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Transmission, Detection and Mapping Resistant Genes for Tomato Spotted Wilt Virus in Tomato

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Abstract

Tomato spotted wilt virus (TSWV) causes serious diseases of many economically important plants including dicots and monocots. TSWV is the only member of an RNA-containing virus group that has membrane-bound spherical particles 70-90 mm in diameter. TSWV is one of the major diseases in tomato transmitted by thrips and became the main threat in tomato cultivation. The life cycle of thrips takes about 20-30 days from egg to adult, again depending on the temperature. Symptoms of tomato spotted wilt differ among hosts and can be variable in a single host species. Stunting is a common symptom of TSWV infection, and is generally more severe when young plants are infected. Virus can be detected by many methods such as ELISA, polymerase chain reaction and recombinant polymerase amplification. Resistance is controlled by a single dominant gene from the resistant sources such as *Solanum chilense* and *Solanum peruvianum*.

Introduction

The tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family and has 2n=24 chromosomes. The tomato originated from Peru, Equator, Galapagos Islands and mountainous sections of Chilli. There are about 200 diseases which include viruses, bacteria, nematodes and fungi in the tomato. Tomato spotted wilt virus (TSWV) is a member of the genus *Tospovirus*, which belongs to the family *Bunyaviridae*. TSWV has a wide host-range that includes tomato, tobacco, pepper, potato, celery, pea, peanut, dahlia, lettuce, chrysanthemum, gerbera, iris, and impatiens, among others. TSWV has been reported in over 800 plant species and is a serious problem throughout the World, particularly in warm, tomato-producing regions. Among the viruses, Tomato Spotted Wilt Virus (TSWV) was first encountered in 1906 and designated as "spotted wilt of tomato" by Brittlebank in 1919.

History

In 1919, Brittlebank published a description of a new tomato disease noticed for the first time in 1915 in the State of Victoria, Australia. He named the disease "spotted wilt of tomato." During the early 1920's it was described from all the states of Australia. Samuel was the first person to characterize the causal agent of the disease as a virus, and named it as "Tomato spotted wilt virus" in 1930 (Tentchev *et al*., 2011).

The disease was first seen outside Australia by K.M. Smith at United Kingdom (Tentchev *et al*., 2011). Subsequently the disease has been recorded from other parts of Europe, South America, North America, Africa, and Asia, so that it can be said to have a worldwide distribution. At the outset, the virus of tomato spotted wilt (TSW) created great interest because of

its vector specificity. It was the only virus that was transmitted by thrips. Furthermore, adult insects could transmit the virus from infected to healthy plants only if they had fedon virus-infected plants when they were in the larval stage.

Strains

Best and Gallus characterized six strains they had separated and which bred true over a period of many years. They used three indicator plants: *Lycopersicon esculentum* (var. Dwarf Champion), *Nicotiana glutinosa,* and *Nicotiana tubacum* (var. Blue Pryor) to characterize the separated strains biologically, making all tests under standardized conditions in insect-proof glasshouses. Since the six variants bred true in terms of these markers over a period of years they have been accepted as naturally occurring strains with genetic continuity, and have been designated by letters A, B, C1, C2, D, and El respectively. The six strains form a graded series in respect to severity of the symptoms they evoke, forming, as it were, a "spectrum," on whichever kind of host plant they are compared.

All strains move systemically in *Lycopersicon esculentum* (var. Dwarf Champion), and in this plant the strains may be classified broadly into three main groups according to the systemic symptoms developed: those producing severe necrosis along with the formation of brown (or purple) pigment (strains A, B, D); those producing only very mildsurface necrosis unaccompanied by pigmentation, often in the form ofringspot or parallel-line patterns (strains C1, C2); and those in which there is neither visible necrosis nor pigmentation (strain E). Strain A is distinguished from all the rest by the fact that it is the only one which produces primary, pigmented, necrotic disc lesions on inoculated tomato leaves, and is the only one which produces apical necrosis (so called "tip blight") in systemically invaded tomato plants. All strains cause a stunting of tomato plants, strains A and B also cause the leaves and leaflets to curl downward, whereas strains C1, C2, and E cause a flattening of the leaves and leaflets and a marked shortening ofthe internodes. Strain D is characterized by the formation of purple pigment along petioles and veins.

Causal Agent, Its Morphology, Transmission and Hosts

The disease is caused by Tomato spotted wilt virus (TSWV), *Tospovirus*. The TSWV genome consists of three single-stranded RNA. It is transmitted by mechanical means and the most important means of natural transmission are thrips. Nine species are reported as vectors: *Frankliniella occidentalis* (western flower thrips); *F. schultzei, F. fusca* (tobacco thrips); *Thrips tabaci* (onion thrips); *T. setosus, T. moultoni; F. tenuicornis, Lithrops dorsalis* and *Scirtothrips dorsalis.* The first four are considered the most important vectors because of their wide distribution and the overlapping host ranges of these species and TSWV.

