Research Article

Conserved Epitope Mapping of Potato Virus Y for Broad-Spectrum Antigen Design: Implications for Plant Immune Recognition and Diagnostic Innovation

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Abstract | Potato virus Y (PVY) is a major plant pathogen affecting solanaceous crops, particularly potato and tomato, leading to significant economic losses. The viral coat protein (CP) is essential for virion stability, host interactions, and immune recognition. Identifying conserved and variable epitopes within the PVY CP provides insights into viral evolution, host adaptation, and potential targets for disease control. This study aimed to investigate the conservation and sequence variability of key PVY CP epitopes across diverse geographic locations and host species. By analyzing the epitope sequences, this study identified the conserved regions suitable for diagnostic tools and the variations indicative of viral adaptation. A comparative sequence analysis of PVY strains from different regions was conducted. Multiple sequence alignments determined identity percentages, conserved motifs, and variations in epitopes, including PVY-CP-A, -D, -F, -G, -H, and -I. Most epitopes exhibited high conservation. PVY-CP-A showed 100 % identity in most strains, with slight variations (91.67 %) in a Greek isolate. PVY-CP-D and -F were largely conserved, with minor variations (87.50 %) recorded in Poland and Bangladesh. PVY-CP-G displayed reduced identity (83.33%) in Poland, China, and Bangladesh, suggesting adaptation. PVY-CP-H was completely conserved, reinforcing its structural importance. PVY-CP-I showed the highest conservation, with minor variations in the UK and China. These findings highlight the conserved PVY epitopes as potential targets for universal diagnostic tools and control strategies. Minor variations suggest regional adaptations, influencing pathogenicity, and host interactions. This study provides valuable insights into PVY evolution, aiding in improved virus detection and diagnosis development.

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Introduction

Potato virus Y (PVY) is one of the most economically damaging viruses affecting potato crops worldwide. Belonging to the *Potyviridae* family, PVY is transmitted primarily by aphids in a non-persistent manner, which significantly increases its spread in agricultural fields (Ali, 2024; Li *et al.*, 2025). PVY infects not only potatoes but also a wide variety of other Solanaceous plants, causing high yield losses and quality deterioration (Kassim *et al.*, 2014; Berindean *et al.*, 2024; Dupuis *et al.*, 2024). The virus's genetic diversity and ability to adapt to different environmental conditions and host plants make it particularly challenging to control (Verma *et al.*, 2016; Rushton *et al.*, 2024). Resistance strategies based on chemical control, crop rotation, or breeding resistant varieties have had limited success, primarily due to the virus's rapid mutation rate and ability to evolve resistance to the conventional management practices (Bai *et al.*, 2019; Zhang *et al.*, 2023). Consequently, there has been growing interest in using alternative strategies, such as the development of plant vaccines or immune-based therapies, to combat PVY infections in agricultural settings (Anikina *et al.*, 2023; Lee *et al.*, 2023).

The viral coat protein (CP) of PVY plays a central role in the virus's lifecycle. It encapsulates the viral RNA genome, protecting it from degradation, facilitates the virus's movement within the host plant, and its transmission by aphids (Bhoi *et al.*, 2022; Machado-Assefh *et al.*, 2023; Pitt *et al.*, 2024). Beyond its structural role, the CP is a major target for the host's immune system, eliciting both humoral and cellular immune responses. The viral CP is highly immunogenic, as it can stimulate the production of antibodies and activate the T-cells that recognize viral peptides presented by major histocompatibility complex (MHC) molecules (Lindbo and Falk, 2017; Sheibani *et al.*, 2023; Sheibani *et al.*, 2024). However, PVY, like many other plant viruses, has evolved several mechanisms to evade immune detection, thus complicating efforts are needed to mount a robust and effective immune response (Liu *et al.*, 2017). Understanding the specific epitopes within the PVY CP that are recognized by the immune system is crucial for developing strategies to overcome this immune evasion.

Epitopes are the specific regions of a protein that are recognized by the immune system, particularly by the T-cells and antibodies. The viral CP, being the most exposed part of the virus, is a key candidate for identifying such epitopes. Several studies on other plant viruses demonstrated that viral CP often harbors conserved epitopes that can be targeted for immune intervention (Cuevas *et al.*, 2012). In PVY, these epitopes are likely to play a pivotal role in modulating the immune response, and their identification could provide valuable insights into how the virus interacts with its host and evades immune surveillance (Tian *et al.*, 2014; Belabess *et al.*, 2024). Furthermore, identifying these epitopes is not only important for understanding the virus's immune evasion mechanisms but also for the development of targeted vaccine strategies, enhancing immune recognition of the virus and preventing infection.

While various epitopes of viral proteins have been identified in related plant viruses, limited studies have comprehensively mapped the immune epitopes within the PVY CP. A previous study has suggested that the PVY CP contains regions capable of binding to MHC molecules, which could trigger the T-cell responses (Rosendahl *et al.*, 2014). Other studies have pointed to the importance of small peptide sequences, ranging from 8 to 15 amino acids in binding to MHC class I and class II molecules, triggering both cellular and humoral immune responses (Afridi *et al.*, 2016; Rock *et al.*, 2016; Rich and Chaplin, 2019). However, the full spectrum of immune-dominant epitopes within the PVY CP remains largely unexplored.

In this context, identifying and characterizing these epitopes from the PVY CP could pave the way for the development of epitope-based vaccines and other therapeutic strategies. Peptide-based vaccines are gaining attention due to their ability to target specific regions of a pathogen, minimizing the risk of immune evasion and providing a more precise immune response (Malonis *et al.*, 2019; Nimisha *et al.*, 2024). These vaccines can stimulate both humoral and cellular immune responses, with potential applications not only in plant pathology but also in broader antiviral vaccine development. Additionally, identifying epitopes that bind to MHC molecules could provide insights into how PVY evades immune detection and how this evasion might be overcome using immunotherapy.

Furthermore, advances in computational tools have

made it possible to predict the binding affinities of viral peptides to host MHC molecules, greatly accelerating the identification of potential vaccine candidates (Croft *et al.*, 2019). By combining experimental and computational methods, it is now possible to more accurately identify and characterize immunodominant epitopes and design vaccines that could effectively combat PVY infection. However, for these vaccines to be effective, further validation is required to confirm the ability of the identified epitopes to induce immune responses *in vivo* and their capacity to bind to MHC molecules (Kreiter *et al.*, 2015).

