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MOLECULAR IDENTIFICATION, PHYLOGENETIC ANALYSIS, AND IMPROVEMENT OF MANAGEMENT STRATEGIES FOR TOMATO VIRUSES

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Abstract. Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops worldwide and is highly susceptible to a wide range of viral pathogens. Tomato viruses can cause severe reductions in plant growth, fruit quality, and yield, resulting in substantial economic losses. The increasing diversity, emergence, and geographic spread of tomato viruses have created a need for rapid and reliable diagnostic methods, accurate molecular characterization, and effective disease management strategies. Molecular techniques, including reverse transcription polymerase chain reaction (RT-PCR), quantitative RT-PCR (RT-qPCR), conventional PCR, sequencing, and high-throughput sequencing, have significantly improved the identification and characterization of viral pathogens. Phylogenetic analysis based on nucleotide and amino acid sequences provides important information about virus evolution, genetic diversity, relationships among isolates, and possible routes of dissemination. This review summarizes current approaches for the molecular identification and phylogenetic analysis of major tomato viruses and discusses integrated strategies for improving their management. Particular attention is given to resistant cultivars, vector control, sanitary measures, molecular diagnostics, biological control, and the potential application of genomic and biotechnological approaches. The integration of molecular surveillance with preventive and sustainable disease management is essential for reducing the impact of tomato viral diseases and improving tomato production.

Keywords: tomato viruses, molecular identification, RT-PCR, RT-qPCR, phylogenetic analysis, genetic diversity, virus management, disease resistance, *Solanum lycopersicum*.

1. Introduction. Tomato (*Solanum lycopersicum* L.) is an important horticultural crop cultivated in both open-field and protected production systems. It is an important source of vitamins, minerals, antioxidants, and other bioactive compounds and has considerable economic significance for agricultural producers and food industries. However, tomato production is affected by numerous viral diseases that can cause significant yield and quality losses.

Tomato viruses are particularly difficult to manage because they can be transmitted through infected planting material, mechanical contact, soil, seeds, and insect vectors. Several economically important viruses are transmitted by whiteflies, aphids, thrips, leafhoppers, and other vectors. In addition, international trade and movement of infected plant material can facilitate the introduction of viruses into new geographical regions.

The symptoms associated with viral infections may include mosaic patterns, leaf curling, chlorosis, necrosis, stunting, deformation, and abnormal fruit development. However, symptoms are often similar among different viruses or may be influenced by environmental conditions and mixed infections. Consequently, visual diagnosis alone is generally insufficient for reliable identification.
Molecular diagnostic methods provide a more accurate approach to virus detection and identification. At the same time, sequencing and phylogenetic analysis allow researchers to determine the genetic

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relationships between viral isolates and monitor changes in virus populations. The objective of this article is to review molecular approaches for identifying tomato viruses, explain the importance of phylogenetic analysis, and discuss improved strategies for controlling viral diseases in tomato production.

2. Major Viral Diseases of Tomato. Tomatoes can be infected by viruses belonging to several taxonomic families. Among the most important pathogens are viruses from the genera *Tobamovirus*, *Begomovirus*, *Potyvirus*, *Tospovirus* (currently classified within the genus *Orthotospovirus*), *Cucumovirus*, and several other genera.

Tomato brown rugose fruit virus (ToBRFV) has become an important emerging pathogen because of its high transmissibility and its ability to cause severe symptoms and fruit quality problems. Other important pathogens include tomato mosaic virus (ToMV), tomato spotted wilt virus (TSWV), tomato yellow leaf curl virus (TYLCV), tomato chlorosis virus (ToCV), tomato yellow ring virus, cucumber mosaic virus (CMV), and several other viruses.

The epidemiology of these viruses varies considerably. For example, tobamoviruses are highly stable and can spread efficiently through mechanical contact and contaminated tools, surfaces, seeds, and plant debris. Begomoviruses are commonly transmitted by whiteflies, whereas orthotospoviruses are primarily associated with thrips vectors. Therefore, virus management must be adapted to the biological characteristics and transmission mechanisms of individual pathogens.

3. Molecular Identification of Tomato Viruses

3.1 Sample Collection and Preparation

Accurate molecular identification begins with appropriate sample collection. Young symptomatic leaves, stems, and fruits are commonly collected from infected plants. Samples should be properly labeled and stored under conditions that minimize nucleic acid degradation.

Total RNA or DNA is extracted depending on the genome type of the suspected virus. RNA viruses require RNA extraction followed by reverse transcription, while DNA viruses can generally be analyzed directly after DNA extraction.

3.2 Polymerase Chain Reaction and RT-PCR

Polymerase chain reaction (PCR) and reverse transcription polymerase chain reaction (RT-PCR) are among the most widely used molecular techniques for plant virus detection.

For RNA viruses, viral RNA is first converted into complementary DNA (cDNA) using reverse transcriptase. The resulting cDNA is then amplified using virus-specific primers. The amplified products can be visualized by agarose gel electrophoresis and subsequently sequenced for confirmation.

RT-PCR is relatively rapid, sensitive, and specific when appropriate primers are available. However, its effectiveness may be reduced when viruses exhibit substantial genetic variability or when primer-binding regions contain mutations.