Being adult thrips unable to acquire the virus, it must be acquired by the larval stage at first; the subsequent adult can then transmit the virus. The virus is retained when the vector moults and the latent (incubation) period is 3-10 days, depending on the vector species (Figure 1). Transmission to a susceptible host plant occurs through feeding activities of adults. TSWV has a host range spanning several hundred species in both monocotyledonous and dicotyledonous plants.

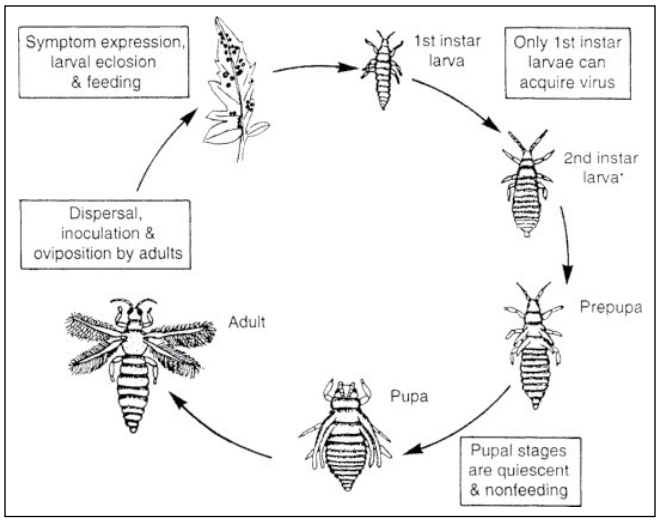


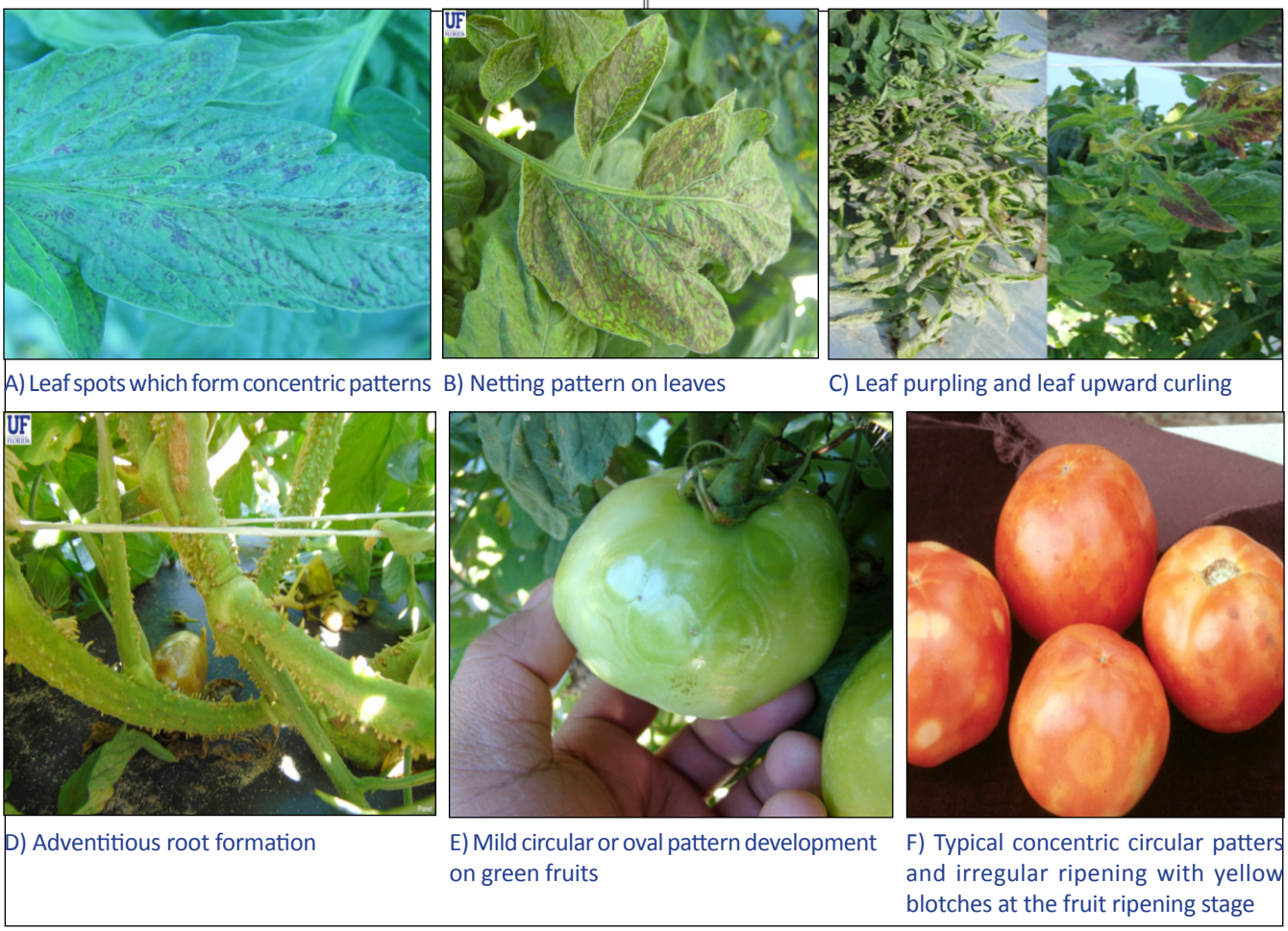
Figure 1: Lifecycle of thrips

Symptoms

On tomato, the first symptoms usually are small, orange-coloured flecks on some middle or lower leaves or on the calyx. As new spots appear, older leaves turn brown, die, and droop. Similar spots or streaks occur on the stems and petioles. The entire plant becomes dwarfed, and with its drooping leaves it resembles a plant affected by a wilt. A marked bronzing of the foliage is typical in Australia and in the western United States. On the green fruits, yellowish spots up to 10 mm in diameter appear, usually with distinct concentric zones of shades of yellow or brown alternating with green and later with pink or red. These zoned fruit spots are the most striking symptom of spotted wilt on red tomato fruits (Figure 2).

Detection

A number of methods have been published for the detection of TSWV in thrips, ranging from ELISA, electron microscopy, nucleic acid dot-blots, immunological squash blots and loop-mediated isothermal amplification (LAMP). Monitoring thrips numbers is important in the control of virus, but does not give information on how many thrips are viruliferous. Monitoring the presence of viruliferous thrips at an early stage of an epidemic leads to



A) Leaf spots which form concentric patterns B) Netting pattern on leaves C) Leaf purpling and leaf upward curling

D) Adventitious root formation E) Mild circular or oval pattern development on green fruits F) Typical concentric circular patters and irregular ripening with yellow blotches at the fruit ripening stage

Figure 2: Symptoms of TSWV

improved control as the vector pressure is low and symptoms may take several weeks to appear on some hosts.