PVY is one of the most economically significant viral pathogens affecting potato crops worldwide, with a wide range of strains and evolving variants that complicate effective detection and management. Traditional virus purification techniques used to produce antisera for diagnostic applications are often time-consuming, labor-intensive, and yield limited antigen quantities (Lacomme and Jacquot, 2017; Chikh-Ali and Karasev, 2023). In this context, the epitope-based strategies offer a promising alternative for generating broad-spectrum diagnostic tools.

The objective of the present study was to identify conserved epitopes within the PVY CP across a wide range of global isolates. The most immunodominant and conserved epitopes were selected for synthetic peptide production, with the goal of developing an efficient and scalable antigen suitable for generating PVY-specific polyclonal antibodies. Such an approach not only enhances serological detection across multiple PVY strains but also provides insights into viral structural conservation and potential plant immune interactions.

Materials and Methods

Viral strains and plant materials

Potato virus Y (PVY) strains originating from diverse geographic regions and host species were collected, including isolates from Egypt (QKI29299.1), Kazakhstan (QGR25617.1), Turkey (CAC19617.1), Poland (AIA59688.1, AIA59686.1, and AIA59685.1), China (AGU99182.1, AGU99163.1, and AGU99138.1), and other countries. Host plants, including potato and tomato, were used to propagate PVY isolates under controlled greenhouse conditions.

Sequence retrieval and epitope prediction

Potato virus CP sequences from various geographic isolates were retrieved from GenBank (https://www.ncbi.nlm.nih.gov/protein/). Potential epitopes were predicted using an immune epitope prediction tools such as IEDB (Bepipred Linear Epitope Prediction 2.0 (Vita *et al.*, 2019) based on the CP sequences.

Epitopes analysis

The identified epitopes were compared across the PVY strains to assess their conservation and sequence variability. The analysis focused on amino acid identity percentages and variations in specific regions. The sequences were aligned using multiple sequence alignment tools (blastp, protein-protein BLAST, (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify conserved and variable regions within key epitopes (PVY-CP-A, -D, -F, -G, -H, and -I).

Phylogenetic analysis

A phylogenetic tree was constructed based on the aligned PVY sequences using MEGA (Molecular Evolutionary Genetics Analysis) software (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This tree was used to assess the genetic relationship among the different PVY strains and identify the potential evolutionary patterns in the epitopes.

Data interpretation

The obtained results were interpreted to determine the functional significance of an epitope conservation and variability. The implications for diagnostic tool development, PVY vaccine design, and future PVY management strategies were discussed.

Results

Identification of epitopes

In this study, several CP epitopes from the viral genome of PVY were identified and characterized, which were crucial for understanding the virus's immune recognition and potential for vaccine development. The identified epitopes, derived from various regions of the viral CP, are listed in Table 1, where each entry represents a unique peptide sequence with its corresponding length.

The identified epitopes spanned a range of lengths ranging from a single amino acid (e.g., peptide 3: "I") to 84 amino acids (peptide 1). This diversity in length suggested that the viral CP contained regions

Table 1: *Coat protein epitopes of Potato Virus Y (AAM81207.1) and their corresponding peptide sequences and lengths.*

No. of protein epitopes	Codes	Start	End	Peptide	Length amino acids
1	A	5	88	IDAGGSSKKDARPEQGSIQSNPNKGKDKDVNAGTSGTHTVPRI-KAITSKMRMPTSKGATVLNLEHLLEYAPQQIDISNTRATQS	84
2	B	90	92	FDT	3
3	C	103	103	I	1
4	D	123	130	GTSPNVNG	8
5	E	139	141	EQV	3
6	F	172	181	EMRNKKEPYM	10
7	G	187	193	IRNLRDM	7
8	H	206	211	TSRTPV	6
9	I	238	263	GISTQEENTERHTTEDVSPSMHTLLG	26

with varying degrees of immunogenicity, which may interact differently with the host immune system. The longest epitope (peptide 1) included a stretch of amino acids from position 5 to 88 and could be a key target for immune responses, given that its substantial size and possible structural conformation may enhance its ability to bind to the major histocompatibility complex (MHC) molecules.

Conservation of PVY coat protein epitopes across regions and hosts

The data presented in this study highlight the identity percentages of amino acid sequences of various epitopes derived from the PVY CP of an Egyptian strain, compared to capsid proteins from different geographic regions and hosts. The results indicated a high degree of conservation for most of the epitopes, with the majority of sequences exhibiting 100 % identity across the different countries and the host plants. This suggests that the regions of the PVY CP under investigation, including Epitope-A, Epitope-D, Epitope-F, Epitope-G, Epitope-H, and Epitope-I were highly conserved across the diverse geographic regions and host species, such as potato and tomato. While most sequences exhibited perfect identity, few strains showed minor variations, particularly in Epitope-G and Epitope-I, where the identity percentages dropped slightly (83.33 % and 96.15 %, respectively).

Sequence variability in the PVY epitope regions

PVY-CP-A epitope

The identities of the PVY-CP-A epitope sequences ranged from 91.67 % to 100 % across the tested samples (Table 2, Figure 1). The majority of sequences exhibited 100 % identity, including those from Egypt, Poland, China, Germany, South Africa, Kazakhstan, and Iran. Some sequences, particularly from Poland, China, and Jordan, showed slightly reduced identity percentages (98.81 %), while the lowest percentage (91.67 %) was observed for a sequence from Greece. The sequences analyzed were primarily from potato hosts (e.g., Egypt, China, Germany, South Africa, and Kazakhstan), with a few from tomato (e.g., Poland, Greece, and Bangladesh). Sequences from the United Kingdom lacked host information.

PVY-CP-D epitope

The PVY-CP-D epitope sequences obtained from Egypt, China, Czech Republic, Germany, Greece, Iran, Jordan, Kazakhstan, South Africa, Turkey, and the United Kingdom displayed 100 % identity (Table 2, Figure 1). However, some sequences from Poland (AIA59677.1, AIA59685.1, AIA59688.1, and AIA59686.1) and Bangladesh (AFO64661.1) exhibited a lower identity percentage (87.50 %). The host distribution included both potato (e.g., Egypt, China, South Africa, Poland, Kazakhstan, and Iran) and tomato (e.g., Greece and Poland). Some sequences lacked host information.

