Tomato Yellow Leaf Curl Virus: A Serious Threat to Tomato Plants World Wide

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Abstract
Tomato (Solanum lycopersicum) is one of the widely grown crops worldwide. It is consumed in various forms and has excellent nutritional values. Presently, this crop is facing a serious threat to its yield and survival because of Geminivirus infection. One of the Geminivirus species hampering tomato productions world-wide is Tomato yellow leaf curl virus (TYLCV). Tomato yellow leaf curl disease is one of the most destructive plant diseases destroying the tomato crops globally. It has spread to many countries worldwide including Southern, Central and Northern parts of America, Southern Asia, Africa and Mediterranean basin. TYLCV genome consists of a circular single stranded DNA of 2.7 kb in size and occasionally contains beta satellite of 1.3 kb. TYLCV genome encodes six open reading frames, of which two are in the viral orientation and four are in the complementary orientation. TYLCV is transmitted by the insect vector Bemisia tabaci, commonly known as the silverleaf white fly. This review provides an overview of Geminiviruses with special emphasis on TYLCV, virus vector relationship between TYLCV and white fly, different strains of TYLCV, its genome organization and replication, present status of its spread in different parts of the world and strategies employed for controlling it. The knowledge about the recent progress in the study of TYLCV would help develop novel strategies for its control in agriculture.

Keywords: TYLCV; Whitefly; Tomato; Geminivirus; Rolling circle replication; RNAi

Introduction
Tomato is one of the most widely grown vegetables globally having potential health benefits even more than that of apple. Lycopene, a flavonoid antioxidant, is one of the unique phytochemicals present in tomato [1]. Antioxidants present in tomato are scientifically proven to provide protection against oxidative damage thus slowing the ageing process. They have been found to confer protection against different types of cancers also. According to the Food and Agriculture Organization (FAO), global tomato production reached 159 million metric tons in 2011, area harvested was 4734356 hectares, yield in hектogram per hectare was 335892, while global trade was 16556760 (1000 US $) in 2010 (FAO stat 2010-11). However, because of its perennial growth on a large scale, tomato is susceptible to a number of pathogens including bacteria, fungi and viruses. Amongst these, Geminiviruses causes great loss in yield of tomato production globally.

Geminiviruses
Geminivirus is one of the most devastating pathogens for tomato agriculture in the tropics and subtropics. Frequent recombination of different geminiviruses coinfecting a plant leads to the development of novel, possibly virulent viruses resulting in epidemics of geminivirus diseases. Geminiviruses have characteristic genomes being circular, single-stranded DNA within twinned quasi-isometric geminate virions from which they derive their name [2]. Geminiviruses have been classified into following seven genera based on their genome organization, hosts, and insect vectors [3].

1. Genus Becurtovirus; type species: Beet curly top Iran virus; transmitted by leafhoppers to dicotyledonous plants
2. Genus Begomovirus; type species: Bean golden mosaic virus; transmitted by whiteflies (Bemisia spp.) to dicotyledonous plants
3. Genus Curtovirus; type species: Beet curly top virus; transmitted by leafhoppers or treehoppers to dicotyledonous plants
4. Genus Eraviruses; type species: Eraviruses; infects monocotyledonous plants
5. Genus Mastrevirus; type species: Maize streak virus; transmitted by leafhoppers, usually to monocotyledonous plants
6. Genus Topocurvirus; type species: Tomato pseudo-curly top virus; transmitted by treehoppers to dicotyledonous plants
7. Genus Turncurvirus; type species: Turnip curly top virus; infects dicotyledonous plants

Among begomoviruses, monopartite Tomato leaf curl virus (TYLCV) and bipartite Tomato leaf curl virus (ToLCV) are the main viruses causing major loss to tomato production. Symptoms associated with TYLCV includes, reduction in leaf size, upward leaf curling, yellowing of young leaves, together with stunted growth and flower abortion [4,5]. Figure 1 shows a tomato plant infected with TYLCV. Host of TYLCV include many plant species such as Acanthaceae, Asteraceae, Canicaceae, Euphorbiaceae, Fabaceae, Malvaceae, Oxalidaceae, Pedaliaceae, Plantaginaceae and Solanaceae. In 2005, three new hosts were identified for TYLCV from Tanzania viz, Achyranthesaspera, Euphorbia heterophylla and Nicandraphysalloides [6].

Virus - Vector Relationship
Bemisia tabaci (silver leaf whitefly), belonging to the family Aleyrodidae, is the natural vector of TYLCV. It transmits TYLCV in a persistent-circulative nonpropagative manner [7]. TYLCV virions are

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TYLCV vertically [13].