3.3 Real-Time RT-PCR

Real-time RT-PCR, or RT-qPCR, provides a quantitative and highly sensitive method for detecting viral nucleic acids. Fluorescent probes or DNA-binding dyes allow amplification to be monitored during each cycle of the reaction.

RT-qPCR can be particularly useful for detecting viruses at low concentrations and for distinguishing closely related pathogens. Multiplex RT-qPCR assays can also allow several viruses to be detected

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simultaneously, which is important because mixed infections are relatively common in tomato production systems.

3.4 Sequencing and High-Throughput Sequencing

Sequencing of PCR products provides information about the genetic composition of viral isolates. Partial gene sequences can be compared with sequences available in international databases to determine the identity of an unknown virus.

High-throughput sequencing (HTS), also known as next-generation sequencing, has substantially expanded the possibilities for plant virus diagnostics. Unlike targeted PCR assays, HTS does not necessarily require prior knowledge of the pathogen. It can therefore detect known viruses, divergent variants, and potentially novel viruses in a single sample.

HTS is especially valuable for investigating samples with unusual symptoms, mixed infections, or viruses that cannot be detected by conventional diagnostic assays.

4. Phylogenetic Analysis of Tomato Viruses

Phylogenetic analysis is an important component of molecular characterization. It enables researchers to reconstruct evolutionary relationships among viral isolates based on genetic sequence data.

The general workflow includes sequence acquisition, quality control, multiple sequence alignment, selection of an appropriate evolutionary model, and construction of a phylogenetic tree. Commonly used methods include Neighbor-Joining (NJ), Maximum Likelihood (ML), and Bayesian inference.

For tomato viruses, phylogenetic analysis can be performed using conserved genes or genomic regions. The choice of the analyzed region depends on the virus. For example, genes encoding coat proteins, replication-associated proteins, movement proteins, or other conserved proteins may provide useful information for comparison.

Bootstrap analysis is frequently used to evaluate the statistical support for individual branches of a phylogenetic tree. High bootstrap values indicate stronger support for a particular grouping of isolates.

Phylogenetic analysis can reveal whether tomato virus isolates from different geographical regions belong to the same genetic lineage or represent distinct evolutionary groups. It can also help identify newly introduced variants and monitor the emergence of genetically divergent populations.

5. Molecular Epidemiology and Genetic Diversity

Genetic diversity plays an important role in the epidemiology and management of plant viruses. RNA viruses, in particular, may accumulate mutations relatively rapidly, resulting in the emergence of new variants.

The comparison of viral sequences obtained from different regions can provide information about virus movement and population structure. Molecular epidemiological studies may identify relationships between isolates collected from different production systems and geographical locations.

Genetic monitoring is especially important for emerging viruses such as ToBRFV. Continuous sequencing and phylogenetic surveillance can help identify new genetic groups and determine whether particular variants are becoming geographically widespread.

Such information is valuable for disease surveillance, quarantine programs, breeding programs, and the development of diagnostic primers and probes.

6. Factors Contributing to Virus Spread

The spread of tomato viruses is influenced by several biological, environmental, and agricultural factors. The most important include:

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1. **Infected planting material:** Seedlings and vegetative material may serve as sources of primary infection.
2. **Insect vectors:** Whiteflies, thrips, aphids, and other insects can efficiently transmit specific viruses.
3. **Mechanical transmission:** Viruses such as tobamoviruses can spread through hands, tools, machinery, clothing, and plant-to-plant contact.
4. **Infected seeds:** Some tomato viruses can be associated with or transmitted through seeds.
5. **Plant residues:** Infected plant debris may remain a source of inoculum.
6. **Poor sanitation:** Inadequate disinfection of tools, greenhouse structures, and equipment can facilitate disease spread.
7. **Global movement of plant material:** International trade can contribute to the introduction of viruses into previously unaffected regions.

Understanding these factors is essential for designing effective management programs.

7. Improvement of Tomato Virus Management Strategies

7.1 Use of Resistant Cultivars

The development and cultivation of virus-resistant tomato varieties represent one of the most sustainable approaches to disease management. Resistance genes obtained from wild tomato relatives or identified through molecular breeding can provide protection against specific viruses.

However, the emergence of new viral variants may overcome existing resistance. Therefore, breeding programs should consider genetic diversity within virus populations and aim to develop varieties with durable and broad-spectrum resistance.

Conclusions. Tomato viral diseases represent a major challenge to sustainable tomato production. The complexity of viral populations, the occurrence of mixed infections, and the emergence of new variants highlight the limitations of symptom-based diagnosis and conventional disease management alone.

Molecular diagnostic methods such as RT-PCR, RT-qPCR, sequencing, and high-throughput sequencing provide powerful tools for accurate virus detection and characterization. Phylogenetic analysis complements these techniques by revealing evolutionary relationships, genetic diversity, and geographical patterns among viral isolates.

Future tomato virus management should focus on the integration of molecular surveillance, rapid diagnostics, resistant cultivars, vector management, strict sanitation, and modern biotechnology. Continuous monitoring of viral genetic diversity is particularly important because newly emerging variants may compromise existing diagnostic assays and resistance sources.

An integrated approach combining molecular identification, phylogenetic surveillance, and preventive disease management offers the most promising strategy for reducing the impact of tomato viruses and ensuring stable and sustainable tomato production.

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