

The Viral Etiology of Tomato Yellow Leaf Curl Disease – A Review

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Abstract

GLICK E., LEVY Y., GAFNI Y. (2009): **The viral etiology of tomato yellow leaf curl disease – a review.** Plant Protect. Sci., **45**: 81–97.

Tomato yellow leaf curl disease (TYLCD) is one of the most devastating plant diseases in the world. As a result of its continuing rapid spread, it now afflicts more than 30 tomato growing countries in the Mediterranean basin, southern Asia, Africa, and South, Central and North America. The disease is caused by a group of viral species of the genus *Begomovirus*, family Geminiviridae (geminiviruses), referred to as *Tomato yellow leaf curl virus* (TYLCV). These are transmitted by an insect vector, the whitefly *Bemisia tabaci*, classified in the family Aleyrodidae. The genome of TYLCV generally consists of a single circular single-stranded (ss) DNA molecule, with only one exception in which two components were identified. It encodes six open reading frames, only one of which codes for the coat protein (CP) that represents a building block of the viral particle. TYLCV, like all other members of the Geminiviridae, has geminate particles, apparently consisting of two incomplete T = 1 icosahedra joined together to produce a structure with 22 pentameric capsomers and 110 identical CP subunits. Close to 50 years of intensive research into TYLCV epidemics has been conducted to find solutions to the severe problem caused by this virus. To date, breeding for resistance appears to be the best approach to controlling this disease, although only partially resistant varieties are commercially available. Since the virus consists of a ssDNA that replicates in the host-cell nucleus, the molecular mechanisms involved in its nuclear import have been the focus of our studies in recent years and results, as well as prospects, are discussed in this review. In addition, we describe our recent finding of a suppressor of gene silencing encoded by one of the TYLCV-Isr genes. This paper provides an overview of the most outstanding achievements in TYLCV research that may lead to more effective control strategies.

Keywords: TYLCV; tomato; geminivirus; ssDNA; whitefly; PTGS

1. Introduction

Tomato (*Solanum lycopersicon*) is one of the most widely grown vegetables in the world grown for its edible fruit. The cultivated tomato originates from wild plants found in the Andean regions of Chile and Peru. The tomato was first domesticated in Mexico and it is believed that the Spanish explorer Cortez may have been the first to transfer it to Europe in the mid 16th century. It was grown for

the beauty of its fruit, which was not often eaten. It was only in the 20th century that its importance as an edible fruit emerged. Today, tomato is grown in practically every country in the world in outdoor fields, greenhouses and nethouses. The tomato plant is very versatile and the crop can be divided into two categories: fresh market tomatoes and processing tomatoes. The latter are grown only outdoors and are mechanically harvested for the canning industry. According to the Food and Agri-

culture Organization (FAO) of the United Nations, global tomato production (processing and fresh) reached 110 million metric tons in 2003, while global trade increased to \$4.3 billion. However, due to its continuous large-scale production throughout the year, it has become susceptible to a number of pathogens, limiting its production. Apart from a number of bacterial and fungal pathogens which cause severe infections on tomato, it is infected by a number of viruses. Among the viral pathogens, whitefly-transmitted geminiviruses have become the most important in the tropics and subtropics. In this virus family (the Geminiviridae), viruses are distinct in having genomes of circular, single-stranded DNA contained within twinned quasi-isometric (“geminata”) virions from which they derive their name. According to STANLEY *et al.* (2005) and FAUQUET and STANLEY (2005), this family is divided into four genera (*Mastrevirus*, *Curtovirus*, *Topocuvirus*, *Begomovirus*) based on the organisation of their genomes, biological properties, type of insect vector (either whitefly, leafhopper or treehopper) and host range (either mono- or dicotyledonous hosts). The largest group in the family belongs to the genus *Begomovirus*, named after its type member *Bean golden mosaic virus*. In 2008 Fauquet and coworkers published updated list of geminiviruses species including 672 characterised and/or described isolates of begomoviruses 200 of which have been reported as pathogens of tomato (FAUQUET *et al.* 2008). The most destructive disease of tomato is caused by the *Tomato yellow leaf curl virus* (TYLCV). TYLCV is the generic name given to a complex of viral species occurring in tropical and subtropical regions that cause severe disease in economically important crops, including tomato, with yield losses of up to 100%. In the Mediterranean basin, based on sequence comparisons, two species of TYLCV are present and have been formally recognised as such by the International Committee on Taxonomy of Viruses [ICTV; (RYBICKI *et al.* 2000)]. They are: *Tomato yellow leaf curl virus-Israel* (TYLCV-Isr) (NAVOT *et al.* 1991) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) (KHEYR-POUR *et al.* 1991). Both species cause severe disease in tomato; however, TYLCV-Isr is currently the most prevalent species in Europe, also affecting pepper (*Capsicum annum*) and probably common bean (*Phaseolus vulgaris*).