Different Strains of TYLCV

Two strains of TYLCV have been identified based on the severity of infection. The mild strain occurred in Israel and Spain, whereas the more widely spread severe one was found in Caribbean and Southern United States [14].

Besides TYLCV, the TYLCV like viruses include Tomato yellow leaf curl Sudan virus (TYLCSDV), Tomato yellow leaf curl Axarquia virus (TYLCAXV), Tomato yellow leaf curl Malaga virus (TYLCMLV), Tomato yellow leaf curl Sardinia virus (TYLCSV), Tomato yellow leaf curl Mali virus (TYLCMLV), Tomato yellow leaf curl China virus (TYLCCNV), Tomato yellow leaf curl Guangdong virus (TYLCGuV), Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV), Tomato yellow leaf curl Thailand virus (TYLCTHV) and Tomato yellow leaf curl Vietnam virus (TYLCVNv; [15]).

Genome Organization of TYLCV

Single stranded circular DNA genome of TYLCV with a size of 2787 nucleotide encodes six partially overlapping open reading frame (ORFs). These ORFs are bidirectionally arranged into two transcriptional units that are separated by an intergenic region (IR) of approximately 300 nucleotides. Two ORFs viz v1 and v2 are in viral sense orientation whereas four ORFs c1, c2, c3, c4 are in complementary orientation [3]. The intergenic region harbors important elements for replication and transcription of the viral genome. Figure 2 show the TYLCV genome organization.

V1 Protein

The V1 protein having molecular weight of 29.9 kDa and 260 amino acids in length is encoded by the v1 gene located on the sense strand of the viral genome. This is the coat protein (CP) of the virus [2,16]. Function of CP is encapsidation of ss-DNA and formation of the virus particle which protects the viral DNA during transmission by the insect vector [17]. A single point mutation in TYLCV-CP cause loss of infectivity or reduces whitely transmissibility indicating that CP is absolutely essential for infection of tomato plants [18]. It also interacts with the proteins which function in the synthesis of cell wall polysaccharides in the tomato plants to facilitate the invasion and cell-to-cell movement [19].

V2 Protein

The V2 protein having molecular weight 13.4 kDa and 116 amino acids in length is encoded by the v2 gene, which is also present on the sense strand of the viral genome. This is also known as the pre coat protein. This gene is a pathogenicity gene as it enhances the severity of infection and hence symptom development [16,20]. Recently, it was shown that V2 protein of TYLCV-Isr suppressed gene silencing by
interacting with the cytoplasmic bodies of the host cell SGS3 protein which is involved in gene silencing [21,22].

**C1 Protein**

It has a molecular weight of 41 kDa, 357 amino acids in length and is encoded by ORF c1 which is on complementary or antisense strand of viral genome. This protein is known as replication protein (Rep) as it is involved in viral replication [23]. It recognizes a specific sequence on viral DNA i.e., origin of replication, binds there, cleaves it for DNA replication and ligates it to the initial formation after completion of replication. This function is performed by the amino terminal domain of the protein [24] whereas the carboxy terminus has a nucleoside triphosphate binding domain required for replication [25]. Moreover, c1 in planta can also raise hypersensitive responses restricting the viral construct to the sites of infection [20].

**C2 Protein**

It has a molecular weight of 15.6 kDa, 134 amino acids in length and is encoded by ORF c2 which is again present on the complementary or antisense strand. This protein resides in the nucleus of host-plant cell and determines pathogenicity [26]. The N terminal half is basic in nature while the C terminal half is acidic, having a role in transcriptional activation. A zinc finger motif lies between the two terminals function as a suppressor of postranscriptional gene silencing (PTGS) in plant cells.

**C3 Protein**

It has a molecular weight of 15.9 kDa, 134 amino acids in length and encoded by c3 gene. Its position is on the complementary or antisense strand of viral genome. It is the geminivirus replication enhancer (Ren) protein. It interacts with C1 protein, resulting in enhancement of viral DNA accumulation, enhance infectivity and symptom expression [27,28].

**C4 Protein**

It has a molecular weight of 10.9 kDa, 98 amino acids long and is encoded by c4 gene present on the complementary strand. This protein is involved in the development of disease symptoms during viral infection [29].

**Replication of TYLCV**

Replication in case of TYLCV follows rolling circle replication (RCR) [30]. Replication occurs in three stages:

- **Stage I:** Viral single stranded DNA enters into the cell and is RNA primed replicated into covalently closed double stranded DNA replicative form (RF).

- **Stage II:** It involves synthesis of additional RF DNA in which the original RF serves as template.

- **Stage III:** This stage of RCR, which occurs late in the replication cycle, is responsible for the accumulation of viral genomes for encapsidation (RF-SS synthesis).

