Plant Tissue Cult. & Biotech. **36**(1): 149-164, 2026 (June) **ISSN 1817-3721, E-ISSN 1818-8745**
DOI: https://doi.org/10.3329/ptcb.v36i1.91168

PTC&B

Molecular Detection and Coat Protein Gene Mutation Analysis of Potato Leafroll Virus and Potato Virus Y in Bangladesh

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Key words: Potato viruses, DAS-ELISA, RT-PCR, Phylogenetic relationship, Bangladesh

Abstract

Potato (*Solanum tuberosum*) production in Bangladesh faces significant threats from viral diseases, particularly Potato Leafroll Virus (PLRV) and Potato Virus Y (PVY). This study was designed to investigate the prevalence and molecular characteristics of these viruses across three major potato-growing districts: Rangpur, Jamalpur, and Gazipur. Twenty-four samples collected during the 2023 harvest were analyzed using DAS-ELISA and RT-PCR techniques. Coat protein (CP) gene-specific primers facilitated amplification and sequencing of viral isolates. Results revealed high infection frequencies, including notable mixed infections. Sequence analysis identified six PLRV and five PVY isolates harboring distinct missense mutations. Phylogenetic analysis indicated close genetic relationships with isolates from India, China, and South Africa, suggesting possible transboundary virus introduction. Identified mutations may influence viral movement, protein function, and transmission efficiency. These findings provide valuable baseline data on the genetic diversity of potato-infecting viruses in Bangladesh, offering critical implications for seed potato certification, disease management, and agricultural biosecurity programs.

Introduction

Potato (*Solanum tuberosum*), the third most important food crop after rice and wheat, feeds 1.3 billion people globally and is cultivated in more than 100 countries, including Bangladesh (Devaux et al. 2021, Shahriar et al. 2013). Viral infections significantly hinder the sustainable cultivation of potatoes by diminishing the crop's quality and quantity

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(Rashid et al. 2020). Given the pervasiveness of viruses in the natural world, it is generally accepted that they provide a significant obstacle to the development of potatoes, particularly in multiferous infection (Kerlan and Moury 2008, Halterman et al. 2011, Kreuze et al. 2019). Globally, more than 50 species of viruses have been identified that negatively affect production. In combined infections, the yield reduction can exceed 80%, ensuing over 55 billion USD losses (Wale et al. 2008, Pallás et al. 2018, Kreuze et al. 2019).

To date, eight distinct potato viruses have been reported in Bangladesh: Potato leafroll virus (PLRV), Potato Virus Y (PVY), Potato Virus X (PVX), Potato Virus A (PVA), Potato Virus H (PVH), Potato Virus M (PVM), Potato Virus S (PVS), and Potato aucuba mosaic virus (PAMV) (Wu et al. 2018, Kreuze et al. 2019, Rashid et al. 2019). PLRV, PVX, and PVY are the most common among them, and they are responsible for yearly yield losses ranging from 15-78%. PLRV and PVY are responsible for yield losses reaching up to 70-80% (Hossain et al. 1989, Hossain et al. 1992, Loebenstein 2008, Salan et al. 2022). Recent studies confirm that PVY can cause yield losses of up to 85% (Baebler et al. 2020). Sustainable potato production is exceedingly hampered due to the unavailability of viral infection-free seeds (Wale et al. 2008). Therefore, virus detection at an early stage is an effective strategy for managing disease progression and ensuring the generation of virus-free seed potatoes (Pallás et al. 2018). Numerous techniques have been developed for detecting plant viruses (Dickinson 2004, El-Araby et al. 2009, Hull 2009). Furthermore, serological assays such as ELISA and molecular methods like RT-PCR continue to be frequently employed in viral detection. Serological assays such as ELISA remain widely used in seed certification programs due to their affordability and ease of use. However, ELISA often underestimates virus incidence, particularly in dormant tubers and mixed infections. In contrast, RT-PCR and real-time RT-PCR provide superior sensitivity, specificity, and strain differentiation (Mackenzie et al. 2015, Jalalypour et al. 2016).

These methods provide advantages such as fast results, high accuracy, specificity, sensitivity, and relatively affordable costs (Koenig et al. 2008, Li et al. 2015, DG and EE 2017). In this study, serology and RT-PCR-based detection were carried out along with subsequent amplicon sequencing to characterize two significant potato viruses (PLRV and PVY) in three vital potato-growing locations in Bangladesh. Although PLRV and PVY are among the most economically important potato viruses in Bangladesh, information regarding their molecular diversity, sequence variation, and phylogenetic relationships remains limited in Bangladesh. The findings will offer valuable opportunities for future research, particularly in developing disease management techniques for generating virus-free seed potatoes.

Materials and Methods

In November-December 2023, a total of 24 potato tuber samples were collected from Rangpur, Jamalpur, and Gazipur, Bangladesh, during the harvesting period (Table 1). The tubers were selected from plants that had exhibited virus-like symptoms in the field, as local potato varieties are frequently affected by viral diseases. The collected samples were subsequently grown under controlled greenhouse conditions at the Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur.

Table 1. Genotype of the samples and their location.