Most of the wild tomato species, such as *Lycopersicon chilense*, *L. hirsutum*, *L. peruvianum*

and *L. pimpinellifolium*, are symptomless carriers (ZAKAY *et al.* 1991). Weeds such as *Datura stramonium* and *Cynanchum acutum* present distinct symptoms, while others, such as *Malva parviflora*, are symptomless carriers.

With the discovery and characterization of a growing number of viruses, and the growing availability of sequence data, virus taxonomy has become progressively more complex, and this is particularly true for geminiviruses. A review article written by FAUQUET *et al.* (2008); provides a recent update on geminivirus taxonomy and classification. Many viruses are referred to generically as tomato yellow leaf curl, although they are known to differ from one another. Therefore, the newly proposed nomenclature suggests adding the location from which the virus was isolated.

Based on the new taxonomy, other species of TYLCV have been characterised in Yemen (TYLCYV), Saudi Arabia (TYLCSAV) and East Asia: TYLCV-C from China (LIU *et al.* 1998) and TYLCTHV from Thailand (ROCHESTER *et al.* 1994), the latter being the only TYLCV that has two genomic components (designated DNA-A and DNA-B). Some related whitefly-transmitted viruses infecting tomato are also called *Tomato leaf curl virus* (ToLCV), and have been found in India and Australia. Whereas ToLCV isolates from Australia, Taiwan and Southern India (Bangalore) have a single genomic component (DNA-A), those from northern India have two (MUNIYAPPA *et al.* 2000). Although cases of recombination between ToLCV and TYLCV have not been reported to date, this possibility should not be ruled out as such a recombination could have a tremendous impact on the severity of the disease. Today, TYLCV is present in most Mediterranean countries and parts of sub-Saharan Africa, Asia, Japan, Australia, Central America, Mexico, and the Caribbean Islands (Table 1). It has also been reported locally in the US, with early reports coming from Florida and Georgia (POLSTON & ANDERSON 1997), and later ones, as the disease continues its spread, coming from Mississippi and North Carolina (Table 1). The disease caused by TYLCV has seriously hampered tomato cultivation and production in India (VASUDEVA & SAMRAJ 1948; BANERJEE & KALLOO 1987; SAIKIA & MUNIYAPPA 1989), where it is widespread in tomato during the summer season in southern India and during the autumn in northern India. In southern India, disease incidence in susceptible cultivars increases rapidly to 100%,

Table 1. Geographical Distribution of *Tomato yellow leaf curl virus*

Country	Type	Reference	Country	Type	Reference
Asia			Europe		
Israel	TYLCV-Isr	AVIDOV (KLEIN) (1940)	Cyprus	TYLCV-Isr	IOANNOU (1985)
Jordan	TYLCV-Isr	MAKKOUK (1978)	<u>Italy</u>		
Lebanon	TYLCV-Isr	MAKKOUK (1976)	Sardinia	TYLCSV	CZOSNEK <i>et al.</i> (1990)
Turkey	TYLCV-Isr	NAVOT <i>et al.</i> (1989)	Sicily	TYLCSV	CREDI <i>et al.</i> (1989)
Saudi Arabia	TYLCSAV	MAZYAD <i>et al.</i> (1979)	Sicily	TYLCV-Isr	ACCOTTO <i>et al.</i> (2003)
Iraq	TYLCV-Isr	MAKKOUK (1978)	Apulia	TYLCSV	SIALER <i>et al.</i> (2001)
Yemen	TYLCYV	BEDFORD <i>et al.</i> (1994)	<u>Spain</u>		
India	ToLCV	VERMA <i>et al.</i> (1975)	Canary Islands	TYLCV-Isr	FONT <i>et al.</i> (2000)
Taiwan	ToLCV	GREEN <i>et al.</i> (1987)	Greece	TYLCV-Isr	AVGELIS <i>et al.</i> (2001)
Thailand	TYLCTHV	CZOSNEK <i>et al.</i> (1990)	Portugal	TYLCV-Isr	LOURO <i>et al.</i> (1996)
China	TYLCV-C	LIU <i>et al.</i> (1998)	The Americas		
Japan	TYLCV-Isr	ONUKE <i>et al.</i> (2004)	Mexico	TYLCV-Isr	ASCENCIO-IBANEZ <i>et al.</i> (1999)
Iran	TYLCV-Isr	Accession AJI32711	Dominican Republic	TYLCV-Isr	POLSTON <i>et al.</i> (1994)
Australia	TYLCV-Isr	DRY <i>et al.</i> (1993)	Jamaica	TYLCV-Isr	MCGLASHAN <i>et al.</i> (1994)
Africa			Cuba	TYLCV-Isr	RAMOS <i>et al.</i> (1996)
Egypt	TYLCV-Isr	CZOSNEK <i>et al.</i> (1990)	Venezuela	TYLCV-Isr	ZAMBRANO <i>et al.</i> (2007)
Sudan	TYLCV-Isr	YASSIN and NOUR (1965)	<u>USA</u>		
Tunisia	TYLCSV	CHERIF and RUSSO (1983)	Florida	TYLCV-Isr	POLSTON <i>et al.</i> (1999)
Nigeria	TYLCV-Isr	DAFALLA (2004)	N. Carolina	TYLCV-Isr	POLSTON <i>et al.</i> (2002)
Namibia	TYLCV-Isr	NONO-WOMDIM (2004)	Puerto Rico	TYLCV-Isr	BIRD <i>et al.</i> (2001)
Swaziland	TYLCV-Isr	NONO-WOMDIM (2004)	Mississippi	TYLCV-Isr	INGRAM and HENN (2001)
Malawi	TYLCV-Isr	NONO-WOMDIM (2004)	Georgia	TYLCV-Isr	MOMOL <i>et al.</i> (1999)
Zambia	TYLCV-Isr	NONO-WOMDIM (2004)	Alabama	TYLCV-Isr	AKAD <i>et al.</i> (2007)
Kenya	TYLCSV	NONO-WOMDIM (2004)	Louisiana	TYLCV-Isr	VALVERDE <i>et al.</i> (2001)
Uganda	TYLCSV	NONO-WOMDIM (2004)			
Burkina Faso	TYLCSV	KONATE <i>et al.</i> (1995)			
Tanzania	TYLCSV	KASHINA <i>et al.</i> (2004)			
Morocco	TYLCV-Isr	PETERSCHMITT <i>et al.</i> (1999a)			
Venezuela	TYLCV-Isr	ZAMBRANO <i>et al.</i> (2007)			

