

A Comparative Analysis of the Amino Acid Composition of Some Viruses Causing Disease Plum Plants

Sattorov Muzaffar*, Fayziyev Vohid

Chair of Biology, Chirchik State Pedagogical University, Tashkent Region, Chirchik, Uzbekistan

Abstract Mutations occurring in phytopathogenic viruses are mainly evident in the gene responsible for their CP amino acid composition and synthesis. Therefore, comparing the CP amino acid sequence of plum pox virus with other isolates is important in solving theoretical and practical problems, such as studying the evolution of viruses. Therefore, in this work, it was aimed to compare the amino acid sequence of CP of PPV with other isolates.

Keywords PPV, Isolate, Nucleotide, Amino acid, Cover protein, Gene, Phylogenetic tree

1. Introduction

Today, various strains and isolates of phytopathogenic viruses are being identified all over the world, and their comprehensive study is considered important in the development of countermeasures. In the territory of our republic, the degree of spread of the virus has been determined in several plants, and scientific research is being carried out. In particular, the level of resistance of different varieties and samples of corn to corn mosaic virus, which infects corn plants, was studied [8].

A number of features of S, A, M, L, Y and X viruses from the phytopathogenic viruses infecting the potato plant, including the spread of a single potato virus X, biological features such as disease symptoms in indicator-test plants [4,5] and the effect on the amount of chlorophyll "a", "b" and carotenoids in the leaves of *Datura stramonium* plant was determined [5].

In addition to these, scientific research work is being carried out on the study of potexviruses that infect various agricultural plants even in the climatic conditions of Uzbekistan. In particular, plum pox virus, which infects fruit trees, was first detected in plum plants in the climatic conditions of Uzbekistan [1,6] and its biological characteristics such as disease symptoms in indicator-test plants [6,9] and the virus the effect on the amount of chlorophyll "a", "b" and carotenoids in plant leaves was determined [5]. To date, several isolates of this virus have been studied by a number of authors [1,8], they were found to be different by their molecular-genetic and biological characteristics [3,6], studied populations of isolates of the virus distributed in different regions a number

of authors proved this situation based on their experience [8]. Basically, such differences are evident in the process of studying the protein coat (CP) gene of the virus [1,8]. This virus was found to be a new isolate of the virus different from other isolates by nucleotide sequence identification of the ORFs responsible for protein coat (CP) synthesis, and was deposited in International GenBank (NCBI) accession numbers MT038048, MT038050.1 and MT038049.1 based on [6].

2. Materials and Methods

To date, a number of methods have been developed for determining the amino acid composition of proteins, and they differ from each other in terms of equipment and levels of complexity [10]. Determining the amino acid composition of the viral protein coat was based on sequencing the nucleotide sequence of the gene responsible for the synthesis of this protein coat [7,9], and the sequence of this method is given below. cDNA synthesized by reverse transcription for diagnosis of PPV by polymerase chain reaction (PCR) PCR-mixture (for 1 reaction): 16.5 MilliQ water, 2.5 µl 10x buffer for Taq DNA-polymerase (Evrogen), 0.5 µl of 10 mM dNTP mix, 2 µl of 25 mM MgCl₂; 0.5 µl forward and reverse primers (10 pM/µl), 0.5 µl Taq DNA polymerase (PK113L, Eurogen) were added. To prevent evaporation, mineral oil was applied to them. PCR was performed in the Tersik amplifier (DNK-Technology, Russia). PCR sample denaturation (94°C, 3 min), 30 - 35 cycles of amplification, polynucleotide chain recovery 5 - 10 min at 72°C and storage at 4°C. The PCR product required for sequencing was obtained in an analogous manner as above, but the reaction was carried out in 50 µl, the necessary components were calculated and mixed in the same way as above. Alternatively, DNA polymerase Encyclo (Evrogen) was used for accurate transcription. The sitting temperature of the primers and the duration of the elongation

* Corresponding author:

sattorovmuzaffar481@gmail.com (Sattorov Muzaffar)

Received: Jul. 10, 2024; Accepted: Jul. 29, 2024; Published: Aug. 8, 2024

Published online at <http://journal.sapub.org/ijvmb>

process are determined by the characteristics of the primers and the length of the synthesized PCR product [1,8].