Real time fluorescent RT-PCR assay based on TaqMan technology had been developed which allows detection of virus with no post PCR processing. The method is quantitative and could be used as a tool for examining tospovirus interaction within the bodies of individual insects. This is the simple and robust extraction method suitable for testing large numbers of thrips. The method incorporates a novel RNA specific internal control to increase the reliability of the result.

Concerns of PCR-based methods are time consuming and limiting applications of rapid diagnostic detection under laboratory and field conditions. So Recombinase Polymerase Amplification (RPA) has been explored for rapid and sensitive molecular detection of various pathogens. An Isothermal reverse transcription recombinase polymerase amplification (RT-RPA) assay, combined with lateral flow strips (LFS), was established for rapid detection of TSWV in pepper infected leaves. The RT-RPA reaction was performed at an optimal condition of 38 °C for 10 min and an LFS incubation time of approximately 5 min. RPA could be monitored in real time using a device and can also be visualized using regular agarose gel electrophoresis or lateral flow strips (LFS) and this RPA-LFS technique can be easily used in the field (Lee *et al*., 2021).

Resistance Source

The resistant varieties against some isolates of TSWV were found in *Solanum esculentum* and *S. pimpinellifolium*. Resistance was also reported in *S. hirsutum, S. chilense* and *S. peruvianum*. However, *S. peruvianum* was found to have broad resistance to all TSWV isolates. Several genes for TSWV resistance have been reported (*Sw1a*, *Sw1b*, *sw2*, *sw3*, *sw4*, *Sw-5*, *Sw-6*, and *Sw-7*). Three recessive genes, *sw2, sw3*, and *sw4,* and the two dominant genes *Sw1a* and *Sw1b* were not used in commercial tomato production because resistance was quickly overcome. *Sw-6* conferred partial resistance to thrips inoculation and showed a narrower range for resistance to viral isolates than *Sw-5*. Recently, *Sw-7* has been identified and introgressed from *S. chilense* LA 1938 into *S. esculentum* and an amplified fragment length polymorphism (AFLP) marker was potentially identified to be linked to the gene and the gene was mapped on Chromosome 12 flanked by the markers

T1263 (45.0 cM) and SSR20 (58.2 cM). *Sw-5* is the most broadly deployed TSWV resistance gene utilized in tomato breeding because of its durability to multiple tospoviruses. The *Sw-5* gene, first identified in *S. peruvianum*, also has provided stable resistance against TSWV isolates from different geographical location (Shi *et al*., 2011).