Sample ID	Genotypes	Location
1	BARI Alu-25 (Asterix)	Gazipur
2	BARI Alu-25 (Asterix)	Gazipur
3	BARI Alu-25 (Asterix)	Gazipur
4	BARI Alu-36	Gazipur
5	BARI Alu-36	Gazipur
6	BARI Alu-53	Gazipur
7	BARI Alu-53	Gazipur
8	BARI Alu-7 (Dimant)	Gazipur
9	BARI Alu-7 (Dimant)	Gazipur
10	Dohazari	Gazipur
11	Lalpakri	Gazipur
12	Shilbilati	Gazipur
13	Shilbilati	Gazipur
14	Ausha	Jamalpur
15	Borojam Alu	Jamalpur
16	Lalpakri	Jamalpur
17	Sadaguti	Jamalpur
18	Ausha	Rangpur
19	Dohazari	Rangpur
20	Indurkani	Rangpur
21	Lalpakri	Rangpur
22	Patnai	Rangpur
23	Sadaguti	Rangpur
24	Sindurkouta	Rangpur

Detection of viral infection in collected leaf samples from the greenhouse was performed using the Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) following the protocol provided by Bioreba, Switzerland, using ELISA Complete Kits. The assay was conducted specifically for the detection of Potato virus Y (PVY) and Potato leafroll virus (PLRV) using the PVY Monoclonal Cocktail Complete Kit 96 (Art. Nr. 112977) and the PLRV Complete Kit 96 (Art. Nr. 110677), respectively.

Subsequently, total RNA was extracted from all 24 samples using the SV Total RNA Isolation System (Promega, USA) according to the manufacturer's instructions. The extracted RNA was stored at −70°C until further use. First-strand complementary DNA (cDNA) was synthesized using the GoScript Reverse Transcriptase System (Promega, USA) and stored at −20°C.

Polymerase Chain Reaction (PCR) amplification was carried out in a total reaction volume of 20 µl, containing 2 µl of cDNA template and 18 µl of PCR master mix. Virus-specific primers previously reported in the literature were used for amplification (Table 2). Following thermal cycling, PCR products were analyzed by agarose gel electrophoresis (1% agarose gel) stained with ethidium bromide (0.5 µl/ml) and visualized under UV light. Amplified products were stored at −20°C for subsequent analysis.

Table 2. The coat protein gene-specific primer sequences of the intended potato viruses.

Sl. No.	Name of the Primers	Primer sequences 5′ to 3′	No. of bp	Tm (°C)	Product size (bp)	Reference
1.	PLRV-CP-F	CGCGCTAACAGAGTTCAGCC	20	60	336	(Bostan and Elibuyuk 2010)
	PLRV- CP-R	GCAATGGGGGTCCAACTCAT	20			
2.	PVY-CP-F	ACGTCCAAAATGAGAATGCC	20	55.6	480	(Nasir et al. 2010)
	PVY-CP-R	TGGTGTTCGTGATGTGACCT	20			

Selected PCR amplicons were subjected to sequencing, including five isolates of PVY and six isolates of PLRV. The expected PCR amplicon sizes were 336 bp for PLRV and 480 bp for PVY. Following Sanger sequencing, low-quality bases at the 5′ and 3′ ends were trimmed to ensure sequence accuracy, resulting in final high-quality consensus sequences of 292 bp for PLRV and 271 bp for PVY, which were submitted to the NCBI GenBank database under accession numbers PQ736923-PQ736933 (Table 3).

Table 3. List of PVY and PLRV isolates with corresponding source samples and GenBank accession numbers.

Isolate	Identified Virus	Genotype-location (ID)	Accession no.
PVYAAR4	PVY	Ausha-Rangpur(18)	PQ736926
PVYBAJ1		Borojam Alu-Jamalpur(15)	PQ736923
PVYAAJ2		Ausha-Jamalpur(14)	PQ736924
PVYAAG3		Asterix-gazipur(1)	PQ736925
PVYLAR5		Lalpakri-Rangpur(21)	PQ736927
PLRVBAJ1	PLRV	Borojam Alu-Jamalpur(15)	PQ736928
PLRVAAR3		Ausha-Rangpur(18)	PQ736930
PLRVLAR4		Lalpakri-Rangpur(21)	PQ736931
PLRVAAG2		Asterix-gazipur(2)	PQ736929
PLRVAAJ5		Ausha-Jamalpur(14)	PQ736932
PLRVSAR6		Sadaguti-Rangpur(23)	PQ736933

Sequence homology analysis was performed using the BLASTn tool against the NCBI database. Multiple sequence alignment was conducted using ClustalW (Thompson et al. 1994), and phylogenetic relationships were analyzed using MEGA11 software (Tamura et al. 2021). Evolutionary relationships were inferred using the Neighbor-Joining method with 1,000 bootstrap replicates. Positions containing gaps and missing data were excluded from the analyses. Additionally, mutation analysis was carried out through bioinformatics approaches by comparing the obtained sequences with reference viral genomes.

Results and Discussion

Precise and rapid detection of viruses is crucial for generating virus-free seed potatoes. Virus-free seed planting is crucial for managing potato viruses in the field and increasing potato production at a commercial level (Faccioli 2001, Oana et al. 2012). Behind the motivation of unveiling the prevalence of PVY and PLRV, the DAS-ELISA result was calculated accurately and found among the 24 germplasm. RT-PCR-based discernment and amplicon sequencing were executed to address the current scenario of two dominant potato viruses in Bangladesh's renowned potato-growing districts (Rangpur, Jamalpur, and Gazipur). Furthermore, the detection results indicated a high frequency of mixed infections in the obtained potato samples (Table 4, Fig. 1, and Table 5).