PVY-CP-F epitope

Sequences from the United Kingdom, Iran, Jordan, South Africa, Germany, Czech Republic, China, Poland, Kazakhstan, Egypt, and Bangladesh all displayed 100 % identity in the PVY-CP-F epitope (Table 2, Figure 2). The sequences were derived from both potato and tomato hosts, demonstrating the conservation of this epitope across the different species. Sequences from Turkey and the United Kingdom lacked host information.

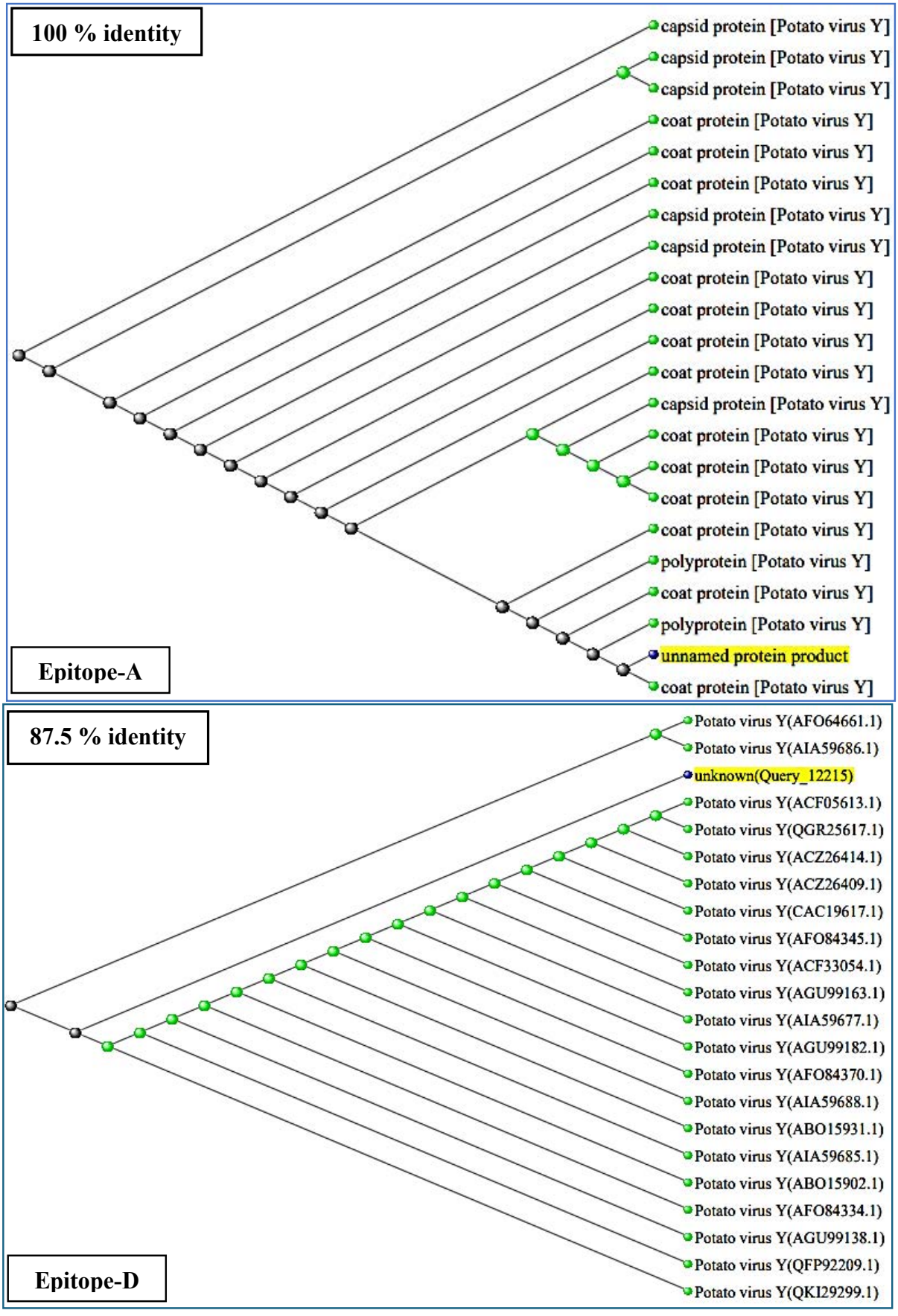

Figure 1: *Phylogenetic analysis of amino acid sequences of Epitopes A and D, derived from the Potato Virus Y coat protein of an Egyptian strain, compared to capsid proteins from diverse geographic regions and hosts, revealing that the ligands themselves demonstrate strong sequence conservation and evolutionary stability, with identity percentages ranging from 87.5 % to 100 %. This underscores their intrinsic potential as universal targets for immune recognition and diagnostic development.*

Table 2: *Identity (%) of amino acid sequences of Epitopes A, D and F derived from the Potato Virus Y coat protein of an Egyptian strain and capsid protein across multiple geographic regions and hosts with 100 % query cover.*

Accession no.	Countries	Hosts	Epitope-A		Epitope-D		Epitope-F	
			E value	Identities (%)	E value	Identities (%)	E value	Identities (%)
ABO15902.1	United Kingdom	----	4e-59	100.00	2e-05	100.00	8e-10	100.00
ABO15931.1	United Kingdom	----	4e-59	100.00	2e-05	100.00	8e-10	100.00
ACF05613.1	Iran	Potato	5e-59	100.00	2e-05	100.00	8e-10	100.00
ACF33054.1	Jordan	Potato	3e-58	98.81	2e-05	100.00	8e-10	100.00
ACZ26409.1	South Africa	Potato	4e-59	100.00	2e-05	100.00	8e-10	100.00
ACZ26414.1	South Africa	Potato	4e-59	100.00	2e-05	100.00	8e-10	100.00
AFO64661.1	Bangladesh	Potato	8e-52	90.48	1e-04	87.50	8e-10	100.00
AFO84334.1	Germany	Potato	3e-59	100.00	2e-05	100.00	8e-10	100.00
AFO84345.1	Greece	Tomato	4e-54	91.67	2e-05	100.00	8e-10	100.00
AFO84370.1	Czech Republic	Potato	4e-59	100.00	2e-05	100.00	8e-10	100.00
AGU99138.1	China	Potato	3e-59	100.00	2e-05	100.00	8e-10	100.00
AGU99163.1	China	Potato	2e-58	98.81	2e-05	100.00	8e-10	100.00
AGU99182.1	China	Potato	5e-59	100.00	2e-05	100.00	8e-10	100.00
AIA59677.1	Poland	Tomato	8e-59	98.81	2e-05	100.00	8e-10	100.00
AIA59685.1	Poland	Tomato	4e-59	100.00	2e-05	100.00	8e-10	100.00
AIA59686.1	Poland	Tomato	3e-59	100.00	2e-05	100.00	8e-10	100.00
AIA59688.1	Poland	Tomato	4e-59	100.00	2e-05	100.00	8e-10	100.00
CAC19617.1	Turkey	—	7e-53	90.48	2e-05	100.00	8e-10	100.00
QFP92209.1	China	Potato	3e-59	100.00	2e-05	100.00	8e-10	100.00
QGR25617.1	Kazakhstan	Potato	5e-59	100.00	2e-05	100.00	8e-10	100.00
QKI29299.1	Egypt	Potato	2e-59	100.00	1e-04	87.50	8e-10	100.00