Analysis of PCR products was determined by electrophoresis on an agarose gel prepared in 1x Tris-acetate buffer (TAE, Thermo Scientific) with ethidium bromide. 3 µl of dye (6x DNA loading dye, Fermentas) was added to 10 µl of the sample and put into the agarose well together with the sample. A mixture of DNA molecules of certain sizes (GeneRuler Plus 100 bp DNA ladder and GeneRuler Plus 1 kb DNA ladder, Fermentas) were used as markers. Electrophoresis was performed using horizon electrophoresis SE-1 (Helikon, Russia) at 80V for 40–60 min. The gel was analyzed at a wavelength of 312 nm on a transilluminator TFP-M/WL (Vilbert Lourmat, France) and photographed using a gel documentation system MultiDoc-It (UVP, UK).

Determination of the sequence of the 3'-end of the OCHV

genome. The PCR product intended for sequencing was separated on a 1-2% agarose gel. After the end of electrophoresis, the gel was illuminated with a transilluminator TFP-M/WL, the desired area was cut with a scalpel and transferred to a 1.5 ml centrifuge tube. DNA was extracted from the agarose on a spin column using the Cleanup Standard kit (cat. no. BC022, Evrogen) according to the instructions provided by the company. Purified DNA was sequenced using the Sanger method at Evrogen using forward and reverse primers. The sequences of the primers used are listed in the table (Table 1).

PZR mahsulotlarining ketma-ketligi amalga oshirilgan sekvens natijasi asosida EditSeq i MegAlign (DNASTAR Lasergene, SSHA) programmasi yordamida aniqlandi.

The sequence of PCR products was determined using the EditSeq and MegAlign (DNASTAR Lasergene, SSHA) program based on the sequence result.

Table 1. Primer used to detect PPV coat protein gene

Praymerlar (Primers)	5'-3'-ketma-ketligi (5'-3' sequence)	Nukleotidlar soni (The number of nucleotides)
Forward (F)	CTTGAATGGGACAGATCAAATGA	23
Reverse (R)	GAGAAAAGGATGCTAACAGGAATC	24

Table 2. Comparative table of PPV isolate protein shell amino acid composition

№	Aminokislotalar (Amino acid)	(Amino acid composition of isolates compared)															
		PPVUzb1 MT038048		PPVUzb2 MT038050		PPVUzb3 MT038049		Kazakhstan LT600780		Japan LC375110		USA OP434483		Turkey MK372971		Bosnia MW412433	
		Aminokislotalarning oqsil qobig'idagi soni va foizi															
		Soni	%	Soni	%	Soni	%	Soni	%	Soni	%	Soni	%	Soni	%	Soni	%
1	Arginine (R)	23	5.49	22	5.24	22	5.35	23	5.48	23	5.48	23	5.48	23	5.54	25	5.97
2	Histidine (H)	9	2.15	9	2.14	9	2.19	9	2.15	8	1.91	8	1.91	8	1.93	9	2.15
3	Lysine (K)	19	4.53	19	4.52	19	4.62	19	4.53	19	4.53	19	4.53	19	4.58	20	4.77
4	Aspartic Acid (D)	24	5.73	24	5.71	25	6.08	24	5.73	25	5.97	25	5.97	24	5.78	24	5.73
5	Glutamic Acid (E)	31	7.4	31	7.38	30	7.3	31	7.4	31	7.4	31	7.4	30	7.23	30	7.16
6	Serine (S)	22	5.25	22	5.24	21	5.11	22	5.25	22	5.25	22	5.25	22	6.3	22	5.25
7	Threonine (T)	31	7.4	30	7.14	31	7.54	31	7.4	31	7.4	31	7.4	30	7.23	32	7.64
8	Asparagin (N)	26	6.2	27	6.43	26	6.33	26	6.21	25	5.97	25	5.97	26	6.27	25	5.97
9	Glutamine (Q)	17	4.06	17	4.05	17	4.14	17	4.06	17	4.06	17	4.06	17	4.1	16	3.82
10	Cysteine (C)	3	0.72	3	0.71	2	0.49	3	0.72	3	0.72	3	0.72	3	0.72	3	0.72
11	Glycine (G)	22	5.25	22	5.24	21	5.11	22	5.25	22	5.25	22	5.25	22	5.3	21	5.01
12	Proline (P)	27	6.44	28	6.66	27	6.57	27	6.44	27	6.44	27	6.44	27	6.51	28	6.68
13	Alanine (A)	40	9.55	39	9.29	38	9.25	40	9.55	40	9.55	40	9.55	40	9.64	40	9.55
14	Isoleucine (I)	20	4.77	22	5.24	20	4.87	20	4.77	20	4.77	20	4.77	20	4.81	20	4.77
15	Leucine (L)	27	6.44	27	6.43	28	6.81	28	6.68	29	6.92	29	6.92	27	6.51	27	6.44
16	Methionine (M)	15	3.58	15	3.57	13	3.16	14	3.34	14	3.34	14	3.34	15	3.61	16	3.82
17	Phenylalanine (F)	12	2.86	12	2.86	12	2.92	12	2.86	11	2.63	11	2.63	11	2.65	12	2.86
18	Tryptophan (W)	5	1.19	5	1.19	5	1.22	5	1.19	5	1.19	5	1.19	5	1.20	5	1.19
19	Tyrosine (Y)	18	4.3	18	4.29	18	4.38	18	4.3	19	4.53	19	4.53	18	4.34	18	4.3
20	Valine (V)	28	6.68	27	6.43	27	6.57	28	6.68	28	6.68	28	6.68	28	6.75	26	6.21
Aminokislotalar soni		419		420		411		419		419		419		415		419	

