Tomato yellow leaf curl disease and plant–virus vector interactions

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ABSTRACT

Tomato yellow leaf curl virus (TYLCV) (Begomovirus, Geminiviridae) is the type member and representative of the complex of viruses associated with the tomato yellow leaf curl disease (TYLCD) with ssDNA genome, a plant-infecting group of viruses that have single or double genomic components enveloped by an icosahedral coat protein. These viruses infect tomatoes and other vegetable and ornamental crops and cause severe losses estimated by billions of dollars each year. Begomoviruses are exclusively transmitted by the whitefly \textit{Bemisia tabaci} in a persistent circulative manner. First epidemics of TYLCV were reported in Israel in the early 1960s and later on the causative agent was identified as TYLCV. Epidemics were often associated with the presence of whiteflies. Since then, extensive research in many laboratories in the world was conducted to better understand the interactions between TYLCV, the tomato plant and its only vector \textit{B. tabaci}. These studies resulted in hundreds of research papers and reviews, and in creating new research disciplines unraveling geminiviruses and their interactions with plants and whiteflies. In this review, we will give the readers an overview presenting the emergence of this field of research, main discoveries that have been made, current research disciplines that are being conducted and future research lines that will bring novel opportunities for controlling TYLCV and whiteflies and preventing their damage.

Keywords: TYLCV, \textit{B. tabaci}. Tomato, virus, transmission, gene silencing

1. INTRODUCTION

Tomato yellow leaf curl disease, limiting disease for tomato production: A historic perspective

During the 1960s a new disease reported in the Jordan valley in Israel caused severe damages to a newly introduced tomato variety to the market. This disease was later called tomato yellow leaf curl disease (TYLCD) (Cohen and Nitzany, 1966). Tomato yellow leaf curl virus (TYLCV) was found to be the causative agent of this disease and was associated with outbreaks of the whitefly \textit{Bemisia tabaci} (Gennadius) populations nearby cotton fields, which were newly grown in this area. These cotton fields helped \textit{B. tabaci} populations to build up to high levels, and outbreaks of the disease were seen afterwards. Although symptoms of TYLCD on plants were observed as early as the 1930s, outbreaks of the disease were not observed until \textit{B. tabaci} populations greatly increased. TYLCV virus was observed as having geminate shape in 1980 (Russo et al., 1980), and a few years later the viral genome was fully cloned and sequenced, and the virus was shown to be a monopartite geminivirus (Navot et al., 1991). Since the late 1990s research regarding TYLCV focused on understanding the interactions between TYLCV, plants that it infects, and its only vector, \textit{B. tabaci}. Research on virus–plant interactions included understanding virus movement,
symptoms induction, replication, and plant response to the virus, while on the virus-vector front research was aimed to understand mechanisms of acquisition, retention, and transmission of TYLCV by *B. tabaci*. TYLCV is known today to occur in several continents around the globe, including Asia, Africa, Europe, and North America (Czosnek and Latterot, 1997). The only vector in all countries is *B. tabaci* and epidemics are often associated with a rise in populations of this vector.

2. TYLCV–*B. TABACI INTERACTIONS*

2.A. Appearance of geminiviruses and *B. tabaci*

*B. tabaci* was first described by P. Gennadius on poinset-tia plants in 1889 as *Aleyrodes tabaci*. A hundred years later, a new group of plant viruses was described and called geminiviruses (Hamilton et al., 1982; Goodman, 1981). They were identified as containing ssDNA genome and are of virions with geminate shape. *B. tabaci* was recognized as a set of sub-species based mainly on biology and host range, and during the 1980 the A biotype was the main biotype in the United States (Brown, 1994). A few years later, physiological disorders in vegetable crops such as silverleafing and irregular ripening were associated with *B. tabaci* (Shuster et al., 1991). However, only in 1990 a survey in Arizona concluded that the B biotype displaced the A biotype; 70% of the populations collected in Arizona were B, and were mainly responsible for these physiological disorders. During the same year, severe cassava mosaic virus was reported from Uganda, associated with whitefly populations outbreaks (Legg and Fauquet, 2004). These populations were later described as belonging to the B biotype. During the 1990s several esterase morphotypes markers were developed to distinguish between the B and the A biotype, and based on these markers the B biotype was reported to spread rapidly (Costa et al., 1993). Based on these markers several “biotypes” were described based on biology and using the esterase morphotypes. The Q biotype of *B. tabaci* was first described as native to the Mediterranean Basin in 1997 (Guirao et al., 1997) and one year later TYLCV was first described on the United States east coast and Yucatan Peninsula (Ascencio-Ibáñez et al., 1999), then into Mexico (Brown and Idris, 2006) and Puerto Rico (Bird et al., 2001), and TYLCV displaced *tomato yellow leaf curl sardinia virus* (another virus species associated with TYLCD) in Spain. By this time reports also described the replacement of the B biotype in Spain by the Q biotype (Moya et al., 2001). A closely related Q was also described in Israel in 2005 (Horowitz et al., 2003, 2005) and another Q was introduced into the United States in 2005 although was still confined to greenhouses (McKenzie et al., 2009).