Table 4. Prevalence of PLRV and PVY in 24 potato samples collected from the different potato cultivation areas of Bangladesh.

Location	No. of Samples	Viral incidence	DAS-ELISA detection N (%)	RT-PCR Detection N (%)
Rangpur	7	PLRV	5(71.4)	6(85.7)
		PVY	4(57.1)	4(57.1)
		PLRV+PVY	3(42.9)	4(57.1)
Jamalpur	4	PLRV	4(100)	4(100)
		PVY	3(75)	3(75)
		PLRV+PVY	3(75)	3(75)
TCRC, Gazipur	13	PLRV	8(61.5)	8(61.5)
		PVY	8(61.5)	9(69.2)
		PLRV+PVY	5(38.5)	5(38.5)

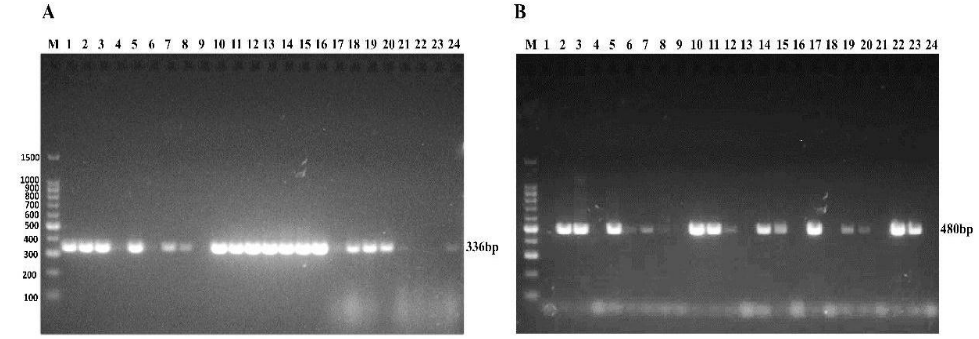

Fig. 1. RT-PCR-based detection of *Potato leafroll virus* (PLRV): (A) and *Potato virus Y* (PVY), (B) in potato samples. Lane M: molecular size marker; lanes 1-24 represent PCR products amplified from the partial coat protein (CP) gene of PLRV and PVY obtained from 24 individual potato plants collected from different locations. The expected product sizes were ~336 bp for PLRV and ~480 bp for PVY.

Table 5. Detection of potato leaf roll virus and potato virus Y in different potato genotypes using ELISA and PCR.

Sample ID	Genotypes	Location	DAS-ELISA		RT-PCR	
			PLRV	PVY	PLRV	PVY
1	BARI Alu-25 (Asterix)	Gazipur	-	+++	-	+++
2	BARI Alu-25 (Asterix)		+++	-	+++	+++
3	BARI Alu-25 (Asterix)		-	+++	-	+++
4	BARI Alu-36		-	-	-	-
5	BARI Alu-36		-	-	-	+++
6	BARI Alu-53		+++	+++	+++	+++
7	BARI Alu-53		+++	+++	+++	-
8	BARI Alu-7 (Dimant)		+++	+++	+++	+++
9	BARI Alu-7 (Dimant)		+++	-	+++	-
10	Dohazari		+++	+++	+++	+++
11	Lalpakri		+++	+++	+++	+++
12	Shilbilati		+++	-	+++	-
13	Shilbilati		-	+++	-	+++
14	Ausha	Jamalpur	+++	+++	+++	+++
15	Borojam Alu		+++	+++	+++	+++
16	Lalpakri		+++	-	+++	+++
17	Sadaguti		+++	+++	+++	-
18	Ausha	Rangpur	+++	+++	+++	+++
19	Dohazari		-	+++	+++	+++
20	Indurkani		+++	-	-	-
21	Lalpakri		+++	+++	+++	+++
22	Patnai		+++	-	+++	-
23	Sadaguti		+++	+++	+++	+++
24	Sindurkouta		-	-	+++	-

Comparison of viral CP sequences revealed that PLRV isolates shared 97.79-100% nucleotide identity with each other, whereas PVY isolates demonstrated the highest nucleotide (nt) identity of 98.98%. A comparison was conducted between the viral CP sequences from this study and the NCBI database. The results revealed that the PLRV sequence from this investigation had the highest nucleotide identity of 100% with Indian isolates (accession no. OQ718426.1, OQ718425.1, OQ718423.1, OQ718422.1, OQ718421.1, MK244366.1, MK244365.1, MK244364.1 and MK244362.1), Pakistani isolates (MF327272.1 and MF276875.1), South African isolates (MN689375.1 and MN689369.1), Irish isolate (MT264739.1), Serbian isolate (MT366794.1) and Chinese isolates (MK451706.1, MK451707.1, and MF062487.1). It also had a 99.63% nucleotide identity with Chinese Tobacco virus 2 isolates (NC_034265.1); 99.26% with Bangladeshi isolates (MK445319.1, MG356503.1, and MG356502.1). Additionally, it had a 98.98% nucleotide similarity with an isolate of China (KR051203.1) and 98.15% identical with Bangladeshi isolates (MK445318.1 and MG356504.1), respectively (Table 6).

