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Araştırma Makalesi

Full-Genome Characterization of Turkish Rose Yellow Vein Virus Isolates

Ali KARANFİL[1]*, Filiz RANDA-ZELYÜT[2], Savaş KORKMAZ[1]

[1]1Department of Plant Protection, Faculty of Agriculture, Canakkale Onsekiz Mart University, Canakkale, Türkiye

[2] Department of Plant Protection, Faculty of Agriculture and Natural Sciences, Bilecik Seyh Edebali University, Bilecik, Türkiye

*Sorumlu Yazar: ali.karanfil@hotmail.com

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ABSTRACT

Rose yellow vein virus (RYVV) is a viral agent recently identified in roses, with a limited known distribution worldwide. However, studies conducted in Türkiye have revealed the presence of the virus in various regions and provinces. As direct chemical control of viral diseases is not feasible, unlike other biotic diseases, molecular characterization of the virus is essential for future resistance breeding or gene silencing studies. To this end, 44 previously identified RYVV isolates from Türkiye were selected for full genome analysis. The full genomes of three isolates were successfully sequenced using overlapping PCR with seven species-specific primer pairs. However, the full genomes of the remaining isolates could not be obtained. The genome size of the three sequenced RYVV isolates was determined to be 9.314 nucleotides (PQ298785-87). A high level of similarity in the nucleotide sequence was observed between the Turkish isolates and the single RYVV isolate available in the GenBank database. However, the fact that the full genome of the other isolates could not be obtained and those obtained were amplified with primer pairs designed based on the single isolate in the GenBank may be due to the presence of different strains of RYVV isolates. Therefore, next-generation sequencing (NGS) should be prioritized in future studies to obtain comprehensive genome information for a broader range of RYVV isolates.

Key words: Rose, RYVV, Bioinformatic

Türk Rose Yellow Vein Virus İzolatlarının Tüm Genom Karakterizasyonu

ÖZ

Gül sarı damar virüsü güllerin son yıllarda tanılanmış bir virüs hastalığı olup dünyada son derece sınırlı bir alanda varlığı bilinmektedir. Ancak ülkemizde gerçekleştirilen çalışmalarda etmenin varlığı farklı bölge ve illerde tespit edilmiştir. Virüs hastalıklarının diğer biyotik hastalıklarda olduğu gibi doğrudan kimyasal mücadelesi mümkün olmadığı için etmenin moleküler karakterizasyonu gelecek yıllarda yapılması muhtemel dayanıklılık ya da gen susturma çalışmaları için temel oluşturmaktadır. Böylece ülkemizde daha önceden tespit edilen 44 RYVV izolatı tüm genom çalışmaları için kullanılmıştır. Tüm genom çalışmalarında türe özgü 7 farklı primer çifti ile overlapping PCR yöntemi uygulanmıştır. Bu izolatların arasından 3 tanesinin tüm genomu elde edilmiştir. Diğer izolatların ise tüm genomu elde edilememiştir. Tüm genomu elde edilen 3 RYVV izolatının 9314 nükleotit büyüklüğünde olduğu belirlenmiştir (PQ298785-87). Genbankasında bulunan tek RYVV izolatı ile Türkiye izolatlarının da birbirleri ile yüksek oranda nükleotit dizisi benzerliği gösterdiği belirlenmiştir. Bununla birlikte, diğer izolatların tüm genomunun elde edilememesi ve elde edilenlerin de GenBankası veri tabanındaki bulunan tek izolata göre tasarlanmış olan primer çiftleri ile amplifiye edilmesinden dolayı RYVV izolatlarının farklı strainlerinin bulunmasından kaynaklanabilir. Bu sebeple gelecek çalışmalarda tam genom bilgilerinin çıkarılması için yeni nesil sekanslama çalışmalarına öncelik verilmesi gerektiği düşünülmektedir.

Anahtar kelimeler: Gül, RYVV, Biyoinformatik

INTRODUCTION

Roses, cultivated in almost every region of Türkiye, are a significant ornamental plant. However, viral diseases pose a major challenge to their cultivation. With advancements in sequencing technologies, new viral diseases continue to be identified, some of which have been detected in rose-growing regions in Türkiye. Although numerous viruses are known to infect roses globally, the presence of many of these pathogens in Türkiye remains unknown. These viral infections can cause severe yield and quality losses, particularly through symptoms on leaves that render the product unmarketable. Currently, no effective agricultural control methods are available to prevent such losses.