Table 3: *Identity (%) of amino acid sequences of Epitopes G, H and I derived from the Potato Virus Y coat protein of an Egyptian strain and capsid protein across multiple geographic regions and hosts with 100 % query cover.*

Accession no.	Countries	Hosts	Epitope-G		Epitope-H		Epitope-I	
			E value	Identities (%)	E value	Identities (%)	E value	Identities (%)
ABO15902.1	United Kingdom	----	7e-06	100.00	0.001	100.00	7e-25	100.00
ABO15931.1	United Kingdom	----	7e-06	100.00	0.001	100.00	8e-24	96.15
ACF05613.1	Iran	Potato	7e-06	100.00	0.001	100.00	7e-25	100.00
ACF33054.1	Jordan	Potato	3e-04	100.00	0.001	100.00	7e-25	100.00
ACZ26409.1	South Africa	Potato	7e-06	100.00	0.001	100.00	7e-25	100.00
ACZ26414.1	South Africa	Potato	7e-06	100.00	0.001	100.00	7e-25	100.00
AFO64661.1	Bangladesh	Potato	0.003	83.33	0.001	100.00	7e-25	100.00
AFO84334.1	Germany	Potato	7e-06	100.00	0.001	100.00	7e-25	100.00
AFO84345.1	Greece	Tomato	3e-04	100.00	0.001	100.00	7e-25	100.00
AFO84370.1	Czech Republic	Potato	7e-06	100.00	0.001	100.00	7e-25	100.00
AGU99138.1	China	Potato	3e-04	100.00	0.001	100.00	7e-25	100.00
AGU99163.1	China	Potato	7e-06	100.00	0.001	100.00	7e-25	100.00
AGU99182.1	China	Potato	0.003	83.33	0.001	100.00	7e-25	100.00
AIA59677.1	Poland	Tomato	7e-06	100.00	0.001	100.00	7e-25	100.00
AIA59685.1	Poland	Tomato	7e-06	100.00	0.001	100.00	7e-25	100.00
AIA59686.1	Poland	Tomato	0.003	83.33	0.001	100.00	7e-25	100.00
AIA59688.1	Poland	Tomato	7e-06	100.00	0.001	100.00	7e-25	100.00
CAC19617.1	Turkey	—	3e-04	100.00	0.001	100.00	7e-25	100.00
QFP92209.1	China	Potato	7e-06	100.00	0.001	100.00	3e-23	96.15
QGR25617.1	Kazakhstan	Potato	7e-06	100.00	0.001	100.00	7e-25	100.00
QKI29299.1	Egypt	Potato	7e-06	100.00	0.001	100.00	7e-25	100.00

