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Comparative Analysis of Amino Acid Composition of the Coat Protein of Tomato Brown Rugose Fruit Virus (ToBRFV) "M-24" Isolate

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Abstract In this study, the nucleotide and amino acid composition of the coat protein (CP) of the Tomato brown rugose fruit virus (ToBRFV) isolate "M-24", collected from tomato in Uzbekistan, was investigated and compared with other available isolates. Bioinformatic analyses revealed that the CP amino acid sequence of the "M-24" isolate is closely related to, but not identical with, several previously reported ToBRFV isolates. Comparative alignments with the Iranian isolates "Yaz.Kha.To" (XSB39591.1 / PP916473.1) and "B" (XQH04660.1 / PQ310342.1) demonstrated several single nucleotide polymorphisms (SNPs) resulting in missense substitutions: five substitution sites relative to XSB39591.1 and three sites relative to XQH04660.1. These small variations may reflect local adaptation or ongoing genetic diversification under biotic and abiotic pressures. The findings emphasize the importance of molecular surveillance and comparative protein-level analyses for understanding ToBRFV epidemiology and potential impacts on virulence and host interaction.

Keywords ToBRFV, Coat protein, Amino acid composition, M-24 isolate, Nucleotide sequence, Comparative analysis

1. Introduction

Plant virus epidemics constitute a major threat to global food security, increasing crop losses and exacerbating food shortage issues [1,2]. Tomato brown rugose fruit virus (ToBRFV), a member of the genus *Tobamovirus*, has emerged in recent years as a significant pathogen of tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*), capable of overcoming commonly used resistance genes such as Tm-22 [3]. Typical ToBRFV infection results in reduced vigor, severe yield losses and fruit quality deterioration including discoloration, deformation and tissue hardening [4]. ToBRFV spreads efficiently via mechanical transmission and can rapidly disseminate through a crop from few infected plants within a single growing season [5]. To date, ToBRFV has been reported from numerous countries worldwide [6].

Tobamovirus virions are rigid, rod-shaped particles with helical symmetry, approximately 18 nm in diameter and 300–310 nm in length, and are non-enveloped. The ToBRFV genome is a positive-sense single-stranded RNA molecule of ~6376–6394 nt encoding four open reading frames (ORFs). ORF1 encodes a 126 kDa protein (p126) with methyltransferase and helicase domains; readthrough of the amber stop codon yields a 183 kDa protein (p183) containing an RNA-dependent RNA polymerase (RdRp) domain. ORF3 and ORF4 encode the movement protein (MP, ~30 kDa) and the coat protein (CP, ~17.5 kDa), respectively. ToBRFV shows highest nucleotide sequence similarity to tomato mosaic virus (ToMV) and tobacco mosaic virus (TMV) (~81–82%) [7]. The coat protein is essential for virion assembly, stability, vector interactions and host responses, and even minor amino acid substitutions can affect biological properties. The protein coat is of fundamental importance for virion assembly, stability, and interaction with the host plant, as well as for the progression of the pathogenic process. Moreover, even minor amino acid substitutions within the coat protein may significantly influence the biological characteristics and infectivity of the virus [8,9,10].

From this perspective, the present study was aimed at the molecular characterization of the coat protein of the Uzbek isolate "M-24" (GenBank accession number PQ660493.1) of ToBRFV. In particular, a comparative analysis of its amino acid composition with that of closely related isolates was conducted, and the impact of the identified substitutions on viral variability and host–plant interactions was discussed.

2. Materials and Methods

Sample collection and initial diagnosis. Symptomatic

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tomato plants were sampled from greenhouse production sites in Uzbekistan. Total RNA was extracted from symptomatic leaf tissue using standard protocols. Reverse transcription was performed to synthesize cDNA followed by PCR amplification of the CP gene using ToBRFV-specific primers (primer sequences and PCR conditions are available on request).

Sequencing. The obtained PCR products were purified using electrophoresis and sequenced by the Sanger method. The nucleotide sequences were edited and assembled using the SnapGene software tools.

Sequence submission and accession. The CP nucleotide sequence from the Uzbekistan isolate was deposited in GenBank under the accession PQ660493.1 (Tomato brown rugose fruit virus isolate "M-24"). The nucleotide and amino acid sequences were compared with other ToBRFV isolates available in the GenBank and EBI databases (including PP916473.1 – Yaz.Kha.To and PQ310342.1 – isolate "B"). Multiple sequence alignment was performed using the Clustal Omega software, and SNPs as well as amino acid substitutions were identified and verified.

3. Results

The ToBRFV genome consists of a single-stranded RNA, and its detection using the RT-PCR method was carried out through the following stages: (a) extraction of viral RNA from the sample; (b) synthesis of complementary double-stranded cDNA from single-stranded viral RNA via reverse transcription; (c) amplification of the cDNA; (d) analysis of the amplicon by gel electrophoresis and visualization under a transilluminator; and (e) sequencing of nucleotides, followed by the study of amino acid sequences using Clustal Omega and MEGA 11 software.