PVY exhibited the highest nucleotide resemblance of 100% with a South African isolate (GQ853630.1), a Chinese isolate (KU043114.1), two isolates from France (KJ741086.1 and MN414539.1), and an isolate from Poland (KJ746442.1). Additionally,

Table 6. List of 25 PLRV isolates used for comparison of coat protein sequences.

Accession	Isolate	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Nucleotide Completeness	Country
OQ718426.1	Samsastipur2_ZoneI	501	100%	4.00E-137	100	624	Partial	India
OQ718425.1	Purnea2_ZoneII	501	100%	4.00E-137	100	624	Partial	India
OQ718423.1	Patna4_ZoneIIIa	501	100%	4.00E-137	100	624	Partial	India
OQ718422.1	Begusarai2_ZoneII	501	100%	4.00E-137	100	624	Partial	India
OQ718421.1	Bhagalpur2_BAU_ZoneIIIb	501	100%	4.00E-137	100	624	Partial	India
MK244366.1	Imphal-MN	501	100%	4.00E-137	100	639	Partial	India
MK244365.1	Arunachal Pradesh	501	100%	4.00E-137	100	602	Partial	India
MK244364.1	Manipur	501	100%	4.00E-137	100	640	Partial	India
MK244362.1	Tripura	501	100%	4.00E-137	100	578	Partial	India
MF327272.1	566-Okara	501	100%	4.00E-137	100	627	Partial	Pakistan
MF276875.1	433a-Sahiwal	501	100%	4.00E-137	100	627	Partial	Pakistan
MN689375.1	18-1095	501	100%	4.00E-137	100	5868	Complete	S. Africa
MN689369.1	18-1111	501	100%	4.00E-137	100	5942	Complete	S. Africa
MT264739.1	P166	501	100%	4.00E-137	100	5884	Complete	Ireland
MT366794.1	38-17	501	100%	4.00E-137	100	627	Partial	Serbia
MF062487.1	EP	501	100%	4.00E-137	100	5837	Complete	China
MK451707.1	Wuyuan	501	100%	4.00E-137	100	627	Partial	China
MK451706.1	Guanghe	501	100%	4.00E-137	100	627	Partial	China
NC_034265.1	Tobacco virus 2	496	100%	2.00E-135	99.63	5979	Complete	China
MK445319.1	Bogra-2	490	100%	9.00E-134	99.26	627	Partial	Bangladesh
MG356503.1	PLRV171	490	100%	9.00E-134	99.26	5788	Complete	Bangladesh
MG356502.1	PLRV165	490	100%	9.00E-134	99.26	5847	Complete	Bangladesh
KR051203.1	RV76	484	100%	4.00E-132	98.89	627	Partial	China
MK445318.1	Bogra-1	473	100%	9.00E-129	98.15	627	Partial	Bangladesh
MG356504.1	PLRV184	473	100%	9.00E-129	98.15	5847	Complete	Bangladesh

Table 7. List of 19 PVY isolates used for comparison of coat protein sequences.

Accession	Isolate	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Nucleotide Completeness	Country
MN414539.1	1A10-1	802	100%	0	100	801	partial	France
KU043114.1	NMDZ	802	100%	0	100	9264	partial	China
KJ741086.1	314	802	100%	0	100	801	partial	France
KJ746442.1	S5	802	100%	0	100	801	partial	Poland
GQ853630.1	NN333B_87_152	802	100%	0	100	807	partial	South Africa
MN414571.1	2G6-4	797	100%	0	99.77	801	partial	France
MN414569.1	2D14-1	797	100%	0	99.77	801	partial	France
MN414568.1	2C15-9	797	100%	0	99.77	801	partial	France
MT264732.1	p097	797	100%	0	99.77	9694	complete	Ireland
MN380533.1	P166	797	100%	0	99.77	752	partial	Egypt
MK244385.1	Ass03	797	100%	0	99.77	796	partial	India
MK244382.1	Tri05	797	100%	0	99.77	699	partial	India
OR480020.1	PVY-T21ML34R-RUS-2021	797	100%	0	99.77	9788	complete	Russia
OQ102071.1	DSMZ-PV-1370	797	100%	0	99.77	9703	complete	Germany
EF027898.1	8086	797	100%	0	99.77	801	partial	United Kingdom
JX088119.1	PVY-BD-B1	797	100%	0	99.77	804	partial	Bangladesh
JX088120.1	PVY-BD-B2	797	100%	0	99.77	804	partial	Bangladesh
MF589764.1	PvY-Jessore	797	100%	0	99.77	805	partial	Bangladesh
HM243482.1	KER.JI.NTN.T	797	100%	0	99.77	1133	partial	Iran

PVY shared an identity of 99.77% with several isolates from France (MN414571.1, MN414569.1, and MN414568.1), Ireland (MT264732.1), Egypt (MN380533.1), India (MK244382.1 and MK244385.1), Russia (OR480020), United Kingdom (EF027898.1), Bangladesh (JX088119, JX088120, and MF589764), Germany (OQ102071.1), and Iran (HM243482.1), respectively (Table 7).