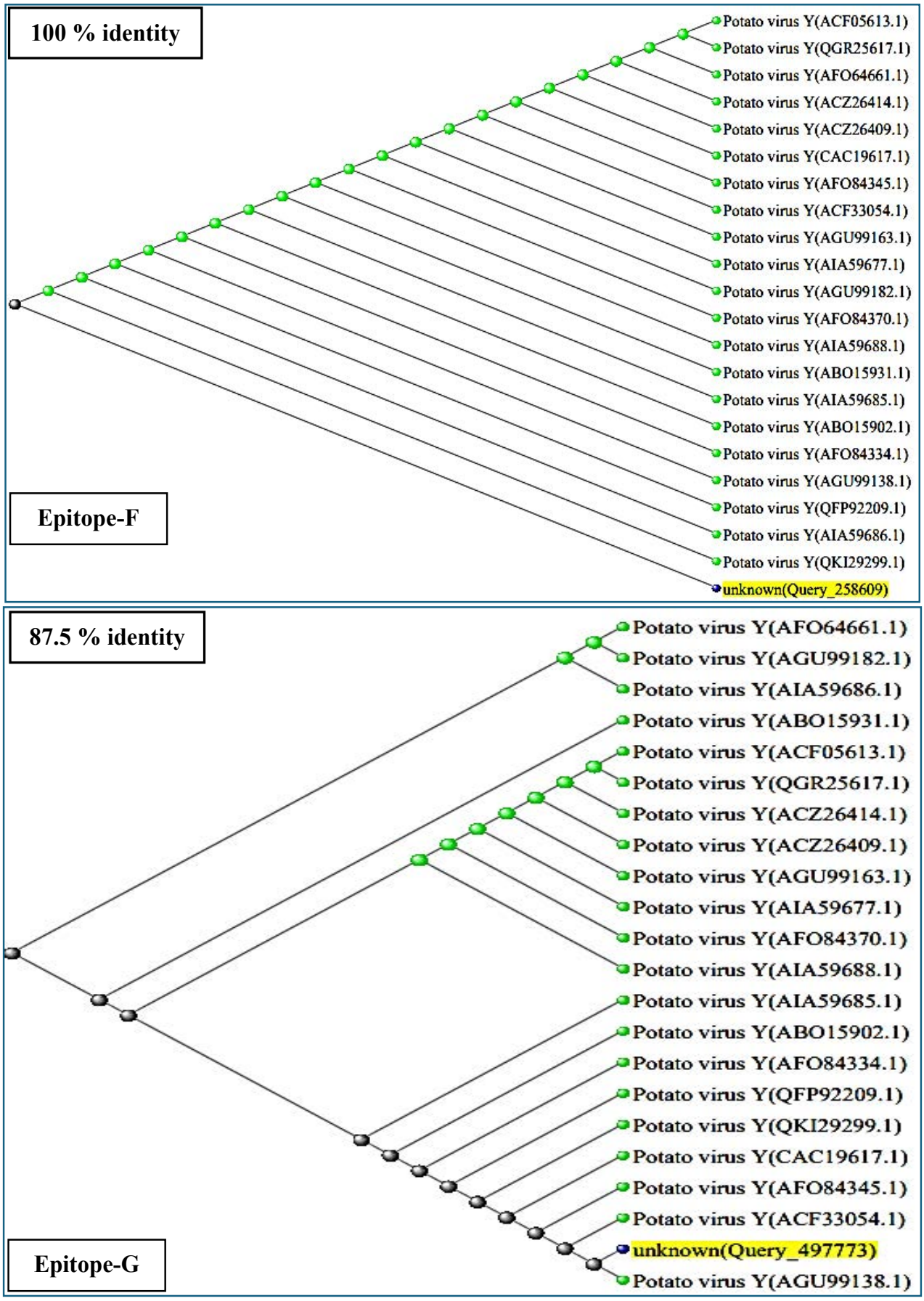

Figure 2: *Phylogenetic analysis of amino acid sequences for Epitopes F and G from the Potato Virus Y coat protein. The epitopes show high sequence conservation across isolates from diverse geographic regions and host plants, with identity percentages ranging from 87.5 % to 100 %. This strong conservation supports their use as reliable targets for broad-spectrum diagnostics and immunological studies.*

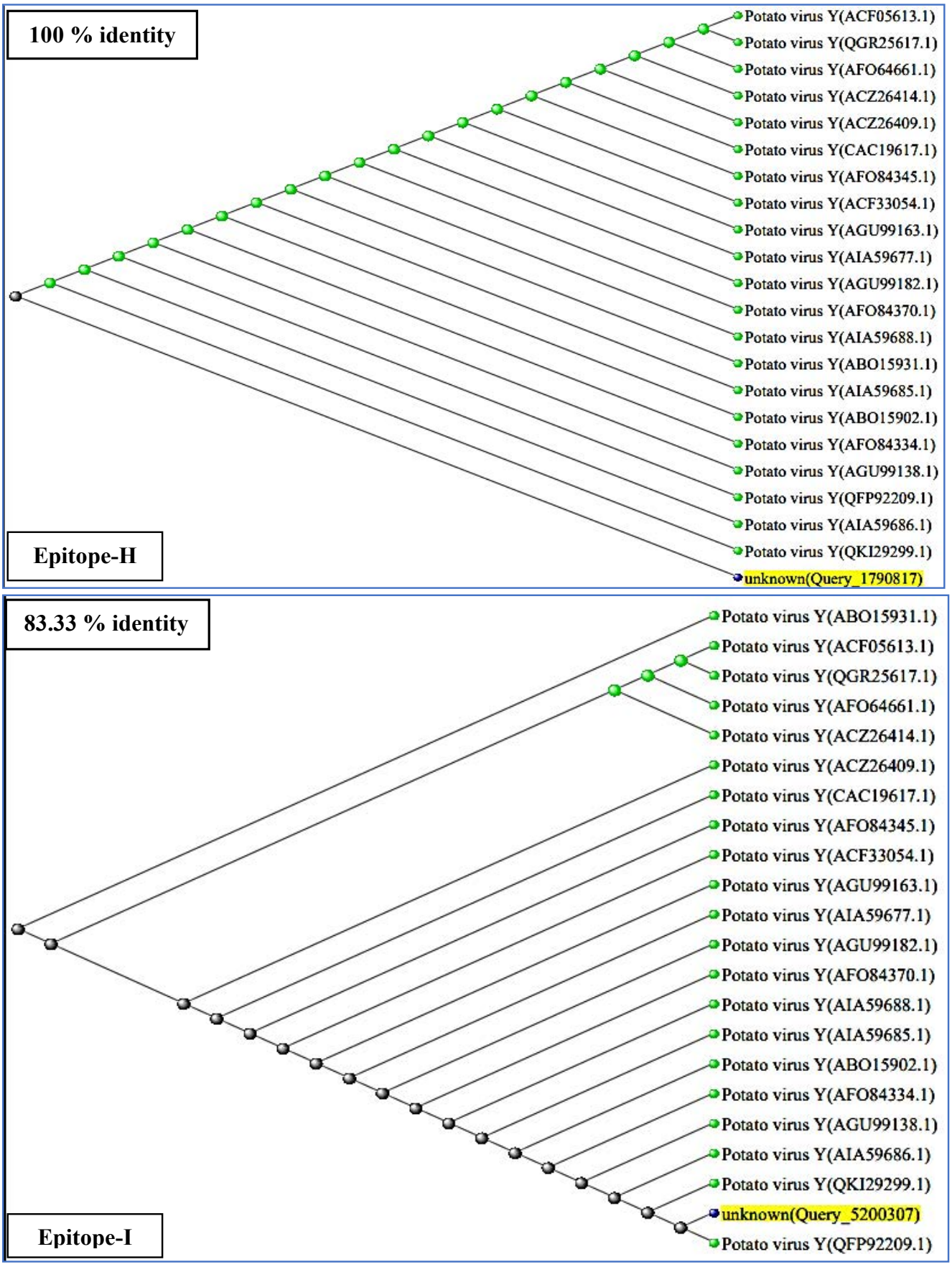

Figure 3: *Phylogenetic analysis of amino acid sequences for Epitopes H and I from the Potato Virus Y coat protein demonstrates strong evolutionary conservation across diverse geographic regions and host species. Identity percentages range from 83.33 % to 100 %, underscoring the stability of these epitopes across global PVY variants. This conservation supports their potential as effective targets for cross-strain immune recognition and diagnostic assay development.*

PVY-CP-G epitope

The PVY-CP-G epitope exhibited 100 % identity across sequences from Egypt, Kazakhstan, China, Poland, the Czech Republic, South Africa, Iran, Turkey, Greece, Jordan, and Bangladesh (Table 3, Figure 2). However, slight reductions in identity (83.33 %) were observed in sequences from Poland (AIA59686.1), China (AGU99182.1), and Bangladesh (AFO64661.1).

PVY-CP-H epitope

The PVY-CP-H epitope sequences from the United Kingdom, Iran, Jordan, South Africa, Germany, Greece, Czech Republic, China, Poland, Kazakhstan, Egypt, and Bangladesh showed complete identity (Table 3, Figure 3). Host information was unavailable for some sequences from Turkey and the United Kingdom.