3. Nucleotide and Amino Acid Sequence Analysis

Nucleotide and their underlying amino acid sequences were compared using the ClustalW v.2.1 (<http://clustalw.ddbj.nig.ac.jp>) program or the version included in the BioEdit package [2,9].

The obtained comparative information was used to determine sequence divergence and identity and phylogenetic analysis. Phylogenetic analysis was performed in the MEGA6 program using neighbor joining or maximum likelihood and Kimura -2 or Tajima-Nei evolutionary models [1,6,9].

Molecular diagnostics of the virus in the MSU "Biochemistry of Plant Viruses" laboratory carried out together with Dr. (Ds), prof. S.N.Chirkov. Therefore, We express our gratitude to Dr.(Ds), prof. S.N.Chirkov.

4. The Obtained Results and Their Analysis

PPV is one of the strongest phytopathogenic viruses, and it is an RNA capture virus. This virus passes from an infected plant to a healthy plant through mechanical, agricultural machinery [5,9,11]. Together with other viruses, it causes serious damage to agricultural plants. To prevent this damage, the creation of virus-resistant varieties is the most economical and effective way to control OCD. However, it takes a lot of time to breed such varieties, and the mutation of one amino acid can lead to the emergence of new strains.

The protein shell is considered one of the important components of the virus, and if the virus particle is visualized in a very simplified way, it can be considered as a shell that surrounds the nucleic acid. As mentioned above, the shell is "capsid" and its sub-elements can be called capsomeres or its morphological subunits. The whole virus particle is called nucleocapsid. In simple viruses such as tobacco mosaic virus, the viral protein coat consists of a single type of polypeptide chain with the same structure. Their amino acid composition is unique to the same protein. In complex viruses (T pair bacteriophages) with dozens of proteins, it is more difficult to determine the composition of all amino acids, because it is characteristic of heterogeneous proteins. In this case, the protein content of each capsid is analyzed [10,11].

The amino acid sequence of isolates of OChV MT038048 Uz1 MT038050 Uz2, MT038049 Uz3 isolated in our country was determined based on the ORFs nucleotide sequence and it is presented below (Table 2):

Based on this sequence, when the amino acid content of the protein shell of different isolates isolated from other regions of the world was compared, it was found that there are a number of differences between them. In particular, Arg - 22 in isolate Uzb2 MT038050 and Uzb3 MT038049, 25 in isolate Bosnia MW412433, and 23 in other isolates. His-Uzb1,2,3. 9 each in isolates MT038048 and Bosnia MW412433, 8 each in Japan LC375110 and other isolates, 20 in Lys-Bosnia MW412433, and 19 in other isolates.

