Tomato spotted wilt virus

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Summary

Tomato spotted wilt virus (TSWV) belongs to the genus *Tospovirus* of the family *Bunyaviridae*. This is the only genus of the family Bunyaviridae that comprises of plant-infecting viruses. In nature, TSWV is transmitted by thrips in a circulative and propagative manner. The viral genome consists of three single stranded-RNAs. The large RNA is in negative sense while the middle and small RNAs have an ambisense genome organization. As a result, the use of reverse genetics as a tool to study structure-function relationships has not been possible. However, genetic reassortment studies and complementation studies using heterologous virus systems provided insights into the functions of various TSWV gene products. The current understanding of the TSWV gene functions indicates that the NSm serves as a movement gene, NSs is a silencing suppressor and the glycoproteins contain determinants for thrips transmission. Management of TSWV has proven to be challenging due to the wide host range of both the virus and its vector, and emergence of resistance breaking strains. However, increased understanding of the biology, genetics, epidemiology and molecular biology of TSWV resulted in development of practical and effective integrated disease management programs for reducing the impact of TSWV in some crops.

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Taxonomy

Tomato spotted wilt virus (TSWV) belongs to the genus *Tospovirus* in the family *Bunyaviridae*. Of the more than 300 species of primarily arthropod-borne viruses described in the family *Bunyaviridae*, a small proportion infects plants. Members of other genera in this family are important pathogens of humans and animals. TSWV is considered as the type member of the genus and hence its name (tomato spotted wilt *virus*) formed the basis for coining the genus name. However, discovery of a second tospovirus, *Impatiens necrotic spot virus* (INSV), was followed by description of more tospoviruses from several parts of the world. To date, there are more than 14 distinct tospoviruses described. Descriptors for classification of new viruses as tospoviruses include genome organization (Figure 1), thrips transmission, host range, and serological and molecular relationships of the nucleoprotein (N) gene. <Figure 1 near here> Excellent reviews on various aspects of tospoviruses have been published in the last few years. This review focuses on summarizing recent advances in our understanding of the tospovirus biology, molecular biology, epidemiology and control.

Biology

TSWV was first described in 1915. The virus has a wide host range, infects more than 900 plant species that include numerous crops and weeds. The virus is mechanically transmissible and is not seed-transmitted. In nature, TSWV is transmitted by several species of thrips. Crops that are affected by TSWV include bean, lettuce, peanut, pepper, potato, tobacco, and tomato. Biologically distinct isolates of TSWV exist in nature. Isolates that differ in thrips transmissibility, symptomatology and symptom severity have been described. (Figure 2). <Figure 2 near here>. Variability in the N gene sequence of TSWV isolates suggested geographic delineation in natural virus populations which may be useful for attribution: tracing the potential source or origin of a particular isolate. TSWV causes systemic infection in most of the crops it infects. Infection at early stages of the plant growth causes the most damage that may include severe stunting of the entire plant which often results in death. TSWV epidemics in peanut, pepper, tobacco, and tomato in southeastern United States caused major economic losses and forced shifts in production practices. Losses due to TSWV outbreaks in peanut were estimated at more than US $100 million in Georgia alone in the USA.

Molecular biology

The morphology and genome structure and organization of tospoviruses share several features with members of other genera in the family *Bunyaviridae*. Particles are near pleiomorphic, 80-120 nm in diameter. The genome of tospoviruses includes three RNAs referred to as Large (L), Medium (M) and Small (S). 5’ and 3’ terminal sequences of the RNAs are conserved. L RNA is in negative sense while M and S RNAs are

ambisense in their genome organization. L RNA codes for the RNA-dependent RNA polymerase (RdRp). M RNA encodes precursors for two structural glycoproteins, G_N and G_C, and a non-structural protein, NSm. The S RNA codes for the nucleocapsid protein (N) and another non-structural protein, NSs. The three genomic RNAs are tightly linked with the N protein forming ribonucleoproteins (RNPs). These RNPs are encased with in a lipid envelope consisting of two virus-encoded glycoproteins, and a host-derived membrane. The genome organization, gene products and their roles are shown in Figure 2. Due to the negative strandedness of the genome, virions contain several molecules of the RNA-dependent RNA polymerase to initiate initial rounds of replication of the virion RNAs. Genome expression is facilitated through synthesis of subgenomic RNAs.