It was reported that the resistance in *S. peruvianum* is controlled by a single dominant gene *Sw-5* which is more stable and less isolate specific, this resistance source has been widely used in tomato breeding programs. The *Sw-5* has been genetically mapped between the markers CT71 and CT220 on chromosome 9. *Sw-5* gene has five alleles along the chromosome 9, named *Sw5-a* to *Sw5-e*, and among them, *Sw5-b* is the functional allele for conferring resistance to TSWV. The presence of *Sw-5* gene in tomato plants confers resistance to TSWV by a hypersensitive defense response that causes local lesions on the leaf, preventing the spread of the virus from the infection site through the plant. In addition, some isolates like TSWV6 from Spain and Italy have been reported to overcome the resistance provided by *Tsw* gene.

As a result of the efforts to obtain a new resistance sources, S*w-7* gene was determined which was conferred by a single dominant gene not linked to *Sw-5* and *S. chilense* was used as a source of this resistance gene. New resistance sources were determined against TSWV from the tomato germplasm which include *S. penellii, S. chmielewski, S. habrochaites*, *S. peruvianum* and *S. sitiens*. The genotypes LA0716, LA1028, LA1777, LA2744 and LA4110 respectively can be used as a resistance source in breeding studies.

S. esculentum cultivars 'Rey de los Tempranos' and 'Manzana' also had TSWV resistance although the derivation of the resistance was not identified. Resistance identified in the uncultivated *Solanum* species has been used to develop resistant cultivars. The TSWV resistance found in the cultivar 'Pearl Harbor' was developed from *S. pimpinellifolium*. 'Anahu' released as a TSWV resistant tomato cultivar which had *S. peruvianum* in its background. The fresh market cultivar 'Stevens' also derives its TSWV resistance from *S. peruvianum*.

Mapping of *Sw-5* Gene using Molecular Markers

Sw-5 mapped between restriction fragment length polymorphism (RFLP) markers CT71 and CT220 near a telomeric region of chromosome 9 and also reported one random amplified polymorphic DNA (RAPD) marker 421R likely within 0.5 cM of *Sw-5*, using a segregating *L. esculentum* backcross population derived from near isogenic lines (NILs). Further RAPD marker 421R linked to *Sw-5* within a distance of 1 cM and the RFLP marker CT220 about 2.4 cM from *Sw-5*. Four RAPD markers was reported and linked to *Sw-5* within a distance of 10.5 cM on chromosome 9 and one sequence characterized amplified region (SCAR) marker developed from the four RAPD markers (Shi *et al*., 2011).

Five alleles (*Sw5-a, Sw5-b*, *Sw5-c*, *Sw5-d*, and *Sw5-e*) and seven *Sw-5* homologs distributed on chromosomes 9 and 12 were identified and cloned. Among them, *Sw5-b* is the functional allele for conferring resistance to TSWV and its sequence was published in GenBank with the accession AY007366 (AY007366 contains the sequence of the *S. esculentum* tospovirus resistance protein A (*Sw5-a*) and tospovirus resistance protein B (*Sw5-b*) genes. The tospovirus resistance protein A of the gene *Sw5-a* is complement to the sequence of the *Sw-5* locus located at AY007366 from 20824 to 24561 base and the tospovirus resistance protein B of the gene *Sw5-b* is complement to the sequence of the *Sw-5* locus located at AY007366 from 29513 to 33253 base. Dominant PCR-based marker representing the *Sw-5* gene sequence co-segregated with the RFLP marker CT 220 in 50 individuals of an F_2 mapping population.

Conclusion

Earlier the disease spotted only in the plants raised on tropical and subtropical conditions, but later the disease spread worldwide and became a major threat for tomato farmers. TSWV is large geographically widespread over the tomato cultivated lands and a large series of hosts, resulting in serious economic losses of 60-100%. TSWV is known to cause an average loss of yield of 1 billion dollars each year and is one of the most intensively studied plant viruses due to the future economic importance of TSWV. Tomato Spotted Wilt Virus (TSWV) is placed in the second position among the top 10 virus diseases.

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