2.B. TYLCV interactions with *B. tabaci* and parameters for acquisition and transmission

TYLCV is only vectored by *B. tabaci* in a persistent circulative manner (Ghanim et al., 2001a). This transmission mode requires that the virus be acquired from the phloem of an infected plant through the insect stylet, pass through the esophagus to the midgut, cross the mid-gut to the haemolymph, circulate in the haemolymph, and reach the salivary glands from which it is ingested into the plant phloem again (Ghanim et al., 2001a,b). Parameters for acquisition and transmission were extensively studied (Ghanim et al., 2001a) and exact times of virus translocation in the insect were determined. Unlike other geminiviruses, TYLCV has unique interactions with its vector, and studies have shown that the virus is transovarially transmitted from females to their offspring through the egg (Ghanim et al., 1998), and it can be transmitted between males and females during sexual intercourse (Ghanim and Czosnek, 2000) in the absence of any source of the virus. These modes of transmission were not described for any plant virus so far. The latent period of the virus in the vector lasts between 8–24 h (Ghanim et al., 2001a), and once the virus circulates and passes the latent period, it can be transmitted to a new plant after 5 min of inoculation access period (IAP) (Atzmon et al., 1998). The virus can also be acquired from an infected plant after 5 min of acquisition access period (AAP) (Atzmon et al., 1998).

2.C. *B. tabaci–TYLCV* anatomical and molecular interactions

TYLCV is acquired as a virion from the plant phloem as a result of different pressure values between the plant cell and the opening–closing cibarial pump in the insect mouth apparatus (Ghanim and Medina, 2007). The virions pass along the food canal in the stylet with sugars and other substances form the phloem and reach the esophagus. The esophagus is a chitin-lined tissue that does not allow food/virion penetration to the haemolymph (Ghanim et al., 2001b). The first tissue through which virions can cross to the haemolymph is a modification of the digestive system called the filter chamber (Ghanim et al., 2001b). The filter chamber is a complicated structure that combines tissue from the midgut, hindgut, and the caeca. Membranes from these organs interdigitate to form this complicated structure that insures direct absorption of “pure” useful substances for the insect into the haemolymph, while more “complicated” food is pushed into the descending midgut by the muscular caeca. It is hypothesized
that the majority of the TYLCV virions are absorbed from the filter chamber into the haemolymph, while a minority of the virions circulate into the descending then the ascending midgut, and cross the midgut epithelial cells to the haemolymph (Ghanim and Medina, 2007). Recent studies using localization methods have shown extensive location of TYLCV virions in the filter chamber area, and their concentration decreases toward the descending and the ascending midguts (Ghanim et al., 2009, and Fig. 1). Unlike aphids and luteoviruses, TYLCV virions cross the epithelial cells in the midgut and not hindgut, and the specificity resides in this area of the digestive system (Czosnek et al., 2002). In the haemolymph, TYLCV virions interact with a 63 KDa GroEL protein produced by the endosymbiotic bacteria of B. tabaci, which protects the virions from proteolysis by the insect’s immune system (Morin et al., 1999, 2000). Virions cross the digestive system into the haemolymph within 1 h, while reaching the digestive system from the stylet lasts 40 min (Ghanim et al., 2001a). The virions circulate in the haemolymph and are detected in the primary salivary glands after 7 h from the AAP. A second recognition barrier is thought to reside on the apical membrane of the primary salivary gland of B. tabaci (Brown and Czosnek, 2002), unlike the aphid-luteovirus system in which recognition resides in the accessory salivary glands (Gildow and Gray, 1993; Gildow and Rochow, 1980). Once the virions reach the secretory salivary cells in the primary salivary glands, they are secreted with the saliva into the salivary duct, and then to the salivary canal in the stylet, from where they are injected into the plant. Not much is known about the molecular interactions between TYLCV and B. tabaci. Different studies were aimed to address replication of TYLCV in B. tabaci, and the popular view is that TYLCV and geminiviruses do not replicate in their vectors. One study showed accumulation of TYLCV transcripts in B. tabaci after acquisition from infected plants but not tomato mottle virus (ToMoV) (Sinisterra et al., 2005).