PVY-CP-I epitope

The PVY-CP-I epitope sequences from Egypt, Kazakhstan, Turkey, Poland, China, the Czech Republic, Greece, Germany, Bangladesh, South Africa, Jordan, and Iran displayed 100 % identity (Table 3, Figure 3). Slight deviations (96.15 % identity) were observed in sequences from the United Kingdom (ABO15931.1) and China (QFP92209.1).

Comparative analysis and evolutionary insights of PVY epitopes

PVY-CP-A epitope

Data in Figure 4 demonstrated the comparative alignment of variations in the amino acid sequences of Epitope-A (PVY-CP-A) derived from the CP of an Egyptian strain of PVY across multiple geographic strains and hosts. The alignment revealed notable sequence similarities and specific amino acid substitutions across the different sequences.

PVY-CP-A 1	PVY-CP-A 61 LNLEHLLEYAPQQIDISNTRATQS 84
IDAGGSSKKDARPEQGSIQSNPNKGKDKDVNAGT	QKI29299.1 65 88
SGTHTVPRIKAITSKMRMPTSKGATV 60	AIA59686.1 65 88
QKI29299.1 5 64	QFP92209.1 65 88
AIA59686.1 5 64	AGU99138.1 65 88
QFP92209.1 5 64	AFO84334.1 65 88
AGU99138.1 5 64	ABO15902.1 65 88
AFO84334.1 5 64	AIA59685.1 65 88
ABO15902.1 5 64	ABO15931.1 65 88
AIA59685.1 5 64	AIA59688.1 65 88
ABO15931.1 5 64	AFO84370.1 65 88
AIA59688.1 5 64	ACZ26409.1 66 89
AFO84370.1 5 64	ACZ26414.1 66 89
ACZ26409.1 6 65	QGR25617.1 82 105
ACZ26414.1 6 65	AGU99182.1 65 88
QGR25617.1 22 81	ACF05613.1 111 134
AGU99182.1 5 64	AIA59677.1 65 88
ACF05613.1 51 110	AGU99163.1 65 88
AIA59677.1 5 L..... 64	ACF33054.1 65 88
AGU99163.1 5 .....S..... 64	AFO84345.1 65 ..... T..... 88
ACF33054.1 5 ..... K..... 64	CAC19617.1 65 ..... T..... 88
AFO84345.1 5 .....K.....P..... K...VAA 64	AFO64661.1 66 89
CAC19617.1 5 ....E....K.....P..... K...VAA 64	
AFO64661.1 6 ....T...Q...P.L..E.E....V..... K..... 65	

Figure 4: *Comparative alignment of amino acid sequence variations in the Potato Virus Y-CP-A epitope, derived from the coat protein of an Egyptian PVY strain, against strains from multiple geographic regions and host species, revealing a high level of sequence conservation. This suggests that the epitope maintains structural and functional integrity across diverse PVY isolates, reinforcing its potential utility in the design of broad-range diagnostic tools and immunological assays.*

The majority of the sequences displayed 100 % identity with the Egyptian PVY strain (QKI29299.1), with sequences from several countries, including China (AGU99138.1, AGU99163.1, AGU99182.1), Greece (AFO84345.1), Poland (AIA59677.1, AIA59685.1, and AIA59688.1), the United Kingdom (ABO15902.1 and ABO15931.1), South Africa (ACZ26409.1 andACZ26414.1), and others showing a high degree of sequence identity (88–100 %). The alignment also showed some notable variations. The sequence from Kazakhstan (QGR25617.1) displayed the most significant variation, with twenty-two amino acid differences compared to the Egyptian strain. Similarly, the sequence from Bangladesh (AFO64661.1) exhibited mutations at positions 61-65.

PVY-CP-D, F, and G epitopes

Data in Figure 5 present the comparative alignment of variations in amino acid sequences of three epitopes (*i.e.*, PVY-CP-D, PVY-CP-F, and PVY-CP-G) derived from the CP of an Egyptian PVY strain, analyzed across multiple geographic strains and hosts. The PVY-CP-D epitope (positions 1-8) showed high conservation across most sequences, with only a small variation in the sequence from Bangladesh (AFO64661.1) and Poland (AIA59686.1), where the amino acid "I" appeared at position 130. The PVY-CP-F epitope (positions 1-10) exhibited remarkable conservation across the strains from multiple countries, with a single amino acid difference at position 182 in the sequence from Bangladesh (AFO64661.1). The PVY-CP-G epitope (positions 1-7) remained highly conserved across most of the analyzed sequences, with only a minor variation in the sequence from Bangladesh (AFO64661.1), where the amino acid "V" appeared at position 193.

PVY-CP-H and I epitopes

Results in Figure 6 demonstrate a comparative alignment of variations in the amino acid sequences of two epitopes; PVY-CP-H and PVY-CP-I, derived from the CP of an Egyptian PVY strain across multiple geographic strains and hosts.

PVY-CP-D 1 GTSPNVNG 8	PVY-CP-F 1 EMRNKKEPYM 10	PVY-CP-G 1 IRNLRDM 7
ACF05613.1 169 176	ACF05613.1 218 227	ACF05613.1 233 239
QGR25617.1 140 147	QGR25617.1 189 198	QGR25617.1 204 210
ACZ26414.1 124 131	AFO64661.1 173 182	ACZ26414.1 188 194
ACZ26409.1 124 131	ACZ26414.1 173 182	ACZ26409.1 188 194
QKI29299.1 123 130	ACZ26409.1 173 182	QKI29299.1 187 193
QFP92209.1 123 130	AIA59686.1 172 181	QFP92209.1 187 193
CAC19617.1 123 130	QKI29299.1 172 181	AIA59688.1 187 193
AIA59688.1 123 130	QFP92209.1 172 181	AIA59685.1 187 193
AIA59685.1 123 130	CAC19617.1 172 181	AIA59677.1 187 193
AIA59677.1 123 130	AIA59688.1 172 181	AGU99163.1 187 193
AGU99182.1 123 130	AIA59685.1 172 181	AFO84370.1 187 193
AGU99163.1 123 130	AIA59677.1 172 181	AFO84334.1 187 193
AGU99138.1 123 130	AGU99182.1 172 181	ABO15931.1 187 193
AFO84370.1 123 130	AGU99163.1 172 181	ABO15902.1 187 193
AFO84345.1 123 130	AGU99138.1 172 181	CAC19617.1 187 192
AFO84334.1 123 130	AFO84370.1 172 181	AGU99138.1 187 192
ACF33054.1 123 130	AFO84345.1 172 181	AFO84345.1 187 192
ABO15931.1 123 130	AFO84334.1 172 181	ACF33054.1 187 192
ABO15902.1 123 130	ACF33054.1 172 181	AFO64661.1 188 V..... 193
AFO64661.1 124 .....I.. 131	ABO15931.1 172 181	AIA59686.1 187 V..... 192
AIA59686.1 123 .....I.. 130	ABO15902.1 172 181	AGU99182.1 187 V..... 192