Sequence analysis conducted using the NCBI BLASTn service confirmed the identity of six (6) distinct isolates of PLRV (PQ736928-PQ736933) and five (5) isolates of PVY (PQ736923-PQ736927). A mutation analysis identified genetic variations at specific positions in the coat protein (CP) gene sequences of PLRV (292 bp) and PVY (271 bp) among different potato varieties, relative to the reference isolates MG356502.1 and MT264732.1, respectively. PLRV exhibited significant changes, including the substitution of T>C (3681), A>G (3692), A>C (3914), and A>T (3917), which were exclusively identified in PQ736933. Furthermore, A>G (3815) and T>A (3824) substitutions were found in PQ736928 and PQ736932, respectively (Fig. 2A). Due to these changes, the amino acid alteration was observed within the CP, including the substitution of Valine (V) to Alanine (A), Threonine (T) to Alanine, Threonine (T) to Proline (P) and Serine (S) to Cysteine (C) was observed in PQ736933 at the position of 1227, 1231, 1306 and 1307, respectively; additionally, alteration of Methionine (M) to Valine (V) and Serine (S) to Threonine (T) was found at positions 1272 and 1275 in PQ736928 and PQ736932, respectively (Fig. 2B and Table 8).

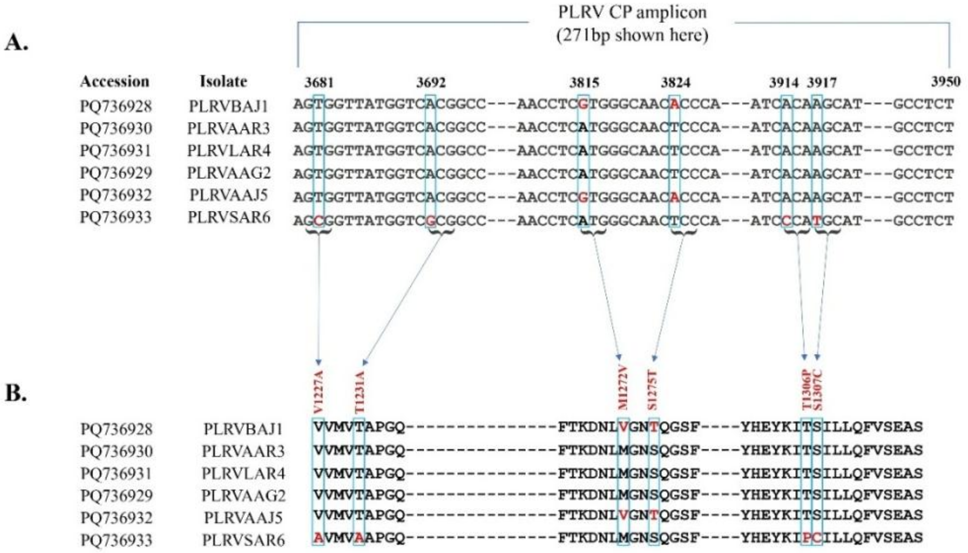

Fig. 2. Illustration of mutation analysis on the PLRV coat protein gene. A point mutation found on the partial coat protein gene: (A) results in a change of the amino acid (B) black letters indicate nucleotide base/amino acid residues conserved among all sequences, where alteration is marked in red. Sequence alignment and translation analyses were performed using MEGA 11, and figure annotations were prepared using Microsoft Power Point.

Table 8. Mutation analysis of the studied viral isolates from across Bangladesh.

Feature		Potato leafroll virus (PLRV)	Potato virus Y (PVY)
Reference genome (GenBank Accession no.)		MG356502.1 (5847 bp)	MT264732.1 (9694 bp)
Study CP gene size		292 bp	271 bp
Mutational evidence	Found in virus isolate	PQ736928, PQ736932, and PQ736933	PQ736923, PQ736924, PQ736925, PQ736927
	CP gene level	T>C (3681), A>G (3692), A>C (3914) and A>T (3917) in PQ736933; A>G 3815, and T>A (3824) in PQ736928, and PQ736932	(G>A)
	Amino acid level	substitution of V1227A and T1231A in PQ736933; M1272V and S1275T in *PQ736928* and *PQ736932*, respectively; T1306P and S1307C in PQ736933	R2950Q
Sequence Similarity		100% with MF327272.1, OQ718426.1, OQ718425.1, OQ718423.1, OQ718422.1, and OQ718421.1	100% with GQ853630.1, MN414539.1, KU043114.1, KJ741086.1 and KJ746442.1