2.D. Recent advances in B. tabaci—TYLCV research

The last five years have witnessed a noticeable increase in the publications that reported the development and use of genomic resources for B. tabaci, with the aim of expanding the methods used to study B. tabaci interactions with biotic and abiotic stress factors such as viruses, plants, and other factors that influence the whitefly’s development. Most notably, a genomic project was launched in 2002 and has sequenced more than 20,000 Expressed Sequence Tags (ESTs) from the adult whiteflies, as well as other developmental stages including nymphs, eggs, and viruliferous adults with TYLCV and tomato mottle virus (ToMoV) (Loshkowitz et al., 2006). Efforts are still underway to sequence more ESTs.
from the whitefly, and a group of researchers around the world has gathered to raise funding for the first whitefly genome sequencing project. However, enough funding for this genome sequence have not yet been secured. This will not be the first hemipteran genome to be sequenced, since the genome sequence of the pea aphid *Acyrthosiphon pisum* has been recently completed and mostly annotated (personal communication), and other model insects such as fruit flies, mosquitoes, the honeybee have been sequenced and extensively studied. This large-scale sequencing of ESTs from *B. tabaci* led to better understanding the genetic makeup of the whitefly relative to other insect models. It was estimated that the genome of the whitefly is about five times the genome of *Drosophila melanogaster* (Brown et al., 2005). Following this sequencing, a spotted DNA microarray containing 6,000 unique ESTs from the whitefly was developed and used to study the response of the whitefly to insecticides (Ghanim and Kontsedalov, 2007), its response to parasitoids (Mahadav et al., 2008), and to heat stress conditions in the B and the Q biotypes (Mahadav et al., 2009). Recent studies using an advanced version of this microarray, which was prepared based on Agilent’s technology, are aimed to study *B. tabaci* response to feeding on plants modified with the contents of defense materials, response to modified contents of nicotine in tobacco plants, and response to the presence/absence of symbiotic bacteria. A recent study has demonstrated that the RNA interference (RNAi) machinery which was shown to be an effective mechanism for silencing mRNA in many organisms including insects was also shown to be active in *B. tabaci* (Ghanim et al., 2007). Although many of the described studies are still underway, the path to considering *B. tabaci* as a genome-enabled organism like model insects which have rich genomic resources is still long, and the efforts to make this insect a genome-enabled one are at their beginning.

### 3. TYLcV–PLANT INTERACTIONS

#### 3.A. Movement and localization of TYLcV in plants

TYLcV is released into the plant phloem by *B. tabaci*. After enough cycles of replication, it invades most of the plant tissues including roots, and symptoms are usually visualized within 2 weeks or less, depending on the environment and the infected host. Transmission Electron Microscopy (TEM) was the first method to be used to visualize virions in the plant tissues (Russo et al., 1980), but since this method is laborious and analyzes small tissue samples, new methodologies have been used over the years to follow the presence of virions and their transcripts in the plant. Most notably, methods such as in situ hybridization using radiolabeled or biotinylated probes and subsequent detection methods were used. To visualize virions, methods for virion preparations from infected host plants were developed and clean virion preparations were observed after negative staining under TEM (Czosnek et al., 1988). Other methods that were used are in situ PCR and immunological detection using specific antibodies, such as the localization of the coat and the C4 proteins to the phloem using specific antibodies (Rojas et al., 2001). Recently, we described a new and relatively easy and inexpensive method for localizing TYLcV in infected tomato plants (Ghanim et al., 2009). The method, based on using short DNA oligonucleotides that are complementary to an RNA sequence from one of the virus transcripts that is modified to harbor a fluorescent molecule on its 3′ or 5′ end,
they are released into the plant, they enter as virions or in a mode of replication inside the plant host. Once TYLCV and other geminiviruses follow almost the same mode of replication. Recent studies also showed the presence of begomoviral DNA in phloem, cambium, and xylem tissues (Rasheed et al., 2006). Although it is accepted that in the infected plant, TYLCV remains confined to the phloem, and encapsidation, a process which may take several days and reach a peak at 11–13 days post inoculation, virions are transported via sieve cells in long-distance movement supported by TYLCV CP, and symptoms are seen after another 2–4 days (Michelson et al., 1997). TYLCV CP mutants were unable to perform such long-distance movement and the infection cycle was affected (Noris et al., 1998). Although it is accepted that in the infected plant, TYLCV remains confined to the phloem, Michelson et al. (1997) showed that viral DNA could be detected in mesophyll tissues and parenchyma cells, maybe as a result of tissue collapse or ageing. Several recent studies also showed the presence of begomoviral DNA in phloem, cambium, and xylem tissues (Rasheed et al., 2006).