Figure 5: *Comparative alignment of amino acid sequences of three epitopes (PVY-CP-D, PVY-CP-F, and PVY-CP-G) derived from the coat protein of an Egyptian PVY strain across multiple geographic strains and hosts showing a high degree of sequence conservation. Identity percentages are mostly at 100 %, with minor variations observed, such as an isoleucine substitution in PVY-CP-D and valine substitutions in PVY-CP-G, indicating strong stability of these epitopes across thr diverse PVY isolates. This conservation supports their potential as reliable targets for broad-spectrum diagnostic and immunological applications.*

PVY-CP-H 1 TSRTPV 6	PVY-CP-I 1
ACF05613.1 252 257	GISTQEENTERHTTEDVSPSMHTLLG 26
QGR25617.1 223 228	ACF05613.1 284 309
AFO64661.1 207 212	QGR25617.1 255 280
ACZ26414.1 207 212	AFO64661.1 239 264
ACZ26409.1 207 212	ACZ26414.1 239 264
CAC19617.1 206 211	ACZ26409.1 239 264
AFO84345.1 206 211	CAC19617.1 238 263
ACF33054.1 206 211	AFO84345.1 238 263
AGU99163.1 206 211	ACF33054.1 238 263
AIA59677.1 206 211	AGU99163.1 238 263
AGU99182.1 206 211	AIA59677.1 238 263
AFO84370.1 206 211	AGU99182.1 238 263
AIA59688.1 206 211	AFO84370.1 238 263
ABO15931.1 206 211	AIA59688.1 238 263
AIA59685.1 206 211	AIA59685.1 238 263
ABO15902.1 206 211	ABO15902.1 238 263
AFO84334.1 206 211	AFO84334.1 238 263
AGU99138.1 206 211	AGU99138.1 238 263
QFP92209.1 206 211	AIA59686.1 238 263
AIA59686.1 206 211	QKI29299.1 238 263
QKI29299.1 206 211	ABO15931.1 238 ..X..... 263
	QFP92209.1 238 .....R..... 263

Figure 6: *Comparative alignment of amino acid sequences of two epitopes (PVY-CP-H and PVY-CP-I) derived from the coat protein of an Egyptian PVY strain across multiple geographic strains and hosts showing high sequence conservation, with identity percentages mostly at 100 % and minor variations ranging between 83.33 % and 96.15 %. This demonstrates the evolutionary stability of these epitopes across diverse PVY isolates, supporting their potential as reliable targets for broad-spectrum diagnostic and immunological applications.*

The PVY-CP-H epitope (positions 1-6) exhibited high conservation across all the analyzed strains and hosts, with no significant variations in most sequences. The PVY-CP-I epitope (positions 1-26) was also largely conserved across the strains, with a substitution observed in the Polish sequence (ABO15931.1), "X: unknown amino acid" appeared at position 263. Additionally, a variation was noted in the Chinese sequence (QFP92209.1), where "R: stands for Arginine" was present at the same position.

Discussion

In this study, we aimed to identify conserved epitopes within the Potato Virus Y (PVY) proteome to inform the design of broad-spectrum diagnostic tools and potential antigen candidates. Through comprehensive epitope mapping, several peptide regions with high conservation and immunological relevance were uncovered, suggesting their utility in enhancing both plant immune recognition and the sensitivity of PVY detection methods. These findings provide insights into viral-host interactions and offer promising targets for the development of cross-strain diagnostic assays and immune-based strategies.

Peptide 1 (positions 5-88) is particularly noteworthy due to its large size (84 amino acids), suggesting that it may function as a dominant epitope for antibody binding and immune recognition. Peptides of this

length are often more effective in eliciting a T-cell response due to their ability to be processed and presented by antigen-presenting cells (APCs) (Eiz-Vesper and Schmetzer, 2020).

Peptides 2, 3, 4, 5, 7, and 8 (ranging from 1 to 10 amino acids) are significantly shorter. These peptides might play an essential role in inducing specific antibody responses or may act as minimal epitopes for T-cell recognition. Shorter peptides are generally known to bind tightly to the MHC molecules, facilitating more efficient immune response activation (Perez *et al.*, 2022; Wu *et al.*, 2023; Song *et al.*, 2024).

Peptide 9 (positions 238-263) is another notable sequence, as it spans 26 amino acids, contributing to antigenic properties through its involvement in both T- and B-cell interactions. Peptides of this length may present a balance between immunogenicity and the ability to form stable complexes with the MHC molecules (Wieczorek *et al.*, 2017).

The identification of these epitopes is significant for vaccine development, as targeting these regions enhances the immune system's ability to recognize and eliminate the virus. In particular, epitopes such as peptide 1 (84 amino acids) could be used in the design of vaccines, which focus on inducing both humoral (antibody-mediated) and cellular (T-cell-mediated) immune responses. The shorter peptides (e.g., peptides 2, 3, 4, 5) might also be suitable for inclusion in peptide-based vaccines aimed at stimulating specific immune responses against the virus (Malonis *et al.*, 2019).

Additionally, these findings underscore the importance of the CP as a critical antigenic target. Previous studies have shown that the viral CP plays a key role in immune evasion, and identifying specific epitopes within this protein could pave the way for more targeted therapeutic strategies (Lindbo and Falk, 2017). Further validation of these epitopes using *in vivo* models and their ability to bind to the MHC molecules is essential for the development of epitope-based vaccines.

These current findings underscore the genetic stability of PVY and its potential for cross-region diagnosis and control. The observed conservation of these epitopes across multiple PVY strains reinforces their potential as targets for diagnostic tools and control strategies aimed at managing PVY infections globally (Gibbs *et al.*, 2017). This supports several previous studies highlighting the importance of these epitopes in PVY's structural integrity and viral function (Quenouille *et al.*, 2013; Trovato *et al.*, 2020).