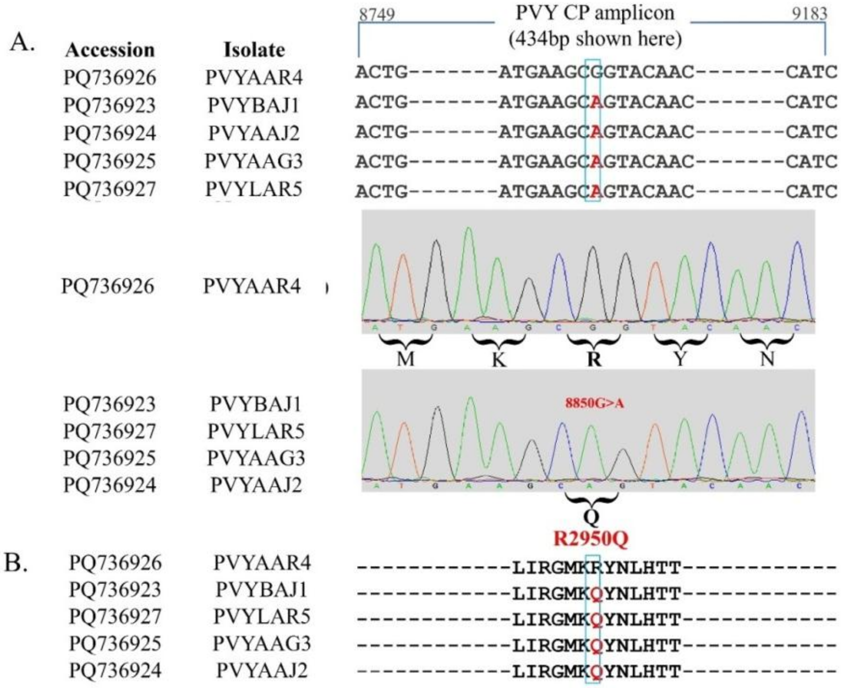

Fig. 3. Demonstration of mutational evidence on the PVY coat protein gene. A point mutation was found in the partial coat protein gene: (A) which changes arginine (R) to glutamine (Q) within gene, (B) black letters indicate nucleotide base/amino acid residues conserved among all sequences, where alteration is displayed in red. Sequence alignment, translation, and mutation analyses were performed using MEGA 11. Chromatograms were visualized using Bio Edit (Hall 1999), and figure annotations were prepared using Microsoft PowerPoint.

A nucleotide substitution (G>A) in the PVY coat protein gene resulted in an amino acid substitution from Arginine (R) to Glutamine (Q) at position 2950 relative to the reference isolate MT264732.1 (Fig. 3A, 3B). The biological significance of this substitution remains unknown and requires further investigation. PLRV shows a 100% resemblance with MF327272.1, OQ718426.1, OQ718425.1, OQ718423.1, OQ718422.1, and OQ718421.1. On the other hand, PVY reveals a 100% similarity with GQ853630.1, MN414539.1, KU043114.1, KJ741086.1, and KJ746442.1 (Table 8).

To elucidate the genetic relationship of PLRV and PVY with similar viruses, coat protein sequences of PLRV and PVY were studied, and NCBI database sequences of relevant viruses were aligned; phylogenetic clustering revealed the genetic relatedness of the isolates and their association with previously reported isolates from different geographic regions. The horizontal distance of the evolutionary tree represents the degree of genetic relationship among the strains.

The present study observed that PLRV from Borojam Alu-Jamalpur (PQ736928) was closely related to Bhagalpur, Patnai, and Begusarai isolates of India (OQ718422, OQ718423, and OQ718421) and Ausha-Jamalpur (PQ736932) also connected to Indian isolates (OQ718425 and OQ718426); whereas Lalpakri-Rangpur (PQ736931) was closely associated with to Bangladeshi isolates (MG356504, MG356503, and MG356502); Asterix-Gazipur (PQ736929) was close to lalpakri and other Bangladeshi isolates; furthermore, PLRV from Ausha-Rangpur (PQ736930) was linked to an Indian (Arunachal Pradesh) isolate (MK244365) and shared ancestral root to Sadaguti-Rangpur (PQ736933) which was close to Indian (Monipur, and Imphal-MN) isolates (MK244364, and MK244366) (Fig. 4). All the studied sequences were 99.31% to 100% similar to reference sequences (Table 6).

PVY isolated from Ausha-Jamalpur (PQ736924) was strongly connected to South African isolate (GQ853630.1), and these were close to a Bangladeshi isolate (JX088119) reported previously; isolate from Asterix-Gazipur (PQ736925) was a close relative of isolates from Poland (KJ746442.1), France (KJ741086.1 and MN414539.1) and China (KU043114.1), respectively; Ausha-Rangpur (PQ736926) was closely associated with an Egyptian isolate (MN380533.1), and these two were linked to Borojam Alu-Jamalpur (PQ736923); all of these shared ancestral root with Lalpakri-Rangpur isolate (PQ736927) which was far distant from previously documented Bangladeshi isolate (MF589764.1) (Fig. 5) (Rashid et al. 2020). All the sequences were 99.77 to 100% similar (Table 6), which suggests a low level of genetic diversity within the analyzed coat protein (CP) region. This high degree of conservation may reflect strong functional constraints on the CP gene, which plays essential roles in virion assembly, virus transmission, and host interactions. The limited sequence variation among the Bangladeshi isolates may also suggest a relatively common ancestry or the widespread circulation of closely related PVY variants within potato-growing regions of the country.