### 3.B. Replication of TYLCV in the plant cell

TYLCV and other geminiviruses follow almost the same mode of replication inside the plant host. Once they are released into the plant, they enter as virions or ssDNA to the nucleus and form a chromatin for further replication, using a polymerase machinery in host cells. This chromatin is the dsDNA wrapped around 13 nucleosomes at maximum. For interactions with factors that drive transcriptions and translations, this chromatin is opened at certain genomic points. The mode of replication is similar to phages and uses a rolling circle mechanism (Jeske et al., 2001). However, since begomoviruses have other intermediate replicating DNA types, it has been observed that these viruses can use other modes of replication: complementary strand replication and recombination-dependent replication (Jeske et al., 2001). The intergenic region of begomoviruses was found to be the initial site in which replication of the ssDNA to dsDNA starts (Donson et al., 1984). After dsDNA is formed, rolling circle replication is used to form ssDNA from dsDNA, while complimentary replication is a mechanism in which rolling circle intermediate products which are not complete are completed by complementary strand replication. The third mode of replication, known as recombination-dependent, is used for repairing ssDNA molecules that do not form properly as a result of improper polymerization or as a result of attack by the plant nucleases. The replication starting site for many geminiviruses, called “replisome”, is the site recognized by the Replicase (Rep) protein of the virus. It is located within 200 bp of the common region of bipartite viruses, or the intergenic region in monopartites (Hanley-Bowdin et al., 1999). This region is easily melted and presented by a hairpin structure. Within this structure is located a defined sequence that is recognized by the Rep protein for the rolling circle replication. Rep is the only protein needed for the initiation of the replication; however, it can perform other tasks such as specific nicking of the DNA, autorepressing its own expression, and activating the DNA-dependent DNA polymerization in the host cell (Hanley-Bowdin et al., 1999). The molecular structure of the Rep protein was extensively studied and the protein was found to include a region specific for DNA binding, nicking, and joining, and another region responsible for ATPase and helicase activities. The Rep protein is known to interact with several plant host proteins (Castillo et al., 2003, 2004). Most interesting of these proteins is the retinoblastoma-related protein (pRBR), which was detected in animal cells as a tumor suppressor protein, and more generally is involved in cell cycle regulation (Durfee et al., 2000; Gutierrez, 2000). The pRBR protein normally interacts with the E2F transcription factor and inhibits the expression of many genes, but when the pRBR protein interacts with the Rep protein, the E2F protein is released and can activate S-phase-specific genes required for viral replication.
3.C. Plant resistance to TYLCV and the involvement of gene silencing