causing yield losses exceeding 90% (SAIKIA & MUNIYAPPA 1989).

TYLCV symptoms (Figure 1) appear several weeks after infection and include severe stunting, a marked reduction in leaf size, upward cupping and chlorosis of the leaf margins, mottling, flower abscission and significant yield reduction (COHEN & ANTIGNUS 1994). The tops of infected plants may resemble a head of broccoli. Most (up to 90%) of the flowers abscise after infection, and thus few fruit are produced. The effects of TYLCV are very similar to those of the *Bean golden mosaic virus* in that if young plants are affected, it is highly likely that fruit will not set. TYLCV can affect

more hosts than ToMoV (*Tomato mottle bigemini-virus*), although this does not take into account crop plants other than tobacco which, like many of the weed hosts, does not show symptoms. In Israel, where tomatoes are grown in the field from the end of March to mid July, weed hosts bridge the gap between tomato seasons.

A number of review articles have comprehensively summarised various aspects of TYLCV biology (COHEN & ANTIGNUS 1994; PICO *et al.* 1996; NAKHLA & MAXWELL 1998; MORIONES & NAVAS-CASTILLO 2000; GAFNI 2003). A book edited by H. Czosnek from Rehovot, Israel (CZOSNEK 2007) has also been published recently covering

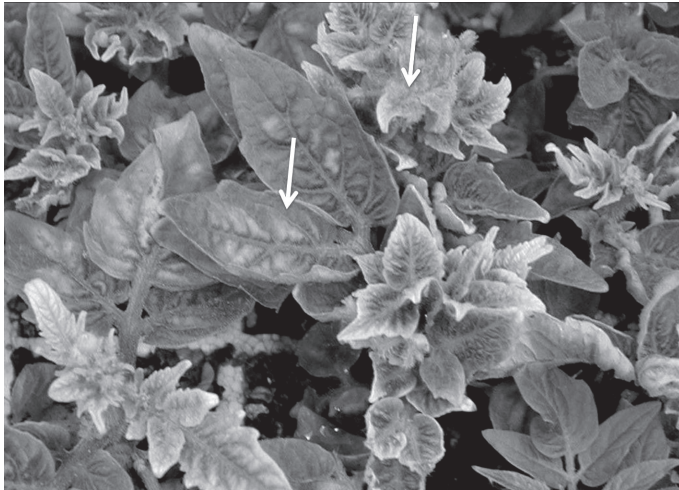


Figure 1. TYLCV-induced disease symptoms on tomato plants. Note the pale color of the upper young leaves (right side arrow) as well as some curling (left side arrow)

many aspects of the disease, the causative viruses and their vector. Nevertheless, accumulation of more new data on the biology of TYLCV and new reports on its spread, as well as recent reports on the introduction of new approaches to combating TYLCV epidemics, contribute to the timeliness of this review.