TYLCV do not have polymerase activity of their own but they depend on host DNA polymerase. Viral proteins involved in replication serve functions such as specificity and initiation factors, replication enhancers, or as regulators of ssDNA versus dsDNA synthesis and/or accumulation.

Initiation of DNA replication requires Rep protein of virus encoded by c1 gene [31]. It recognizes and performs site specific nicking at nonamer sequence TAATATTAC [32]. It binds to adenine residue at 5' end [33]. Nicking and closing activities reside in the amino-terminal region of the protein [34]. C3 protein acts as replication enhancer. C2 protein regulates the replication process. V2 protein plays a direct role in regulating a switch from RF-RF synthesis to RF-SS synthesis. Intergenic region harbour the 13 bp sequence which contains two 5-bp direct repeats separated by a central core of 3 bp (5' - GGTAGTAAGGTAG). This specifically interacts with Rep protein and is inseparable for DNA replication of virus [35].

**Present Status of Spread of TYLCV**

Global trade in agricultural products is leading to the spread of many viruses. The prime example is TYLCV. Global spread of TYLCV started around eighties, soon after TYLCV-Mild and TYLCV-Israel strains developed. TYLCV undergoes inter and intra specific recombination which results in formation of new strains over the period of time. TYLCV is distributed globally but there is no evidence of Middle East and Western Mediterranean harbouring any epidemiologically relevant TYLCV variants arising from recombination. Present day TYLCV diversity region is around Iran where intensive recombination and TYLCV evolution is taking place. Since Iran is epidemiologically isolated so virus will not spread from here to other places and hence are not global threats. Mediterranean basin is recognized as the place from where the spread of new viruses is taking place to the world, creating a serious threat to tomato production [36].

Various strategies are adopted for TYLCV control and eradication to protect tomato plants. Some of these include:

**Physical and agronomic measures**

This strategy includes catching the whitely by hand, shovels, racquets or other tools and use of physical barriers like plastic membrane, net-house or insect preventing net to protect immature plants. Crop rotation is also used to break the inhabitance of insects.

**Use of inorganic insecticides**

Arsenic compounds, copper compounds or lead compounds are used, although they cause serious ailments in people. DDT was used to control the vector and showed significant success at the beginning but later development of resistance to these insecticides developed. B. tabaci developed resistance in Israel due to extensive use of insecticides [37].

**Use of biological insecticides**

Raw crushed plant leaves, extracts of plant parts, and chemicals purified from the plants are used to control pests. Pyrethrum, neem, tobacco, garlic, and pongamia formulations are most commonly used biocontrol agents [38].

**Breeding for resistance**

Resistance to disease is sought by breeding methods which can be breeding for resistance to insect vectors and breeding for resistance to virus.

The Mi-1 gene from wild tomato S. peruvianum is introduced into many varieties of cultivated tomato (S. lycopersicum). It confers resistance to both B- and Q-biotype of B. tabaci in 2-month-old tomato plants [39]. Progress in the breeding for TYLCV resistance has been slow, due to the complex genetics of the resistance and the presence of inter-specific barriers between the wild and domesticated tomato species [40].
Transgenics against TYLCV

Transgenic tomato plants can be developed using genetic engineering which will give resistant plants to TYLCV. This can be achieved using transgenic plants expressing coat protein from monopartite TYLCV exhibiting delayed symptom development. This is dependent on the expression levels of transgenic CP [41]. Truncated Rep gene which is capable of expressing the N terminal 210 amino acids of the Rep protein is used for developing resistance against TYLCV. Transgenic plants expressing truncated Rep Protein from Tomato yellow leaf curl Sardinia virus (TYLCVSY) resulted in inhibition of virus replication in protoplasts and induction of resistance when expressed at high levels. Antisense technology is used to prepare transgenic tomato which will offer resistance to TYLCV. RNA-mediated resistance was engineered against the monopartite TYLCV in tomato [42]. Antisense technology is based on blocking the information flow from DNA via RNA to protein by the introduction of an RNA strand complementary to the sequence of the target mRNA.

RNA interference approach

RNA interference (RNAi) phenomenon is the guiding activity of small RNAs for RNA induced silencing complex (RISC) to slice the corresponding RNA transcripts, thus, leading to the silencing of the genes. Methylation of a Tomato leaf curl virus (ToLCV)-derived transgene promoter and consequent transgene silencing have been observed on ToLCV infection Seemannpili et al. [43] suggesting that virus-derived siRNAs are also generated during geminivirus infection. Nowadays research is going on to develop artificial micro RNA for providing resistance against bipartite ToLCV which can be similarly used for TYLCV [44].

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