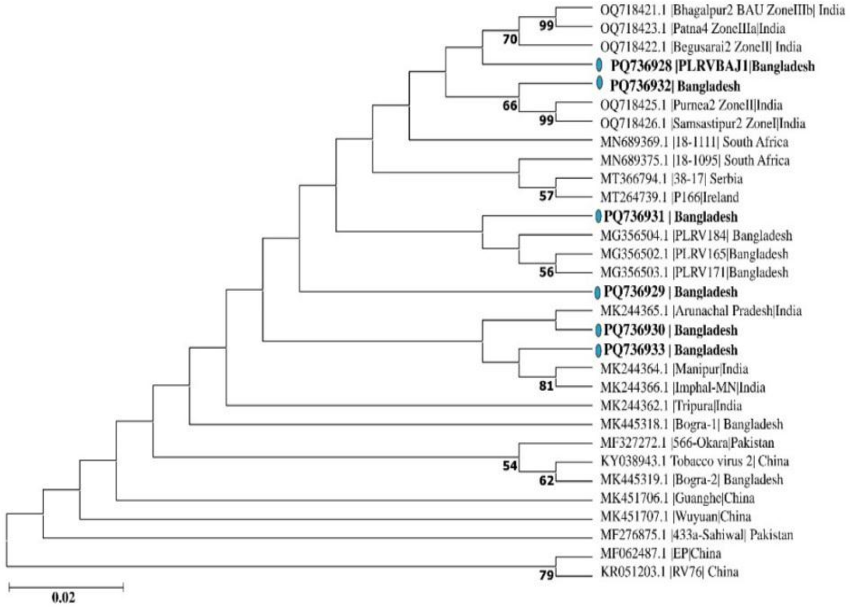

Fig. 4. Phylogenetic relationship of PLRV from this study and the NCBI database. The tree was formed in MEGA11 via the neighbor-joining method with 1000 bootstrap replicates; only values greater than 50% are displayed at the nodes.

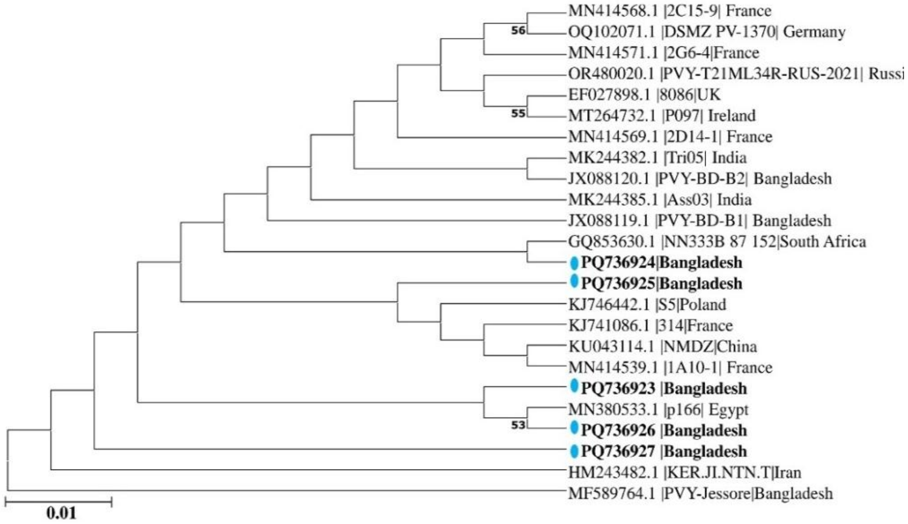

Fig. 5. Phylogenetic connection of PVY from this study and the NCBI database. The tree was constructed in MEGA 11 via the neighbor-joining method with 1000 bootstrap replicates; only values greater than 50% are displayed at the nodes.

Viruses pose the most significant challenge to potato cultivation worldwide, especially in Bangladesh (Peiman and Xie 2006, Rahim et al. 2022). Documenting specific viruses in the seed lot and field is necessary to increase potato production in Bangladesh, as virus-free seed tubers are the main obstacle. However, few Bangladeshi studies have examined potato virus molecular identification and infection status (Rashid et al. 2019, Rashid et al. 2020, 2021). Therefore, DAS-ELISA and RT-PCR-based detection and sequencing were applied to disclose the present condition of three prominent cultivation areas (Rangpur, Jamalpur, and Gazipur districts) in Bangladesh.

The detection result revealed that samples were infected with PLRV and PVY, previously documented in Bangladesh (Kumar et al. 2020, Rashid et al. 2020, Rahim et al. 2022). Furthermore, the results provided additional evidence that mixed infection was quite prevalent, which aligns with previous research findings (Shattock 2002, Wang et al. 2011, Zhang et al. 2017). The co-infection of PLRV and PVY can result in significant financial losses due to the reduction in both the size and quality of tubers, as opposed to when PLRV or PVY infects alone (Wang et al. 2011). Zhang et al. (2017) divulged a significant number of PLRV, PVY, and PVX co-infections. The above three potato viruses are devastating in Bangladesh, causing yearly production losses of 15-78%(Salan et al. 2022b).