2. Economic impact

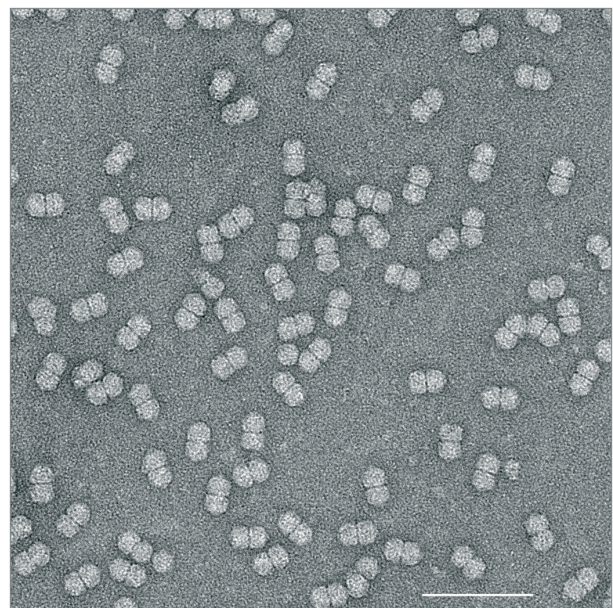
Reductions in tomato crop value have been associated with tomato yellow leaf curl disease (TYLCD) since it was first described by Avidov in the late 1930s in Israel, in association with outbreaks of the whitefly *Bemisia tabaci* [AVIDOV (KLEIN) 1940]. Twenty years later, in 1959, an entire tomato crop was destroyed by a disease with TYLCV-like symptoms in the Jordan Valley (COHEN & ANTIGNUS 1994). COHEN & HARPAZ (1964) published the first description of this new disease, transmitted by *B. tabaci*. It has since become an economically important disease, spreading, during the second half of the 20th century, to all of the countries in the Mediterranean basin, as well as Africa, Asia and the New World (Table 1).

Around 7 million hectares of crop plants in 40 countries are subjected to begomovirus attack by TYLCV or by mixed infections in 15 of those countries (MARTINEZ *et al.* 2003). The treatment in industrial countries includes mainly the use of insecticides against the insect vector and the introduction of more tolerant crop varieties. The implementation of physical barriers and growing tomatoes under greenhouse conditions has also cut the damage to an average 20%, conservatively estimated at more than \$300 million, in Europe and

the US (GIANESSI *et al.* 2002, 2003). The situation is much worse in developing countries, where the use of hybrid seeds and insecticides, as well as the practice of growing tomatoes only in greenhouses, are not options due to their high cost.

3. Virus structure

Like all other known members of the Geminiviridae, TYLCV is a small DNA virus characterised by its unique capsid morphology, which consists of double incomplete icosahedral virions (Figure 2). The TYLCV coat protein (CP) encapsidates a sin-



Bar = 100 nm (Courtesy of Katharina Kittelmann)

Figure 2. Electron microscopy image of purified begomovirus particles

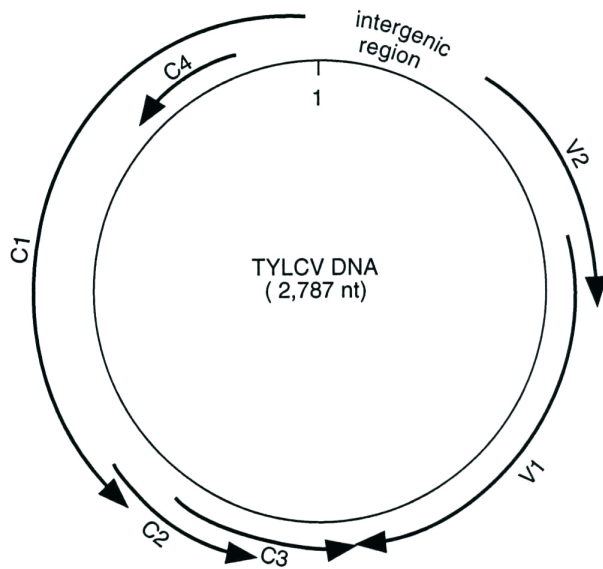


Figure 3. Genomic organisation of TYLCV. Open reading frames are designated V (viral orientation) or C (complementary sense orientation)

gle circular single stranded (ss) DNA genome of 2787 nt in size (Navot *et al.* 1991) (Figure 3). The geminate particles shown in Figure 2 are approximately 20 × 30 nm in size, made up of two incomplete icosahedra with a T = 1 surface lattice containing a total of 22 capsomers, each with five units of the 30.3-kDa CP (260 amino acids each, total MW of the particle 3 330 000). Early electron microscopic investigations revealed a first detailed model for *Chloris striate mosaic virus* (HATTA & FRANCKI 1979; FRANCKI *et al.* 1980), which was refined by electron microscopy and image reconstruction for the Nigerian strain of *Maize streak virus* (MSV) (ZHANG *et al.* 2001). Both viruses belong to the genus *Mastrevirus* (family: Geminiviridae), which comprises viruses infecting monocotyledonous hosts and vectored by different species of leafhoppers (RYBICKI *et al.* 2000). In contrast, TYLCV belongs to the genus *Begomovirus*, most members of which possess bipartite genomes, dicotyledonous hosts, and one whitefly species (*Bemisia tabaci* Genn.) as a vector. Detailed information on the geminate structure of *African cassava mosaic virus* (ACMV), a begomovirus, shows that there are differences between MSV and ACMV that are suggested to account for the alternative transmission mode (KITTELMANN & JESKE 2008). Studies aimed at revealing the structure of TYLCV are now in progress (AGBANDJE-MCKENNA, personal communication).

