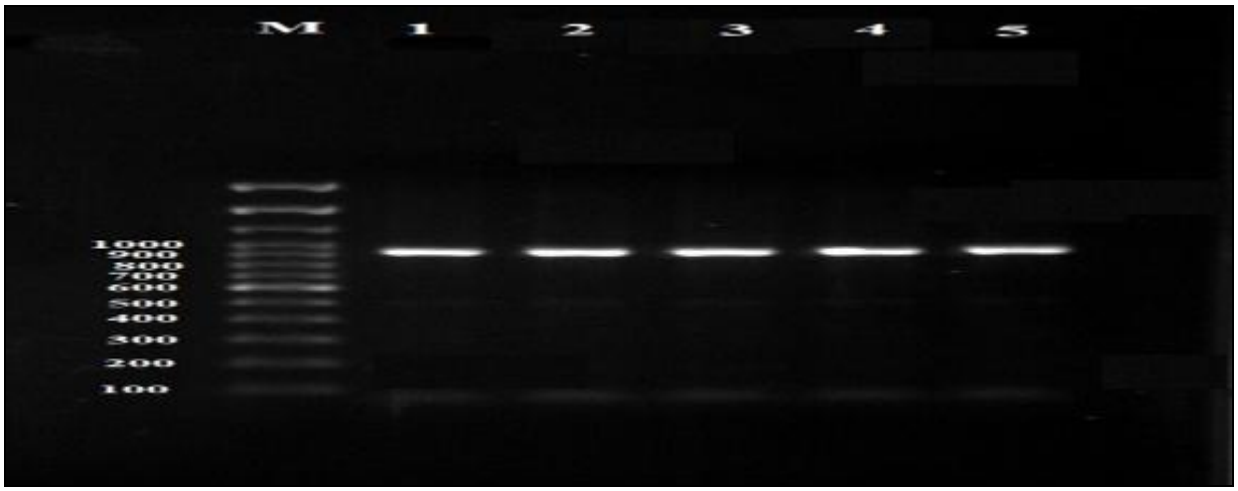

Figure 2. RT-PCR analysis of the BCMV coat protein (CP) gene detected in samples collected from *Phaseolus vulgaris L.* in Tashkent Region. Electrophoresis was performed on a 2% agarose gel. The image shows RT-PCR analysis results for leaf samples taken from *Qora ko'z* (1,2,3) and *Ravot* (4,5,6) cultivars. M - O'GeneRuler 1 kb DNA ladder (Fermentas). The RT-PCR conditions using BCMV1/BCMV2 primers proceeded as described by K. Culal and A. Çat (2022).

These samples then received mechanical inoculation into BCMV-specific test indicator plants, and in the studied plants, the disease symptoms observed bore analysis (Figure 3). Observations on the test indicator plants enunciated that *Amaranthus spinosus* and *Datura stramonium* exhibited yellow-green mosaic symptoms, while *Capsicum annuum* showed swelling between the leaf veins, resembling symptoms of BCMV infection (Figure 3).

The PCR product excision from the gel underwent purification before submission for sequencing. The nucleotide sequence obtained from the sequencing results entailed analysis using the NCBI BLAST database, confirming its identity as BCMV. Based on the verification, the sequence succeeded in depositing in the