Asp-Uzb3 25 in MT038049 isolate, Japan LC375110 and USA OP434483, 25 in isolates, 24 in other isolates, Glu-Uzb1. 30 in MT038049 isolate, 30 in Turkey MK372971 and Bosnia MW412433 isolates, 31 in other isolates, 21 in Ser - MT038049 isolate, and 22 in other isolates. Thr -Uzb2 MT038050 isolate 30, Turkey MK372971 isolate 32, Bosnia MW412433 isolate 31, Asp-Uzb2 MT038050 isolate 27, Japan LC375110, USA OP434483 and Bosnia MW412433 isolate 25 each and 26 in lar. Glu - Bosnia MW412433 16, and the other isolates 17. Cys-Uzb3 is 2 in isolate MT038049 and 3 in other isolates. Gly- Uzb3 MT038049 and Bosnia MW412433 isolates from 21, and the remaining isolates from 22. Pro-Uzb2. 28 each in isolate MT038050 and isolate Bosnia MW412433, and 27 each in the remaining isolates. Ala-Uzb1. 40 in isolate MT038048, 38 in isolate Uzb3 MT 038049, and 40 in the remaining isolates. Iso-Uzb2 isolate MT038050 had 22 and the remaining isolates had 20. Leu - Uzb3. MT038049 and Kazakhstan LT600780 isolates 28, Japan LC375110 and USA OP434483, 29 isolates, and 27 Meth-Uzb1 isolates in the remaining isolates. MT038048, Uzb2. 15 in MT038050 and Turkey MK372971 isolate, 28 in Kazakhstan LT600780 isolate, 14 in Japan LC375110 and USA OP434483 isolates, 13 in Uzb3 MT038049 isolate, 16 in Bosnia MW412433 isolate, Phen- Japan LC375110 and USA OP434483. 11 in isolates Turkey MK372971 and in the remaining isolates from 12. Tyr - Japan LC375110 and USA OP434483, 19 in isolates and 18 in the remaining isolates. Val - Uzb1. 28 in isolate MT038048, 27 in isolate Uzb3 MT038049, 26 in isolate Bosnia MW412433, and 28 in the remaining isolates. In the remaining single Tryptophan (W) isolate, all were found to be in the same number (Table 2).

In general, as a result of the analysis, it was found that the amino acid composition of the isolate isolated in our country is similar to that of many isolates, but not the same. The virus has 98.42% homology and is located in one branch of the phylogenetic family tree. Its ancestor, located in Kazakhstan, underwent changes during its evolution and was able to separate after being exposed to the climatic conditions of our country.

REFERENCES

- [1] Шевелева Анна Александровна. Молекулярно-биологические свойства российских изолятов вируса оспы сливы // Москва, 2020. –с. 110.
- [2] Glasa M., Shneyder Y., Predajna L., Zhivaeva T., Prikhodko Y. 2014. Characterization of Russian Plum pox virus isolates provides further evidence of a low molecular heterogeneity within the PPV-C strain. J. Plant Pathol., 96: 597 – 601.
- [3] Fayziev V, D.Javlieva, Z. Kadirova, S. Chirkov, U. Jurayeva, A. Vakhobov (2020) Study of some biological properties necrotic isolate of potato virus X and phylogenetic analysis. International Journal of Psychosocial Rehabilitation, Vol.24, Issue 09. – P. 455-465. ISSN: 1475-7192.
- [4] Fayziev V., Jovlieva D., Juraeva U., Shavkiev J., Eshboev F.

- (2020) Effects of PVXN-UZ 915 necrotic isolate of Potato virus X on amount of pigments of *Datura stramonium* leaves. *Journal of critical reviews*, Vol 7, Issue 9. – P. 400-403. ISSN-2394-5125. DOI: <http://dx.doi.org/10.31838/jcr.07.09.82>.
- [5] Fayziyev V, Vakhabov A (2019) The study of the biological properties of potato virus X in common environmental conditions of Uzbekistan// *European Sciences review*. № 1–2 (January–February). Volume 2, p. 46-50.
- [6] Sattorov M, Sheveleva A, Fayziev V, Chirkov S (2020) First report of *Plum pox virus* on plum in Uzbekistan. *Plant Disease* (early view). <https://doi.org/10.1094/PDIS-03-20-0456-PDN>.
- [7] Sobirova Z.Sh., Fayziev V.B., Abduraimova Kh.I. (2020) Effect of The Virus of The Yellow Dwarf Corn Mosaic Growth and Development of Varieties of Corn in Various Phases. *Journal of Advanced Research in Dynamical and Control Systems*, 12(6).
- [8] Chirkov, S., et al. 2016. *Arch. Virol.*, 161: 425. <https://doi.org/10.1007/s00705-015-2658-x>.
- [9] Zagrai L., Zagrai I., Ferencz B., Gaboreanu I., Kovacs K., Petricele I., Popescu O., Pamfil D., Capote N. 2008. Serological and molecular typing of plum pox virus isolates in the north of Romania. *J. Plant Pathol.*, 90(S1): 41 - 46.
- [10] Zhang S., Ravelonandro M., Russel P., McOwen N., Briad P., Bohannon S., Vrient A. 2014. Rapid diagnostic detection of plum pox virus in *Prunus* plants by isothermal AmplifyRP using reverse transcription-recombinase polymerase amplification. *J. Virol. Meth.*, 207: 114 - 120.
- [11] Rodamilans B., Valli A., Garcia J.A. 2019. Molecular plant-*Plum pox virus* interaction. *Mol. Plant-Microbe Interact.*, 33: 6 – 17.