In the case of begomoviruses, ACMV, TYLCV and other, transmission by insects is dependent on the CP (BRIDDON *et al.* 1990; HOFER *et al.* 1997; HOHNLE *et al.* 2001), and it is therefore conceivable that the capsid structure may have been adapted to the different receptors of the particular insects.

3.1. DNA β

In the last decade, progress was made in etiological studies of TYLCV-related disease with the report of the existence of DNA β , a single-stranded circular satellite DNA molecule associated with *Tomato leaf curl China virus* (ToLCCNV) (ZHOU *et al.* 2003). Expression of the β C1 protein results in a considerable increase in symptom severity of the virus. This protein is suggested to act as a suppressor of gene silencing (CUI *et al.* 2005). The DNA β molecule is about 1.3 kb in length (depending on the type) and appears to encode a single protein with similarity to the replication-associated protein (Rep) of nanoviruses. Analysis of DNA β molecules revealed that except for a conserved hairpin structure and a TAATATTAC loop sequence, they show little similarity to either DNA-A or DNA-B molecules of begomoviruses. The DNA β requires a helper virus for replication and encapsidation (MANSOOR *et al.* 2003; BRIDDON & STANLEY 2006), the latter can be provided by the TYLCV CP when co-infected. It also requires the DNA-A of TYLCV for insect transmission and movement in plants. Co-agroinoculation of the DNA-A component of TYLCV with its associated DNA β showed its involvement in symptom induction in tobacco and tomato (ZHOU *et al.* 2003). DNA β has also been found to be associated with TYLCTHV (LI *et al.* 2004).

4. Genome organisation and protein functions

The ssDNA genome of TYLCV encodes six partially overlapping open reading frames (ORFs) that are organised bidirectionally (Figure 3): two of these ORFs (*v1* and *v2*) are in the virion sense orientation, and four of them (*c1*–*c4*) are in the complementary orientation. Between the two transcription units resides an intergenic region of about 300 nt which contains key elements for replication and transcription of the viral genome, organised in a typical iterative structure.

4.1. V1 protein

The V1 protein (Mr = 30.3 kDa; 260 amino acids) is encoded by the *v1* gene residing on the (+) strand of the viral genome. This protein is the CP, which represents the only known building block of the virus particle (LAZAROWITZ 1992). It is rich in arginine, valine, serine and lysine and expected to have a positive charge at neutral pH based on charge analysis. The CP of geminiviruses is involved in a number of processes during the life cycle of the virus. As already mentioned, its primary function is the encapsidation of ssDNA and formation of the virus particle to protect the viral DNA during transmission by the insect vector (AZZAM *et al.* 1994). The CP of monopartite geminiviruses is absolutely essential for viral movement (BOULTON *et al.* 1989). The CP of TYLCV is essential for infection of tomato plants, suggesting that monopartite geminiviruses move within the plant in the form of viral particles. Point mutations in TYLCV CP cause loss of infectivity or loss of whitefly transmissibility (NORIS *et al.* 1998). Analyses of the CP's homotypic interaction capacity have shown that the full-length CP has a strong tendency to interact with itself. To study the amino acids involved, mutations were introduced at positions 135 (replacing glutamine with histidine) and 153 (replacing aspartic acid with glutamic acid). These mutations caused an over 90% reduction in the CP-CP interaction (HALLAN & GAFNI 2001). Moreover, truncated versions of the CP at either the N or C terminus failed to interact with each other, suggesting that the interaction probably takes place between the N-terminal amino acids of one CP and the C-terminal amino acids of the other in dimer formation (HALLAN & GAFNI 2001). As is the case in all other known monopartite geminiviruses, a functional CP is also essential for host-plant infection and insect transmission. Two amino-acid replacements in the CP of TYLCV from Sardinia, Italy (proline and histidine for glutamine and glutamine in positions 129 and 134, respectively), abolished virus transmission by *B. tabaci* but not its ability to systemically infect plants (NORIS *et al.* 1998).

Thus far, no evidence exists to support suggestions that the CP possesses an enzymatic function; nevertheless, it is able to interact with other proteins. Interaction with karyopherin α was suggested to play a role in its nuclear import (KUNIK *et al.* 1999), interaction with whitefly GroEL in its

hemolymph was shown to be a necessary condition for circulative transmission (MORIN *et al.* 2000), and it was also shown to bind ssDNA, suggesting its role as a shuttle for the viral genome, targeting it to the plant-cell nucleus for transcription and replication (PALANICHELVAM *et al.* 1998).