Begomoviruses, and especially TYLCV, have gathered great interest in terms of the damage and the epidemics they cause worldwide (Varma and Malathi, 2003). Thus many efforts were directed to develop plants resistant to these viruses (Vidavski, 2007). Other methods for controlling geminiviruses included managing the vectors of the viruses and eradicating the source of inoculums, such as weeds that can be infected with these viruses. Resistance to begomoviruses that was looked for included naturally-occurring resistance in wild tomato accessions as well as engineered resistance using viral genes expressed in transgenic plants. The latter approach was used in a variety of plants and against many virus families (Beachy, 1993). Many reports showed the effectiveness of using the CP of the virus to trigger resistance, as was shown when the V1 (CP) gene was expressed in tomatoes (Abel et al., 1986; Kunik et al., 1994), and the expression of the Rep protein in transgenic *Nicotiana benthamiana* plants (Noris et al., 1996). In all cases, resistance that was introduced using viral genes in transgenic plants did not persist, and the viral infection overcame the resistance. To solve this problem, another approach was undertaken and was based on expressing anti-sense RNA sequences directed against viral genes (Baulcombe, 1994). However, in these cases also the resistance was broken by the virus. After the discovery of the Post Transcriptional Gene Silencing (PTGS) mechanism in plants (Napoli et al., 1990) and the RNA interference (RNAi) mechanism in nematodes (Fire et al., 1998), researchers came to the conclusion that expressing exact molecules directed to destroy mRNA sequences is a better way to achieve stable resistance against many plant viruses. These mechanisms were later discovered in many organism including many plants, insects, and mammals. The RNAi mechanism is based on the activation of the RNA-dependent RNA polymerase (*RdRP*) process that directs anti-sense RNA polymerization to form dsRNA molecules with the target mRNA, and leads this dsRNA to be cleaved by a specific endoribonuclease called Dicer (Waterhouse et al., 1998). This cleavage produces small dsRNA fragments called small interfering RNA (siRNA), which are then directed by a RNA Induced Silencing Complex (RISC) to the target mRNA molecule for degradation. This mechanism appears to be an important way for self-protection against plant DNA and RNA viruses (Voinnet and Baulcombe, 1997; Poogin et al., 2003; Pruss et al., 2004). The approach of using viral sequences to induce RNAi in tomato plants against TYLCV was used by Yang et al. (2004). This group introduced sequences form the TYLCV genome in sense and anti-sense orientations into transgenic plants and tested their ability to block TYLCV infection after inoculation with high numbers of viruliferous whiteflies under field conditions. A number of transgenes that contained introduced sequences from the TYLCV genome showed high levels of resistance (Yang et al., 2004). A recent study used a construct that triggers PTGS against several viruses of the TYLCD complex based on conserved sequences of these viruses (Abhary et al., 2006). Transgenic assays conducted with this construct showed high levels of resistance and absence of viral DNA by hybridization and PCR after more than 3 weeks of inoculation with viruliferous whiteflies carrying the tested viruses. Furthermore, a positive correlation between resistance and the accumulation of TYLCV-specific siRNAs was observed in silenced plants (Abhary et al., 2006). Despite this very effective way to combat viruses by plants, some plant viruses appear to have developed defense strategies against PTGS (Voinnet, 2001; Baulcombe, 2002). Zrachya et al. (2007a) showed that the TYLCV V2 gene acts as a suppressor of gene silencing in the plant. This suppression allowed a Green Fluorescent Protein (GFP) transgene to be normally expressed in infiltrated tobacco leaves with a GFP transgene; however, other TYLCV genes did not perform the same action. Interestingly, this suppression had no apparent effect on the accumulation of GFP-specific short interfering RNAs (siRNAs), suggesting that TYLCV V2 targets a step in the RNA silencing pathway which is subsequent to the Dicer-mediated cleavage of dsRNA (Zrachya et al., 2007a). The same research group has analyzed the effect of siRNAs derived from a construct targeting the CP (V1) gene of TYLCV (Zrachya et al., 2007b). This study demonstrated that 7 weeks after inoculation with the virus, transgenic tomato plants with the construct expressing siRNAs against TYLCV CP did not show any disease symptoms, while non-transgenic control plants developed disease symptoms 2 weeks after inoculation with the virus (Zrachya et al., 2007b). A recent study showed that the TYLCV suppressor of gene silencing V2 protein interacts directly with the tomato *SISGS3* gene, a homologue of the *Arabidopsis SGS3* gene, which is known to be involved in the RNA silencing pathway. This interaction was necessary for inducing RNA silencing, because a point mutation in the V2 gene necessary for the interaction with *SISGS3* aborted the ability of the V2 gene to induce suppression of gene silencing (Glick et al., 2008).

4. CONCLUSIONS AND FUTURE DIRECTIONS

The past 50 years or so of investigating TYLCV–plant
and TYLCV—B. tabaci interaction have resulted in hundreds of research papers aimed at understanding the biological, molecular, and cellular events underlying these interactions and eventually finding a solution for controlling this disease and other viral diseases. Recent findings involving the RNAi mechanism and TYLCV V2 gene in these mechanisms, might lead to the development of tomato lines stably resistant to the virus. These technologies are made possible thanks to the discovery of the RNAi machinery in many organisms, which—although it includes using genetically modified organisms (GMO)—exhibits a great advancement toward overcoming the disease. Many questions remain to be answered in this context, such as whether the developed lines using these technologies will be stably resistant in the field under much more serious sources of inoculums provided by viruliferous whiteflies. On the ethical front, the question remains whether this approach will be acceptable in communities that oppose using GMOs. Recent projects have shown that silencing could be induced against an insect pest through plants that express siRNAs that target an insect gene. This was shown to be possible against some insects (Baum et al., 2007; Mao et al., 2007), and has yet to be proven against sap-sucking insects. If inducing silencing in an insect vector through the plant is possible, transmission of TYLCV, and other plant viruses that require an insect vector, could be interrupted by targeting an insect protein involved in the transmission process.

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