One notable viral disease affecting roses is rose yellow vein virus (RYVV), a recently identified member of the Caulimoviridae family. The complete nucleotide sequence and genome organization of RYVV have been characterized, revealing a genome size of 9,314 base pairs (bp) with eight open reading frames (ORFs). Among these, ORFs 1, 2, and 3 share 22-38% amino acid similarity with known members of the Caulimoviridae family, while the remaining ORFs show no significant similarity with any known viruses (Mollov et al., 2009, 2013). RYVV is reported to be transmissible by grafting, but not by aphids or mechanical means. Common symptoms in infected roses include vein banding and central vein chlorosis (Lockhart, 2011).

Outside of the United States, RYVV was first identified in a single rose sample in New Zealand (Perez-Egusquiza et al., 2012), and its presence was subsequently confirmed in multiple regions of New Zealand (Milleza et al., 2013). In Türkiye, RYVV was first reported by Karanfil et al. (2018), with subsequent studies revealing its presence in various regions and provinces (Karanfil, 2022).

The most effective approach to managing viral diseases involves sanitation practices that promote healthy plant cultivation. Resistance breeding, cross-protection, and vector control are also considered among the most effective management strategies. However, for these methods to be effectively implemented, a comprehensive understanding of the pathogen's biological and molecular characteristics is required. Data from molecular characterization studies, particularly those conducted at the full genome level, can provide valuable insights into the genetic diversity, recombination, and emergence of pathogens. Consequently, analyses of genetic diversity, phylogeny, recombination, and evolutionary divergence of viral agents are crucial. Given this context, molecular characterization is essential, in addition to the detection of plant viral diseases. This study conducted comprehensive genome analyses of newly identified RYVV isolates, which were recently detected in Türkiye and are known to be confined to a limited geographical area globally.

MATERIAL and METHOD

Virus Isolates

The RYVV isolates used in this study were obtained from a previous study conducted by Karanfil (2021). A total of 44 samples previously identified as infected with RYVV were used.

PCR Studies

Total nucleic acid isolation from the selected isolates was performed following the method described by Li et al. (2008) to determine their full genome sequences. Since the RYVV genome had been previously sequenced, the full genome of a new isolate was determined using the existing genome sequences available in GenBank. Seven different primer pairs were designed to specifically target conserved regions of these isolates and amplify overlapping fragments of 100-150 base pairs (bp) around each region (Table 1). The full genome of RYVV was obtained using overlapping PCR.

For primer design, the CLC Main Workbench (Qiagen, USA) sequence analysis software was used. The designed primers were checked for secondary structures and complementarity using the "Sequence Manipulation Suite: PCR Primer Stats" online tool. A BLAST analysis confirmed that the primers did not bind to non-target sequences. The validated primers were synthesized by a commercial service provider.

Table 1. Primer pairs used to determine the full-genome sequence of rose yellow vein virus

Primer Code	Primer Sequence	Amplificon Length
RYVV _39_F1 (8696, 8715)	GAGATTTTAGTAGTCAGGCA	1924 bp
RYVV _40_R1 (1295, 1313)	GAGAATGGGTAGTTTAGAG	
RYVV _41_F2 (1088, 1105)	GGATGACAGAAGGAAAGG	1508 bp
RYVV _42_R2 (2578, 2595)	GGCAGTATTAGGGAGAGA	
RYVV _43_F3 (2325, 2341)	TAACGAAATGGACACCT	1481 bp
RYVV _44_R3 (3789, 3805)	TTGTTTATGGGCTCTGT	
RYVV _45_F4 (3640, 3656)	ACCTCAATGCTCAATCT	1503 bp
RYVV _46_R4 (5126, 5142)	ATCCAAACGGCATAACT	
RYVV _47_F5 (4904, 4920)	AAGAGGAAAAGCTAGGA	1449 bp
RYVV _48_R5 (6335, 6352)	GCTTTCAGGGAATTTTGG	
RYVV _49_F6 (6224, 6240)	GCAAAAGGTAAGAACAC	1593 bp
RYVV _50_R6 (7800, 7816)	CATCTTGTTGGAGACTT	
RYVV _51_F7 (7578, 7595)	GCAAGACTGAAGAAGAGG	1429 bp
RYVV _52_R7 (8990, 9006)	GTGGGGGTTTAAAGTGG	

Cloning

Seven DNA fragments specific to the RYVV genome, amplified by PCR, were cloned using the pGEM T-Easy Vector System-II kit (Promega, USA), following modifications to the method described by Çevik et al. (1995) and Jiang et al. (2008). After cloning, at least two colonies were selected for each DNA fragment, and plasmid isolation was performed. The plasmids were then digested with the EcoRI enzyme, and one plasmid was selected for each genomic fragment that was confirmed to carry the desired gene segments. The DNA sequences were determined in both directions using the cycle sequencing method on an automated DNA sequencing device provided by a commercial service.