4.2. V2 protein

The V2 protein (Mr = 13.5 kDa; 116 amino acids) is encoded by the *v2* gene, which also resides on the (+) strand of the viral genome. It is also referred to as the “pre-coat” protein. In another monopartite geminivirus, MSV, it has been shown to be involved in cell-to-cell viral spread (LAZAROWITZ *et al.* 1989), whereas in ToLCV, it is associated with the accumulation of ssDNA (RIGDEN *et al.* 1993). Therefore, *v2* of TYLCV is considered to be a “pathogenicity gene”, and its product to be involved in movement. Expression of the ToLCV *v2* gene in *Nicotiana benthamiana* was shown to cause severe stunting of the plant, suggesting a role in symptom development (SELTH *et al.* 2004). Recently, V2 protein of TYLCV-Isr was shown to exhibit suppression of gene silencing (ZRACHYA *et al.* 2007a) and to interact in cytoplasmic bodies with the host cell SGS3 protein which is involved in gene silencing (GLICK *et al.* 2008). Mutations introduced in the *v2* coding region eliminated the suppressive activity, indicating that this region's importance in pathogenicity might be, at least in part, due to its gene-silencing activity.

4.3. C1 protein

The C1 protein (Mr = 41 kDa; 357 amino acids) is encoded by the *c1* gene, residing on the (–) complementary strand of the viral genome. This protein is better known as the Rep protein, so called for its involvement in viral replication. Rep is the only viral protein that is absolutely required for viral DNA replication as it is responsible for this step during the rolling-circle amplification stage. The Rep protein exhibits sequence-specific DNA-binding activity (HEYRAUD-NITSCHKE *et al.* 1995; JUPIN *et al.* 1995), as well as site-specific endonucleolytic activity. It has also been suggested to be a member of a superfamily of helicases and proof for such activity has been provided for the TYLCSV Rep (CLEROT & BERNARDI 2006). The solution nuclear magnetic resonance (NMR) structure of the catalytic domain of TYLCSV Rep has

been published (CAMPOS-OLIVAS *et al.* 2002), making this the only geminivirus protein to date for which structural data of true atomic resolution are available.

4.4. C2 protein

The C2 protein (Mr = 15.6 kDa; 135 amino acids) is encoded by the *c2* gene, residing on the (–) complementary strand of the viral genome. This protein is a pathogenicity determinant and is localized in the nucleus of the host-plant cell (VAN WEZEL *et al.* 2001). It contains a novel zinc finger motif within its central core region and was suggested to function as a suppressor of post-transcriptional gene silencing (PTGS) in plant cells (VAN WEZEL *et al.* 2002b). Later, it was shown that the nuclear localization signal (NLS) which is essential for targeting the C2 protein to plant nuclei is required for this protein's induction of necrosis and suppression of PTGS (DONG *et al.* 2003).

4.5. C3 protein

The C3 protein (Mr = 15.9 kDa; 134 amino acids) is encoded by the *c3* gene, residing on the (–) complementary strand of the viral genome. This protein has been found to enhance viral DNA accumulation approximately 50-fold. C3 interacts with the plant-host proteins retinoblastoma-related (RBR) and proliferating cell nuclear antigen (PCNA) (CASTILLO *et al.* 2003), as well as with the virus encoded protein C1 (see above).

4.6. C4 protein

The C4 protein (Mr = 10.9 kDa; 98 amino acids) is encoded by the *c4* gene, residing on the (–) complementary strand of the viral genome. C4 is considered an important symptom determinant (KRAKE *et al.* 1998) and recently, it has been suggested to function together with C1 in the induction of necrosis in *N. benthamiana* (VAN WEZEL *et al.* 2002a), resembling a hypersensitive response.

5. Vector biology

Relationships between plant viruses and their insect vectors are complex and much more than passive associations (MATTHEWS 1991). Some plant viruses are carried in the insect's feeding apparatus