Considerable progress has been made in our understanding of the functional roles of various TSWV genes. The 331-kDa RdRp coded by the L RNA serves as a multifunctional, replication-associated protein and is believed to function cooperatively with host-encoded factors. Screening of a cDNA library of *F. occidentalis* using TSWV fragments of TSWV RdRp, a putative transcription factor that binds to TSWV RdRp was isolated which was shown to bind to TSWV RNA and enhance TSWV replication. Mammalian cells expressing this putative transcription factor supported TSWV replication. Since TSWV is transmitted by several thrips species it remains to be seen if similar transcription factors exist in other thrips vectors.

Assignment of functions to various TSWV gene products was done using indirect approaches since a reverse genetics system based on an infectious cDNA clone is not yet possible for a negative sense RNA virus such as TSWV. Using indirect approaches, the

functions of the M RNA-encoded glycoproteins and NSm were recently deciphered. The absence of a gene similar to NSm in other genera of the family *Bunyaviridae* suggests that NSm serves a function that facilitates TSWV infection of plants. Virus movement in plants is mediated by a virus-encoded movement protein and a similar function was suggested for NSm and subsequent experimental evidence supported this hypothesis. Recently, the role of NSm in TSWV lifecycle was investigated *in vitro* and *in planta*. Virus-encoded movement proteins tend to bind to viral RNAs and facilitate virus movement through plasmodesmata. *In vitro*-expressed NSm interacted with N protein and bound ssRNA in a sequence-nonspecific manner. Members of DnaJ family were found to bind NSm in a yeast-two-hybrid system. Transgenic *Nicotiana tabacum* plants expressing NSm produced symptoms suggestive of TSWV infection. Biochemical analyses of this plant response showed the accumulation of callose, an indicator of the triggering of plant defense response. Constitutive expression of the NSm and its subsequent interference with the plasmodesmata's transport functions could have resulted in abnormal growth pattern seen in these transgenic plants. More direct evidence of the role of NSm in virus movement was obtained by heterologous complementation studies using an infectious cDNA clone of *Tobacco mosaic virus* (TMV) and replacing TMV genes with the NSm gene of TSWV. The NSm gene complemented a movement deficient mutant of TMV in tobacco plants and facilitated the long distance movement of the TMV-TSWV NSm hybrid. The NSm protein, when expressed *in planta*, mediated tubule formation in infected protoplasts.

TSWV mutants lacking the envelope were not thrips-transmissible indicating that the determinants for thrips transmission are localized on the glycoprotein-containing

envelope. Using two distinct isolates of TSWV that differ in thrips transmission, genetic reassortants (=pseudorecombinants) were generated by coinoculating plants with both isolates. The resulting reassortants were evaluated for their ability to be thrips transmitted and the source of the genomic components of each of these reassortants was determined. Only those reassortants that had the M RNA derived from the thrips-transmissible isolate retained thrips transmissibility confirming the functional contribution of M RNA to thrips transmission (Figure 3) <Figure 3 near here> . Sequence comparisons of the glycoprotein genes of these two isolates showed that a point mutation played a critical role in determining the thrips transmissibility. The roles of G_N and G_C in TSWV-thrips vector interaction are beginning to be elucidated and are discussed under Transmission and Epidemiology. Moreover, the M RNA was shown to carry determinants for host adaptation and overcoming host plant resistance as well as pathogen-derived resistance in tomato and tobacco. In the absence of a reverse genetics system, complementation studies and genetic reassortment studies such as those mentioned above offer alternate approaches for studying structure-function relationships of various TSWV genes.