Sequence Analysis

The obtained DNA sequences were analyzed using the Contig Assembly Module in the CLC Main Workbench program. The overlapping regions of the DNA fragments, along with the previously obtained 5' and 3' ends, were aligned to construct contigs, which were then assembled to determine the full genome sequence of RYVV. This resulted in the full genome sequences of the RYVV isolates in Türkiye. Multiple sequence alignments and phylogenetic analyses were performed based on nucleotide (nt) and amino acid (aa) sequences to compare the full genome sequences with other RYVV isolate (Table 2) available in the GenBank database (Muhire et al., 2014).

Table 2. Information on the single rose yellow vein virus isolate available in the GenBank

Accession number	JX028536
Isolate Code	RYVV-MN1
Host	Rose cv. Dr. Merkley
Country	USA
Genome Length	9.314

Phylogenetic Analysis

Phylogenetic tree was constructed using the neighbor-joining method with the Kimura two-parameter (K2) algorithm, utilizing multiple sequence alignment files obtained through Clustal W. To statistically validate the accuracy of the constructed phylogenetic tree, a bootstrap analysis with 1,000 replicates was performed, applying a minimum 50% bootstrap threshold to exclude randomly formed clusters and branches. Cauliflower mosaic virus (CaMV; M90541) was used as an outgroup to enhance the accuracy of the constructed phylogenetic tree.

RESULTS and DISCUSSION

Only three Turkish RYVV isolates (CAN-RYVV, EDI-RYVV, and TEK-RYVV were obtained from Çanakkale, Edirne, and Tekirdağ provinces, respectively. Their accession numbers are PQ298785-87, respectively) had their full genomes successfully amplified (Figure 1) and sequenced.

Figure 1. Agarose gel (1.5%) electrophoresis results. Samples 1, 2, and 3 correspond to amplicons from isolates obtained from the provinces of Çanakkale, Edirne, and Tekirdağ, respectively. NC: Negative control

The genome size of these three isolates was determined to be 9.314 nucleotides (nt). Sequence similarity analyses for gene regions revealed a high degree of similarity between these isolates and the single RYVV isolate in GenBank (Figure 2). Overall, the nucleotide-level similarity rates across all gene regions ranged from 88% to 100%, while amino acid-level similarity rates ranged from 91% to 100%.

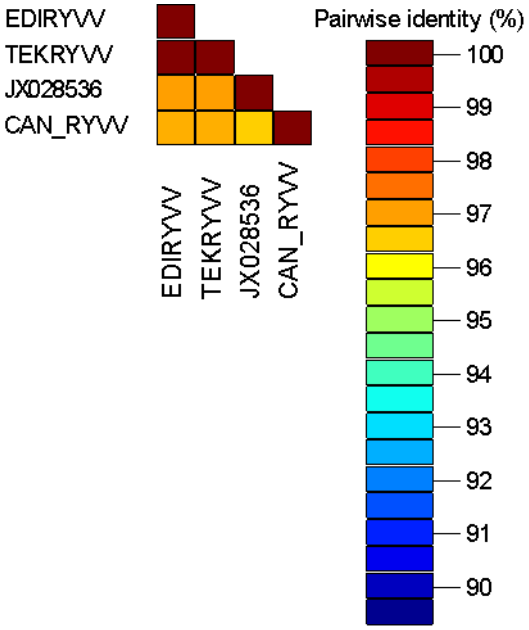

Figure 2. Sequence similarity rates of Turkish rose yellow vein virus isolates based on the full genome nucleotides sequences

When examining the phylogenetic relationships of the three isolates, the isolates were found to be closely related to each other (Figure 3). However, given that there is only one isolate with a full genome sequence in GenBank, further studies involving isolates from different regions of the world are needed for more definitive conclusions.