and can be acquired and inoculated within seconds or minutes (non circulative transmission). Others circulate through the body of the insect and once acquired, can be transmitted only after a latent or incubation period of hours to days (circulative transmission) (GRAY & BANERJEE 1999). TYLCV is transmitted by the whitefly *B. tabaci*. Whiteflies are small piercing and sucking insects of the family Aleyrodidae, order Homoptera, which have been associated with agriculture and with the transmission of plant viruses for many years (CZOSNEK *et al.* 2001). In the last 25 years, whiteflies have expanded their range tremendously from tropical and subtropical regions to more temperate ones, a change associated with the rising economic importance of begomoviruses worldwide (MOFFAT 1999). *Bemisia* is an ideal agent for viral spread because of its high rate of reproduction, its ability to disperse, and its obligate use of particular plants. There are several biotypes of whiteflies: those that transmit TYLCV do so in a circulative manner and they belong to the “B” group, which originated in the Middle East and was introduced into the New World in the early 1990s. Adults and crawlers (first instar) are the only stages during which *B. tabaci* is able to acquire and transmit TYLCV (MEHTA *et al.* 1994; COHEN & NITZANY 1966). The parameters of viral acquisition and transmission by adults have been studied in depth (COHEN & NITZANY 1966; ZEIDAN & CZOSNEK 1991; MEHTA *et al.* 1994; ATZMON *et al.* 1998). These studies and others have shown that even single insects are able to acquire TYLCV and transmit it to tomato plants. The minimum effective acquisition-access and inoculation-access periods are approximately 10 to 20 min each. The rate of transmission increases with longer acquisition- and inoculation-access periods. A minimum 8 h (latent period) from the beginning of acquisition is required for *B. tabaci* to be able to infect tomato test plants. In a one insect/one plant inoculation test, female *B. tabaci* were more efficient (~95%) than males (~25%). Viral DNA can be detected in single insects by PCR after 5 min of access feeding, and in tomato plants as early as 5 min after inoculation feeding (ATZMON *et al.* 1998). A GroEL homologue produced by the insect's coccoid endosymbionts is involved in the circulative transmission of the virus (MORIN *et al.* 1999). TYLCV is associated with the insect vector throughout its adult life. Insects that emerged during a 24 h period and were reared on a non-host plant after a 24 h acquisition

period retained TYLCV for their entire 35 to 40 day lives (RUBINSTEIN & CZOSNEK 1997). During that period, transmission rates decreased from 100% to 15%. Although the viral DNA could be detected throughout the insect's life, the capsid protein was undetectable after 12 days. The long term association of TYLCV with the insect led to reductions of ~20% in its life expectancy and of ~50% in the number of eggs laid (RUBINSTEIN & CZOSNEK 1997). TYLCV can be transmitted through the egg for at least two generations (GHANIM *et al.* 1998; GHANIM & CZOSNEK 2000). As the whitefly vector is now widely recognised for its importance in carrying many viruses with a huge impact on agriculture, a functional genomics project has been established in recent years aimed at constructing a *B. tabaci* sequence database which will provide an important tool for the identification of whitefly genes involved in development, behavior, and *B. tabaci* mediated begomovirus transmission (LESHKOWITZ *et al.* 2006).

6. Virus replication

Replication of TYLCV, like that of all members of the Geminiviridae, occurs in the nuclei of infected cells, using a combination of a rolling circle mechanism and recombination mediated replication (GUTIERREZ 1999; HANLEY-BOWDOIN *et al.* 2000, 2004; JESKE *et al.* 2001; GUTIERREZ *et al.* 2004). On the one hand, this mechanism resembles the way in which ssDNA phages such as ϕ X174 replicate and on the other, the way in which mammalian DNA tumor viruses activate the host genes required for DNA replication. Because of its type of replication, it gives rise to a concatameric double-stranded (ds) DNA intermediate, the replicative form (RF), which is later converted to genome sized circular DNA fragments. The dsDNA intermediates are transcribed in the nuclei of infected plant cells, providing the proteins required for the initiation of replication and for recruitment of the host replication machinery. TYLCV encodes two proteins required for efficient viral replication: C1 (Rep.) which serves as the initiation factor that mediates origin recognition and DNA cleavage/ligation to begin and end the rolling circle replication process, and C3, which facilitates the accumulation of high levels of viral DNA, possibly by modifying C1 activity and/or aiding in the recruitment of the host replication enzymes.

As both replication and transcription occur in the nucleus, import of the viral DNA and/or virions into and out of the host-plant cell nucleus is essential for successful completion of the virus's life cycle. Therefore, movement of the viral genome into and out of the nucleus, as well as from cell to cell and throughout the plant, is critical for viral infection.

7. Virus movement

When a geminivirus first enters the host-plant cell, there are no viral proteins other than the CP. Movement to the nucleus, where TYLCVs, like all other geminiviruses, transcribe and replicate their genome, must therefore be entirely dependent on the CP and the exploitation of host transport mechanisms. Microinjection and transient-expression experiments have provided insight into the mechanism by which the CP may function in the intracellular movement of the TYLCV genome. These experiments localised the CP to the nuclei of insect and plant cells (KUNIK *et al.* 1998). Transport of the TYLCV CP into the nuclei was shown to be an active, energy-dependent process that could be blocked by the GTP analogue GTP γ S. The latter is known to compete with GTP, while making no energy contribution. By testing the nuclear import of the entire CP and deletion mutants, a functional NLS was shown to reside between amino acids 3 and 20 of the TYLCV CP, and to resemble the bipartite class of NLSs with the following amino acid sequence: ¹MSKRPG-DIIISTPVSKVRRRLNFDSPYSS²⁹.

The experiments also showed that a supplementary NLS resides in the TYLCV CP between residues 36 and 61. This latter domain can facilitate nuclear import but is not, in and of itself, sufficient for nuclear accumulation (GAFNI & KUNIK 1997; GAFNI 1998; KUNIK *et al.* 1998).