The S RNA-encoded proteins, N and NSs, play important roles in the TSWV infection cycle. The N protein, as part of the RNP, serves as the structural protein and may also have some regulatory role in modulating the transcription and replication. Additionally, the involvement of the N protein in particle assembly was suggested based on its interaction with one of the two viral glycoproteins. When co-expressed with G_N and G_C in mammalian BHK21 cells, N protein displayed selective interaction with G_C and the subsequent localization of the N and glycoprotein complex in the Golgi

apparatus. The nonstructural protein, NSs, encoded by the S RNA was shown to be a suppressor of RNA silencing.

Diagnosis

TSWV produces a wide range of foliar symptoms. Symptomatology varies depending on the strain, host species and genotype and is also influenced by environmental factors such as temperature. In most cases, accurate diagnosis is facilitated by serological or molecular techniques. Purified TSWV is a good immunogen and virus-specific antisera and ELISA kits are commercially available. Molecular techniques based on RT-PCR have been developed that can detect the virus in plants and thrips vectors. Sensitive, rapid real-time PCR methods are also available. An NSs-specific monoclonal antibody was produced which was shown to be effective in identifying adults that are capable of transmitting the virus since detection of NSs in thrips is an indication that the virus had multiplied in the vector.

Transmission and Epidemiology

TSWV is transmitted by several species of thrips in a circulative and propagative manner. These include *Franklienella occidentalis,F. schultzei, F. Intonosa, F. bispiosa, F. fusca,Thrips setosus, and T.tabaci* The virus is not known to be seed transmitted. Hence, susceptible crops with overlapping production seasons and prevalence of weed hosts and thrips vectors constitute the most important factors for TSWV epidemics. TSWV has to be introduced into a crop by viruliferous thrips and patterns of disease spread mostly suggests primary spread with little secondary spread within the crop.

Considerable progress has been made in our understanding of thrips-TSWV interactions especially in the case of TSWV-*F. occidentalis* association. For the adult thrips to become a transmitter, the larva has to acquire the virus by feeding on an infected plant. First and second instar larvae are capable of acquiring the virus though the former is more efficient. The virus starts replicating in the larva and survives through the developmental stages. The emerging adult transmits the virus and continues so for life. There is no evidence of transovarial transmission. Thus, the virus has to be acquired by each generation of thrips. Therefore, tospoviruses are capable of replicating in both their host plants and thrips vectors. This intimate biological association between tospoviruses and their thrips vectors had created possibilities for evolution and shifting preferences and specificities between individual tospoviruses and thrips species. New vector species of TSWV began to emerge in the past decade. For example, *F. bisponsa* was reported as a vector of TSWV. For TSWV epidemics to occur, thrips vectors should complete a life cycle on virus-infected host plants. Infected plants that do not support thrips lifecycle can be considered as a dead-end in disease epidemiology. The economic impact of TSWV on a number of important crops combined with the fact that thrips-borne inoculum is the most important contributory factor to virus outbreaks resulted in extensive research efforts to understand the basis of TSWV-thrips interactions and the virus and insect factors that contribute to the specificity of virus-vector relations. Of more than 5,000 thrips species described, only seven of them are known to transmit TSWV, suggesting complex interactions of recognition, acquisition, replication and movement at the virus-vector association level. Much is now known about the interaction between TSWV and *F. occidentalis*.

The virus upon acquisition was shown to move through the midgut and subsequently reaches the salivary glands. It is hypothesized that the close proximity of midgut and salivary glands in the thrips larval stage facilitates the virus movement whereas the virus fails to do so as the thrips reaches adult stage. This may explain the inability of adult thrips to transmit the virus if the virus is acquired for the first time in its adult life. The specificity of TSWV and thrips vectors may be due to the presence of a receptor in the vector species which may be absent in non-vector species. A 50-kDa protein and a 94-kDa protein were identified as potential thrips proteins involved in interaction with TSWV. However, cloning the gene for the putative receptor remains to be accomplished. A soluble form of *in vitro*-expressed TSWV G_N protein was used to study its role in recognition by thrips vectors. When thrips were fed with the purified G_N, the protein could be detected in the midgut epithelial cells of the larvae which subsequently resulted in the prevention of TSWV acquisition, suggesting that G_N may be involved in virus recognition by the thrips vector. While G_N potentially mediates the specific recognition between TSWV and its thrips vector, sequence comparisons and biochemical analysis of G_C indicated that it facilitates the fusion and subsequent uptake of the virion into vector cells.