After total RNA was extracted from tomato plant samples infected with the virus, a Master Mix was prepared using specific primers, enzymes, and other PCR components, and standard PCR was performed. The cloned ToBRFV PCR amplicons were sequenced, and the nucleotide sequences were determined. The nucleotide sequence of the ToBRFV coat protein (CP) gene, isolated from tomato under the climatic conditions of Uzbekistan, was deposited in the International GenBank – NCBI database under the name "Tomato brown rugose fruit virus isolate 'M-24'" (PQ660493.1). This nucleotide sequence was compared on the https://www.ebi.ac.uk/ website with the "M-24" isolate and closely related isolates from the NCBI database, namely the Iranian "Yaz.Kha.To" and "B" isolates (Figure 1).

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PP916473.1      ATGAGCTACACCATCGCCACCCCCAGCCAGTTCGTGTTCCTGAGCAGCGCCTGGGCCGAC
PQ660493.1      ATGAGCTACACCATCGCCACCCCCAGCCAGTTCGTGTTCCTGAGCAGCGCCTGGGCCGAC
PQ310342.1      ATGAGCTACACCATCGCCACCCCCAGCCAGTTCGTGTTCCTGAGCAGCGCCTGGGCCGAC
                ************************************************************

PP916473.1      CCCATCGAGCTGATCAACCTGTGCACCAACAGCCTGGGCAACCAGTTCCAGACCCAGCAG
PQ660493.1      CCCATCGAGCTGATCAACCTGTGCACCAACAGCCTGGGCAACCAGTTCCAGACCCAGCAG
PQ310342.1      CCCATCGAGCTGATCAACCTGTGCACCAACAGCCTGGGCAACCAGTTCCAGACCCAGCAG
                ************************************************************

PP916473.1      GCCAGGACCACCGTGCAGAGGCAGTTCAGCGAGGTGTGGAAGCCCGTGCCCCAGGTGACC
PQ660493.1      GCCAGGACCACCGTGCAGAGGCAGTTCAGCGAGGTGTGGAAGCCCGTGCCCCAGGTGACC
PQ310342.1      GCCAGGACCACCGTGCAGAGGCAGTTCAGCGAGGTGTGGAAGCCCGTGCCCCAGGTGACC
                ************************************************************

PP916473.1      GTGAGGTTCCCCGACAGCGGCTTCAAGGTGTACAGGTACAACGCCGTGCTGGACCCCCTG
PQ660493.1      GTGAGGTTCCCCGACAGCGGCTTCAAGGTGTACAGGTACAACGCCGTGCTGGACCCCCTG
PQ310342.1      GTGAGGTTCCCCGACAGCGGCTTCAAGGTGTACAGGTACAACGCCGTGCTGGACCCCCTG
                ************************************************************

PP916473.1      GTGACCGCCCTGCTGGGCGCCTTCGACACCAGGAACAGGATCATCGAGGTGGAGAACCTG
PQ660493.1      GTGACCGCCCTGCTGGGCGCCTTCGACACCAGGAACAGGATCATCGAGGTGGAGAACCAG
PQ310342.1      GTGACCGCCCTGCTGGGCGCCTTCGACACCAGGAACAGGATCATCGAGGTGGAGAACCCC
                ************************************************************

PP916473.1      GCCAACCCCACCACCGCCGAGACCCTGTACGCCACCAGGAGGGTGGACGACGCCACCGTG
PQ660493.1      GCCAACCCCACCACCGCCGAGACCCTGGACGCCACCAGGAGGGTGGACGACGCCACCGTG
PQ310342.1      GCCAACCCCACCACCGCCGAGACCCTGGACGCCACCAGGAGGGTGGACGACGCCACCGTG
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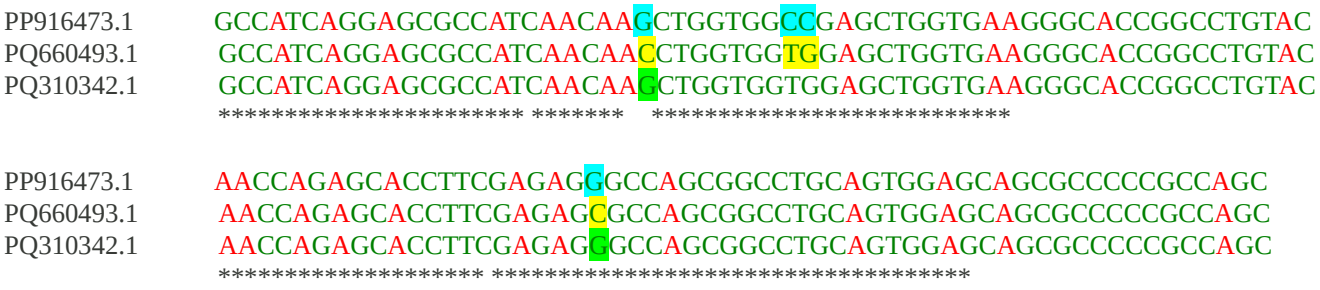