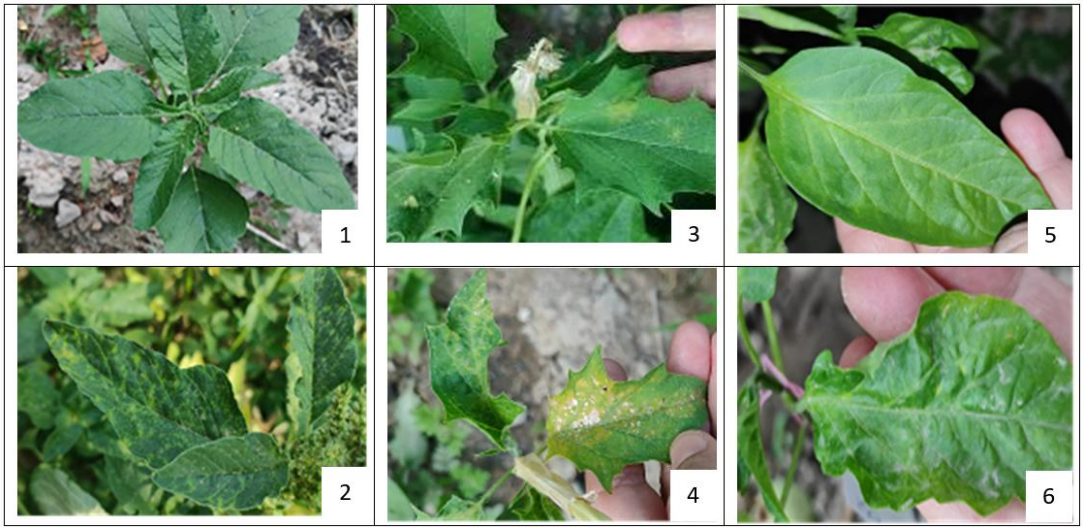

Figure 3. BCMV disease symptoms in test-indicator plants: Symptoms on healthy and infected leaves of *Amaranthus spinosus* (1 and 2), *Datura stramonium* (3 and 4), and *Capsicum annum* (5 and 6).

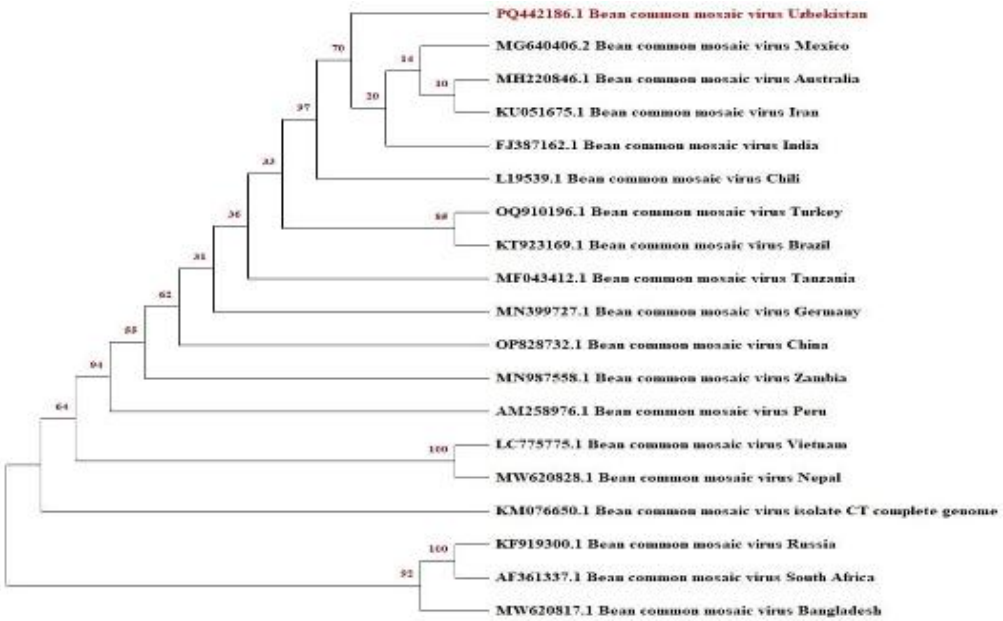

Figure 4. Phylogenetic tree of the BCMV-Uz-AY1 isolate.

NCBI database under the name Bean common mosaic virus isolate 'UZ-1' having the assigned accession number PQ442186.1**:** NCBI Link. The said sequence served as the basis for constructing the phylogenetic tree of the BCMV-'UZ-1' isolate. The identified isolate's nucleotide sequence attained comparison with other isolates available in the NCBI database before being subjected to bioinformatics analysis, proceeding to generate its phylogenetic tree using the MEGA11 software (Figure 4). As a result, determining the relationship of the virus isolate with isolates already identified in other regions was successful. This, in turn, provides insight into the origin and evolution of the isolate.

DISCUSSION

A comparative analysis and comparison of the present results with past findings confirmed that the dark green and yellow mosaic patterns, evident on bean leaves during monitoring, were the characteristics of BCMV. Furthermore, the disease symptoms observed in the test indicator plants, including *Vigna unguiculata* and *Nicotiana benthamiana*, appeared to be consistent with BCMV infection. The RT-PCR assay using CP gene-based primers designed for molecular identification of the virus proved to be an effective method for identifying the BCMV. Additionally, Jingru *et al*. (2024) investigated potential host plants for BCMV by using seven different healthy indicator plants. Their study demonstrated that *N. benthamiana, N. occidentalis, N. tabacum, N. glutinosa, Chenopodium quinoa, C. amaranticolor,* and *Datura stramonium* developed disease symptoms within 10–14 days after inoculation with BCMV. The RT-PCR analysis further confirmed the infected plants had definite effects from BCMV (Mustafa and Abdullah, 2023; Jingru *et al*., 2024).