The high identity percentages observed in the analyzed PVY epitope regions indicate significant conservation across the diverse geographic locations and host plants, suggesting that these epitopes play critical roles in PVY's structural integrity and host-virus interactions. The PVY-CP-A epitope exhibited remarkable conservation with most sequences showing 100 % identity. The few variations, such as the 91.67 % identity observed in a Greek sequence, could reflect regional adaptations. This stability highlights the epitope's potential as a target for diagnostic tools and control measures (Moury *et al.*, 2017).

Similarly, the PVY-CP-D epitope displayed 100 % identity in most sequences, with slight variations observed in Poland and Bangladesh (87.50 %). These minor differences might be attributed to regional strain evolution or host interactions (Cuevas *et al.*, 2012). The presence of sequences from both potato and tomato underscores the virus's polyphagous nature (Karasev and Gray, 2013a). The complete conservation of the PVY-CP-F epitope across multiple regions reinforces its importance in viral stability and host interactions. The absence of significant variations suggests that this region is under strong evolutionary pressure to remain unchanged, making it an ideal target for universal diagnostic methods (Tsedaley, 2015).

Epitope-G showed high conservation across most samples, with a few sequences from Poland, China, and Bangladesh exhibiting reduced identity (83.33 %). These differences might indicate ongoing evolutionary adaptations, warranting further investigation into their impact on pathogenicity and host interactions (Revers and García, 2015).

The PVY-CP-H epitope was completely conserved across all the analyzed regions. This consistency supports its essential role in viral fitness and adaptability across different host plants. The presence of sequences without host information suggests that some studies focused solely on viral genetic material rather than host specificity (Lefeuvre *et al.*, 2019).

The PVY-CP-I epitope displayed the highest degree of conservation, with nearly all sequences exhibiting 100 % identity. The minor variations observed in sequences from the United Kingdom and China may represent adaptive changes, but the overall stability of this epitope indicates its importance for viral survival. Its conservation makes it a promising target for broad-spectrum control strategies (Elena *et al*., 2014; Lacomme and Jacquot, 2017).

The comparative analysis of PVY CP epitopes across various geographic strains and hosts provides crucial insights into the evolutionary dynamics and functional significance of these regions. The high degree of conservation observed in most epitopes indicates that these regions play a pivotal role in the viral life cycle, contributing to structural stability, host interactions, and/ or immune evasion.

The conservation of PVY-CP-A, PVY-CP-D, PVY-CP-F, PVY-CP-G, PVY-CP-H, and PVY-CP-I across the diverse geographic strains highlights their potential functional constraints. Such conservation is indicative of evolutionary pressure to maintain these regions for essential viral functions, such as stability, host receptor binding, or immune system evasion (Moury *et al*., 2017). The observed 88-100 % sequence identity across the multiple countries and host plants further underscores the significance of these epitopes in PVY pathogenicity and transmission. The observed strong conservation also suggests that these epitopes could be exploited for broad-spectrum detection tools or vaccine development.

Despite the overall conservation, some regional and host-specific variations were identified. The strain from Kazakhstan (QGR25617.1) exhibited the most significant divergence in PVY-CP-A, recording 22 amino acid differences compared to the Egyptian strain, indicating local adaptation or evolutionary divergence due to environmental factors. Similarly, minor substitutions in sequences from Bangladesh (AFO64661.1) and Poland (AIA59686.1) suggest the potential influence of regional agricultural practices, host resistance mechanisms, or environmental selection pressures. These findings align with several previous studies highlighting the genetic plasticity of PVY, enabling it to adapt to different environmental conditions and hosts (Karasev and Gray, 2013b; Green *et al*., 2018).

The presence of conserved epitopes across both potato and tomato hosts indicates the broad host range of PVY and its ability to maintain key functional regions despite adapting to different plant species. The minor variations observed, such as those in PVY-CP-D and PVY-CP-G from Bangladesh may reflect localized evolutionary pressures influencing virulence, host specificity, or immune escape strategies (Revers and García, 2015). Understanding these minor but potentially significant mutations could help predict the emerging PVY variants with altered pathogenicity or resistance to existing management strategies (Gibbs *et al*., 2020).

The high degree of sequence conservation in these epitopes suggests their utility in developing universal diagnostic tools. Since epitopes such as PVY-CP-F and PVY-CP-H exhibit minimal sequence variation, they could serve as reliable targets for serological assays like ELISA or molecular detection methods such as polymerase chain reaction (PCR) (MacKenzie *et al*., 2015; Lacomme and Jacquot, 2017). Furthermore, their conservation makes them promising candidates for monoclonal antibody-based detection strategies or epitope-based vaccine development, providing broad-spectrum protection against PVY infections.

Conclusions and Recommendations

This study highlights the high conservation of key epitopes within the CP of Potato virus Y (PVY) across diverse geographic regions and host species. These conserved epitopes, including PVY-CP-A, -D, -F, -G, -H, and -I are crucial for the virus's structural integrity and infectivity. Minor sequence variations observed in specific viral strains suggest local adaptations, reinforcing PVY's ability to evolve in response to environmental pressures. The current findings underscore the potential of using these epitopes as targets for developing universal diagnostic tools and vaccines, offering promising strategies for broad-spectrum management of PVY infections in agriculture. This study recommends using the highly conserved PVY coat protein epitopes, especially PVY-CP-A, -H, and -I, as targets for developing broad-spectrum diagnostic tools and resistance strategies. Regular monitoring of variable epitopes, such as PVY-CP-G, is advised to detect the region-specific adaptations. Expanding the analysis to include more diverse geographic isolates and combining sequence data with structural and immunological modeling

will enhance the understanding of PVY evolution.

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Novelty Statement

This study presents the first comprehensive mapping of conserved epitopes across the PVY proteome, identifying candidate peptides with strong immunogenic potential. By highlighting the regions suitable for the broad-spectrum antigen design, our findings lay the groundwork for next-generation diagnostic tools and immune-based interventions in plant virus management.

Author's Contribution

AHA: Methodology, data collection, data analysis, writing the original draft.
MKA: Conceptualization, data analysis, revision and editing of the manuscript.
ASS: Design and data analysis, revision and editing of the manuscript.
AAM: Data collection, data analysis, revision and editing of the manuscript.

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Ethical approval was not necessary for this study, as it is entirely computational and does not involve human participants, animals, or living plants.

Conflict of interests
The authors have declared no conflicts of interest.

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