Figure 1. Comparative analysis of the coat protein nucleotide sequence of the "M-24" isolate with other isolates

Comparison of the nucleotide sequences revealed that the coat protein nucleotide sequence of the "M-24" isolate, obtained from Uzbekistan, is closely related to but not identical with the compared isolates, as determined by bioinformatic analyses. For example, when compared with the Iranian "Yaz.Kha.To" (PP916473.1) isolate, six SNP mutations were identified in the "M-24" isolate. When compared with the "B" (PQ310342.1) isolate, four SNP mutations were detected (Figure 1).

In the next stage of the study, the amino acid composition of the coat protein of the locally isolated Uzbek "M-24" isolate was analyzed. The amino acid composition of the coat protein was determined based on the nucleotide sequence of the CP gene and compared with the amino acid sequences of the aforementioned Iranian isolates on the https://www.ebi.ac.uk/ website (Figure 2).

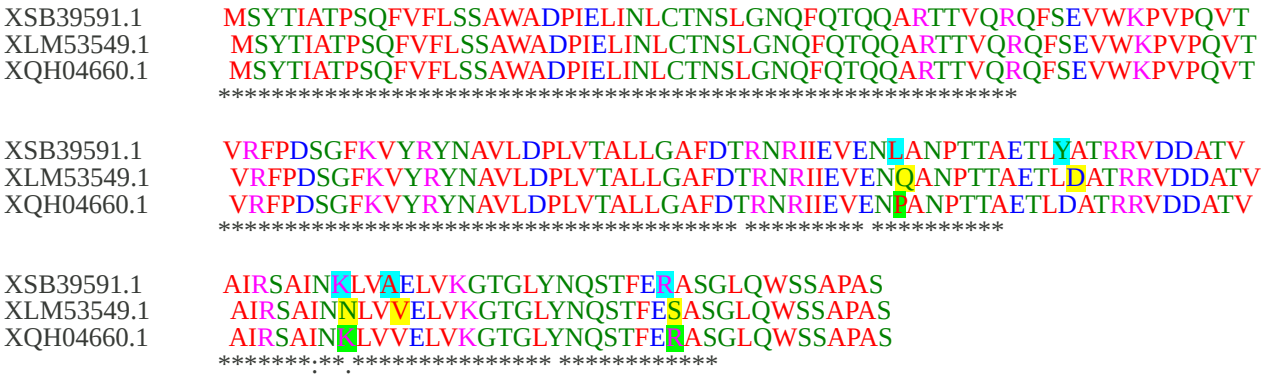

Figure 2. Comparative analysis of the coat protein amino acid sequence of the "M-24" isolate with other isolates

As shown in the comparative table, when the "M-24" isolate was compared with the Iranian "Yaz.Kha.To" (XSB39591.1) isolate, five missense mutations were identified. Specifically, leucine (L) was substituted by glutamine (Q), tyrosine (Y) by aspartic acid (D), lysine (K) by asparagine (N), alanine (A) by valine (V), and arginine (R) by serine (S).

Furthermore, when the "M-24" isolate was compared with the Iranian 2 – "B" (XQH04660.1) isolate, three missense mutations were identified. Specifically, proline (P) was substituted by glutamine (Q), lysine (K) by asparagine (N), and arginine (R) by serine (S).

4. Conclusions

The results of this study demonstrated that the Uzbek "M-24" isolate of Tomato brown rugose fruit virus (ToBRFV) exhibits a high degree of similarity in the nucleotide and amino acid sequences of the coat protein (CP) gene with other isolates obtained from various regions. However, they are not completely identical, as several single nucleotide polymorphisms (SNPs) and missense mutations were observed. These molecular variations may arise under the influence of various abiotic factors (e.g., temperature, agronomic practices, environmental stresses) as well as biotic factors (e.g., plant cultivars, mixed infections, virus–host coevolution.

The detection of such mutations is of particular importance, as even minor amino acid substitutions in the viral coat protein may influence virion assembly, stability, systemic movement, and interactions with host defense mechanisms. Consequently, these genetic changes could potentially alter the biological properties of the virus, including its pathogenicity and ability to overcome resistance conferred by genes such as *Tm-22*.

From an applied perspective, the observed variability emphasizes the necessity of continuous molecular surveillance of ToBRFV populations circulating in Uzbekistan and other regions. Detailed characterization of local isolates will provide critical insights into the evolutionary dynamics of the virus and contribute to the design of sustainable disease management strategies. Furthermore, the identification of

genetic diversity among isolates underscores the need to develop and implement robust diagnostic tools, as well as to breed and deploy tomato cultivars with durable and broad-spectrum resistance against ToBRFV.

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