In the presented research, the developed dynamics of disease symptoms caused by the BCMV-Uz1 isolate receive confirmation through RT-PCR and analysis using the test indicator plants. The results revealed the Uzbekistan-isolated strain of the virus initially caused small yellow chlorotic spots on the leaves of *Amaranthus spinosus* and *Datura stramonium*. However, with the progress of the disease, these symptoms developed into a mixed pattern of yellow-green mosaic and chlorotic spots (Figures 3, 2, and 4). In *Vigna sinensis,* the infection led to leaf curling, blistering, and downward folding, while in *Capsicum annuum*, it caused the hardening of the leaf epidermal layer (Figures 5 and 6).

The disease development in this form has also been notable in past studies (Mihálik *et al*., 2023; Abduvaliev *et al*., 2024; Akhmadaliev *et al*., 2024). Additionally, it allows for the identification of virus spread in local endemic plants (Khojimatov *et al*., 2020; Mustafaev and Khujanov, 2020; Khujanov, 2021; Khamraeva *et al*., 2022). Determining the disease symptoms and development dynamics in indicator plants serves as a basis for further research, including virus isolation, purification from mixed infections, propagation, and obtaining a homogeneous pure preparation.

For molecular identification, the RT-PCR analysis of the virus validated that both evaluated cultivars, Ravot and QoraKo'z, sustained BCMV infection. Additionally, the virus RNA isolated from the cultivar Ravot served as the basis for the first-ever deposition of the BCMV-Uz1 isolate in the NCBI database under Uzbekistan's climatic conditions. These results also provided a basis for constructing the phylogenetic tree of the virus.

The phylogenetic tree revealed that the first molecularly identified virus from common bean plants in Uzbekistan occupies a distinct position within the tree. It was remarkably closely related to isolates MG640406 (Mexico), MH220846.1 (Australia), and KU051675.1 (Iran), indicating a common evolutionary origin (Figure 4). Research in this field has provided valuable insights into the origin and evolution of viruses (Bakhtiyorova *et al*., 2024; Yusubakhmedov and Fayziev, 2024).

According to past phylogenetic tree studies, symptoms of the bean common mosaic virus (BCMV) included green mosaic patterns, thickening of the interveinal leaf epidermis, chlorotic spot patterns, rough mosaic textures on leaves, and a mixture of yellow and green mosaic symptoms. These symptoms were distinct in the plants of the common bean (*P. vulgaris*). The symptomatic bean leaf samples, when collected, underwent analysis using real-time PCR**,** leading to the identification of a new isolate named BCMV-22 'Huhe.' Its genome has a length of 10,062 bp. Its successful deposit in the GenBank received the accession number OR778613.

In China, the BCMV isolate had the recorded name of AzBMV (KP903372), and these isolates have a close association. Furthermore, among 99 BCMV isolates published in the NCBI database, recognizing homology for BCMV-22 'Huhe' occurred (Jingru *et al*., 2024). Similarly, the research conducted in Uzbekistan also led to the molecular identification of several phytopathogenic viruses, which attained depositing in the NCBI

database (Adams, 2019; Aishwarya *et al*., 2024).

The virus bioinformatics analysis revealed that the identified isolate shares 99% homology with the isolate LI9539.1 (Chile) and 97% homology with other phylogenetically related isolates, including LC775775.1 (Vietnam), MW620828.1 (Nepal), KF919300 (Russia), and AF361337 (Africa) (Figure 4). Thus, in turn, it led to the conclusion that the BCMV-Uz1 isolate likely originated from North America and spread to Eastern Asia, specifically Nepal, through waterborne trade routes. From Nepal, it spread to Vietnam, then to China via seed transmission, and eventually entered Uzbekistan through various pathways.

CONCLUSIONS

In the relevant studies, the symptoms observed in bean plants included the yellow and green mosaic pattern mixture, dark green spots (green mosaic), chlorotic vein patterns, leaf epidermis hardening, chlorotic spotted patterns, and leaf mosaic roughening. These were authentic BCMV characteristics through molecular identification. Moreover, the disease symptoms examination in test-indicator plants was successful. The nucleotide sequence of the BCMV CP gene isolated from the bean cultivars '*Ravot*' and '*QoraKo'z,*' upon analysis, led to the identification of a new Uzbek isolate, BCMV-Uz-AY1. This isolate proceeded to be deposited in the NCBI database under the accession number PQ442186.1. The phylogenetic tree determined the relationship of the new Uzbek isolate with other isolates and its evolutionary history.

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