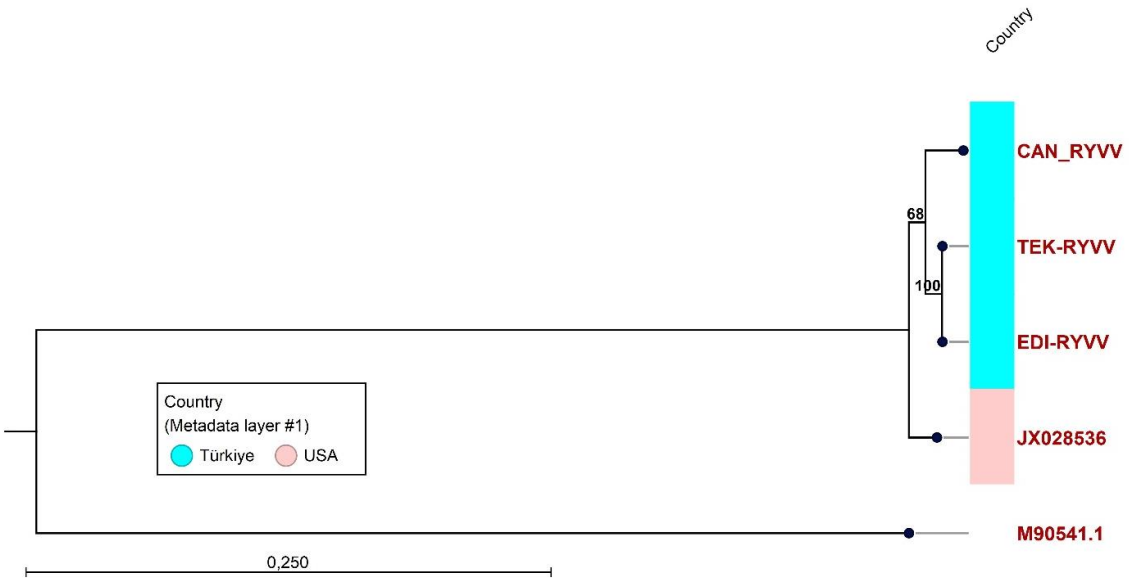

Figure 3. Phylogenetic relationship of Turkish rose yellow vein virus isolates based on the full genome nucleotide sequences (Cauliflower mosaic virus was used as an outgroup, MG90541)

Several viral diseases that cause infections in roses have been identified in studies conducted in Türkiye (Sipahioğlu et al., 2001; Sertkaya, 2010; Çulal-Kiliç et al., 2017; Karanfil et al., 2018; Karanfil, 2021). However, among these, RYVV is considered particularly important since it is relatively new to the scientific community and was reported for the first time outside New Zealand and the United States in Türkiye (Mollov et al., 2013; Perez-Egusquiza et al., 2013; Karanfil et al., 2018). Additionally, it is the only virus with a DNA genome among the rose viral diseases identified in Türkiye (Karanfil, 2021). Previous studies reported RYVV in the Çanakkale and Ankara provinces, suggesting a potentially broader distribution (Karanfil et al., 2018). The recent study confirms the pathogen's presence in different provinces, including those in the Thrace region (Çanakkale, Tekirdağ, Edirne, and Kırklareli), indicating a possible presence in neighboring countries like Greece and Bulgaria. The pathogen appears to be spreading throughout all sampled regions within Türkiye (Karanfil, 2021). Thus, efforts to determine the full genome of RYVV are considered critical.

CONCLUSION and RECOMMENDATIONS

Several isolates failed to have their full genomes sequenced, possibly due to genetic diversity, as indicated by Dr. Mollov (personal communication, cited in Karanfil, 2021). Therefore, next-generation sequencing (NGS) analyses should be employed to better detect and characterize RYVV isolates.

This study represents the first instance in which the full genome sequences of a rose viral disease have been determined in Türkiye, establishing the presence of Türkiye-origin isolates in the GenBank database. Future studies may focus on elucidating the genetic diversity of more RYVV isolates.

Although RYVV infection has been detected in many regions of Turkey, there are still areas and provinces where the presence of the virus has not yet been investigated. Therefore, it is important to explore and confirm the presence of the virus in these unstudied locations. Additionally, this approach would allow for an investigation into the genetic diversity of RYVV isolates from different regions and provinces.

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YAZAR ORCID NUMARALARI

Ali KARANFİL http://orcid.org/0000-0002-4503-6344

Filiz RANDA-ZELYÜT http://orcid.org/0000-0002-1366-4389
Savaş KORKMAZ http://orcid.org/0000-0001-8227-3800

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