Management

TSWV has been an economically important constraint for several crops over the past several decades in several parts of the world. Due to the non-predictive nature of the outbreaks combined with the lack of forecasting, adoption of preventive measures have not always been practical. The disease cycle has proven to be extremely difficult to break

because of the wide and often overlapping host range of both the virus and the thrips vectors.

However, multidisciplinary research in the past decade has led to identification of factors that contribute to TSWV epidemics and development of practical control recommendations which resulted in reduced impact of TSWV in several crops. An integrated management approach must be taken as no single control tactic was found to be effective by itself. The crucial element of the management program is growing virus resistant or virus tolerant cultivars. TSWV-resistant cultivars with desirable agronomic traits are now available for peanut, pepper and tomato. Resistance breaking strains of TSWV continue to pose a threat, potentially limiting the durability of resistant cultivars. Resistance governed by multigenes should be used where available to make the resistance more durable. Production practices such as growing on plastic mulch or reflective mulch significantly reduced the disease incidence in certain crops. Novel strategies utilizing chemicals such as acibenzolar-S-methyl that induce systemic acquired resistance are found to be effective in reducing the incidence of TSWV (Figure 4). <Figure 4 near here>. Thrips management based on selective use of insecticides during the early part of the cropping season combined with resistant cultivars resulted in reduced incidence and increased yield in tomato. Ability to forecast the epidemics by making use of information such as the seasonal dynamics of TSWV transmitters in the vector populations will help growers make appropriate management decisions. Pathogen-mediated resistance due to post transcriptional gene silencing was shown to be effective in several crops. Expression of single chain antibodies, TSWV N gene or various modifications of the N gene were found to be the most effective conferring resistance.

Genetic engineering can be very useful in situations where natural sources of host plant resistance are not available or difficult to transfer to existing agronomically desirable cultivars. Consumer acceptance and other market-related issues have hampered the efforts to commercialize this technology.

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Legends to Figures

Figure 1. Genome organization of *Tomato spotted wilt virus*. Functions of each gene are noted on the right side.

Figure 2. Biological variability of *Tomato spotted wilt virus* isolates. Response of *Nicotiana tabacum* to a severe strain (top) and a mild strain (bottom).

Figure 3. Genetic reassortment as a tool for the functional analysis of tospovirus genes. Localization of thrips transmission determinants on the M RNA of *Tomato spotted wilt virus*.

Figure 4. Protective effect of acibenzolar-S-methyl against *Tomato spotted wilt virus* (TSWV). Both leaves were mechanically inoculated with TSWV. Leaf on the right was treated with acibenzolar-S-methyl prior to inoculation with TSWV. Reproduced with permission from the American Phytopathological Society.