Sequence analysis using the NCBI BLASTn service identified six PLRV isolates and five PVY isolates. Comparative sequence analysis revealed several nucleotide substitutions and corresponding amino acid changes in the coat protein (CP) gene relative to the reference sequences (Table 8). The PLRV nucleotide sequences were found to be 97.79% similar to each other, which was higher than the previous report (95.69%) (Rashid et al. 2020), where PVY sequences were 97.61%. PLRV from Sadaguti-Rangpur (P6) demonstrated notable nucleotide base substitutions T>C (3681), A>G (3692), A>C (3914), and A>T (3917), whereas A>G (3815) and T>A (3824) alterations were found in P1 and P5, respectively (Fig. 2A and Table 8). These alterations prompted amino acid substitutions in the CP, including Valine (V) to Alanine (A), Threonine (T) to Alanine (A), Threonine (T) to Proline (P), and Serine (S) to Cysteine (C) in P6 at positions 1227, 1231, 1306, and 1307, respectively. In addition, a substitution of Methionine (M) with Valine (V) and Serine (S) with Threonine (T) was observed at positions 1272 and 1275 in P1 and P5, respectively (Fig. 2B and Table 8). This study aligns with a previous report that documented several mutations in different segments of the CP of PLRV (Lee et al. 2005, Kaplan et al. 2007). Several nucleotide substitutions and corresponding amino acid changes were identified in the PLRV and PVY coat protein genes. Because the coat protein is involved in virion assembly, movement, and vector-mediated transmission, these sequence variations may be relevant to future functional studies. However, the biological consequences of the observed substitutions remain hypothetical and require experimental validation.

The phylogenetic study provides valuable information on the genetic relationships among virus isolates and their evolutionary divergence (Hull 2001, Gibbs and Ohshima 2010). The nucleotide sequence-based distance tree (neighbor-joining) revealed that the PLRV strain obtained from Borojam Alu-Jamalpur showed a close genetic similarity to isolates from India (OQ718422, OQ718423, and OQ718421). Similarly, the Ausha-

Jamalpur isolate exhibited a robust genetic relationship with Indian isolates (OQ718425 and OQ718426). Lalpakri-Rangpur exhibited a close genetic relationship with isolates from Bangladesh (MG356504, MG356503, and MG356502), whereas Asterix-Gazipur showed similarity to Lalpakri and other Bangladeshi isolates. Additionally, the PLRV from Ausha-Rangpur was linked to an Indian isolate from Arunachal Pradesh (MK244365) and shared an ancestral connection with Sadaguti-Rangpur, which was closely associated with Indian isolates from Manipur and Imphal (MK244364 and MK244366) (Fig. 4).

PVY isolated from Ausha-Jamalpur showed a close relationship with a South African isolate (GQ853630.1), which in turn was intimately linked to a previously reported Bangladeshi isolate (JX088119-NTN strain) (Akhter et al. 2019). The isolate from Asterix-Gazipur was tightly connected to isolates from Poland (KJ746442.1), France (KJ741086.1 and MN414539.1), and China (KU043114.1). Similarly, Ausha-Rangpur exhibited a close genetic similarity to an Egyptian isolate (MN380533.1), and both were closely related to Borojam Alu-Jamalpur. These isolates shared a common ancestral root with the Lalpakri-Rangpur isolate, significantly distinct from the previously documented Bangladeshi isolate (MF589764.1) (Fig. 5). The results provide baseline molecular information on PLRV and PVY populations in Bangladesh and contribute to the understanding of their genetic diversity and phylogenetic relationships. Additionally, numerous studies around the world used the same methods for detecting the potato virus as our study (Rota-Stabelli and Telford 2008, El-Araby et al. 2009, Abbas et al. 2012, Al-Saikhan et al. 2014, Rashid et al. 2021).

Viruses are a significant obstacle to potato production worldwide, as the risk of virus migration is increasing daily due to the growing global trade, including in Bangladesh. Therefore, fast and accurate detection methods are needed to stop these movements and to produce virus-free seed potatoes. The current investigation demonstrated the existence of PLRV and PVY in three vital potato farming regions (Rangpur, Jamalpur, and Gazipur districts) in Bangladesh. Moreover, our study also presented the occurrence of viruses and their evolutionary relationship, along with mutational evidence. Our findings will facilitate further investigation into the biological characteristics of the aforementioned viruses, such as their impact on potato quality and yield, host range, and distribution. Additionally, our findings will aid in developing effective techniques to ensure virus-free potato production.

This study represents a targeted baseline molecular characterization of PLRV and PVY from three major potato-growing districts of Bangladesh. Although the sample size was modest and only two potato viruses were investigated, the results provide valuable information on the occurrence, genetic diversity, and phylogenetic relationships of these economically important viruses. Other potato viruses reported in Bangladesh were beyond the scope of this study. Future surveys involving larger sample sizes, additional geographic regions, and a broader range of potato viruses would provide a more comprehensive understanding of potato virus diversity and epidemiology in

Bangladesh. The molecular characterization and phylogenetic information generated in this study provide a valuable resource for virus surveillance and diagnostic programs in Bangladesh. The identified PLRV and PVY sequences can support the development and validation of molecular diagnostic assays, facilitate seed potato certification, and improve the production of virus-free planting materials. Furthermore, the findings may assist plant breeders, pathologists, and agricultural extension agencies in monitoring virus diversity and designing effective disease management strategies to reduce yield losses in potato cultivation.

Acknowledgments

Special thanks to the authority of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur-1701, Dhaka, Bangladesh. This research was funded by the Krishi Gobeshona Foundation (KGF), Bangladesh, Project ID TF 153-C/23.

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(Manuscript received on 17 May, 2026; revised on 12 June, 2026)