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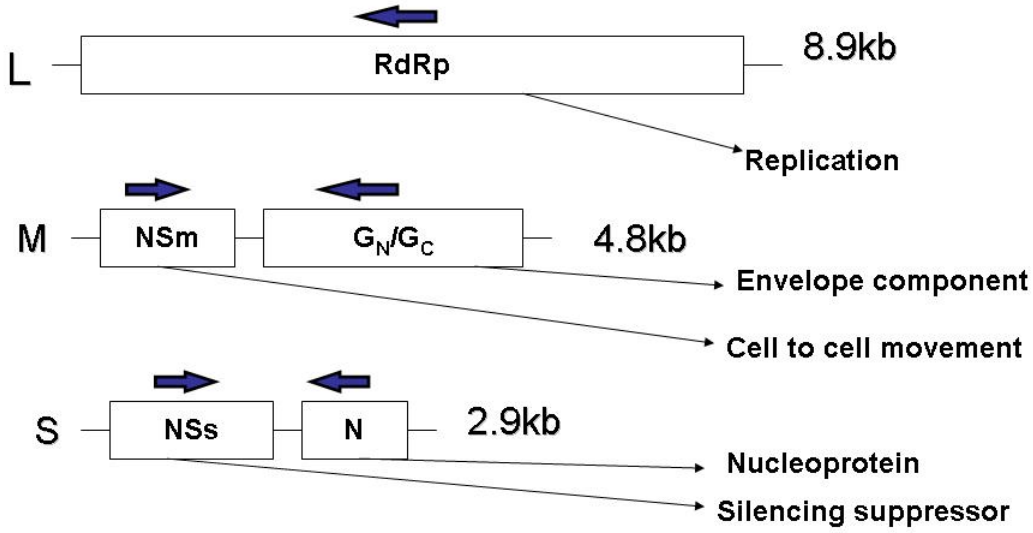

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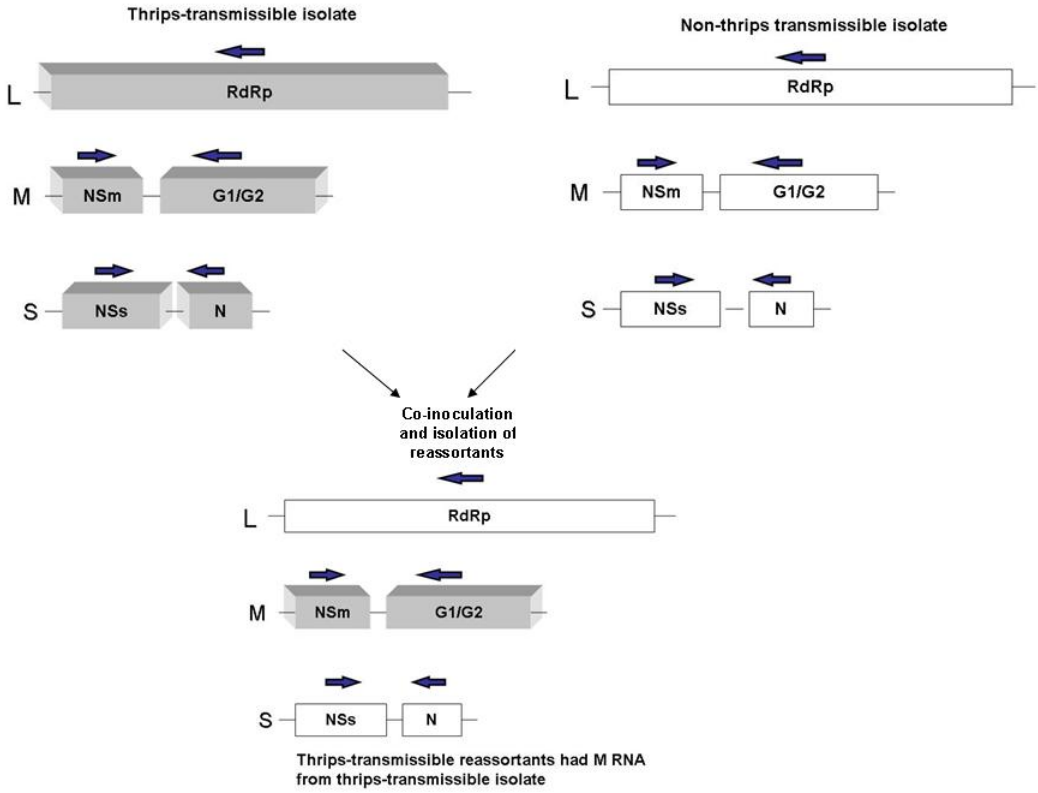

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