A recent study of a very closely related TYLCV from the Dominican Republic confirmed the karyophilic nature of the TYLCV CP (ROJAS *et al.* 2001). In those experiments, Oregon Green (OG)-labeled TYLCV CPs were microinjected into tomato and *N. benthamiana* mesophyll cells. In half of the cases, the OG-CP accumulated within the nucleus of the microinjected cells.

Because of its aforementioned karyophilic nature, it seems self-evident that TYLCV CP would interact with karyopherin α (a protein that serves as a

nuclear shuttle for NLS-bearing proteins) because of its NLS. To examine this hypothesis, a tomato karyopherin α homologue had to be identified. To this end, my group first isolated a tomato cDNA clone encoding this protein, LeKAP α 1. Next, the interaction of LeKAP α 1 with TYLCV CP was demonstrated in a yeast two-hybrid system (KUNIK *et al.* 1999). The results indicated that LeKAP α 1 specifically interacts with CP, most likely mediating its nuclear import by a karyopherin α -dependent mechanism. For the TYLCV CP to be considered a nuclear shuttle protein for the viral genome, another prerequisite, namely ssDNA-binding activity, had to be met. This activity was demonstrated by gel-shift assay (PALANICHELVAM *et al.* 1998).

Upon entry into the nucleus, geminiviruses replicate, producing both single-stranded and double-stranded forms of the viral genome. Once viral DNA has begun to replicate in the nucleus, the newly synthesised CP carries out at least two distinct functions: (i) nuclear export of the infectious form of the virus, and (ii) encapsidation of ssDNA into virions. However, to move from cell to cell, the infectious form of the virus must be able to leave the plant-cell nucleus and be transported to the plasmodesmata, and through them to adjacent cells, followed by transport to and entry into the nucleus of these neighboring cells. A functional analysis aimed at characterizing the proteins involved in the intracellular movement of TYLCV showed that the CP, together with two other proteins, V2 and C4, are involved in the delivery of viral DNA, as virions or as nucleoprotein complexes, to the plant-cell periphery (ROJAS *et al.* 2001). To be transported to adjacent cells, the virus must overcome the barrier to cell-to-cell movement presented by the cell wall. To this end, plant viruses encode movement proteins (MPs) that can interact with plasmodesmata, the plasma-membrane-lined channels that interconnect plant cells, to facilitate cell-to-cell transport of the infectious form of the virus (HEINLEIN & EPEL 2004; LUCAS & LEE 2004; RUIZ-MEDRANO *et al.* 2004). ROJAS *et al.* (2001) suggested that it is the C4 protein, through a putative N-terminal myristoylation domain, which acts to deliver the viral DNA to plasmodesmata and to mediate cell-to-cell transport into neighboring, uninfected cells.

Base on recent accumulated data, a model for geminivirus intra- and intercellular movement was suggested by GAFNI and EPEL (2002), in which the

movement of monopartite geminiviruses, including TYLCV, is strictly dependent on the viral CP.

8. Methods to control the disease

Several methods have been developed to control TYLCD, such as the use of healthy transplants, chemical and physical control of the vector, crop rotation, and breeding for resistance to TYLCV (NAKHLA & MAXWELL 1998). The most effective and environmentally sound management remains planting resistant or tolerant lines. Thus, breeding for TYLCV resistance is probably the most important long term goal for lasting TYLCV management. At present, only partially resistant Fl hybrids are commercially available. Moreover, a prevalent problem is associated with the definition of resistance. As stated by LAPIDOT and FRIEDMANN (2002), a host plant is resistant to TYLCV if it can suppress its multiplication and consequently suppress the development of disease symptoms. Lower virus accumulation in a resistant host has been associated with the latter's resistance, as well as with the effect of infection on total yield and yield components (LAPIDOT *et al.* 1997). Classical breeding has attempted to introduce TYLCV resistance in tomato cultivars. However, resistance appears to be controlled by one to five genes and crosses have produced only tolerant hybrids. It is unfortunate that after over 25 years of breeding programs, the best commercially available cultivars show only tolerance to the virus and meanwhile, the disease continues to spread. Therefore, the production of transgenic tomato plants appears to be a more promising way of obtaining resistance to TYLCV. Several strategies have been used to engineer plants resistant to viral pathogens, based on the concept that the introduction and expression of viral sequences in plants can interfere with the virus's life cycle. This strategy is also referred to as pathogen derived resistance.

8.1. Breeding for resistance

Breeding for resistance in cultivated tomato varieties is the best approach to controlling viral disease (LAPIDOT *et al.* 1997; POLSTON & ANDERSON 1997; LAPIDOT & FRIEDMANN 2002). Genetic resistance or tolerance to TYLCV has been introgressed in tomato in order to develop resistant cultivars since the early 1970s, and some

such cultivars are already commercially available. The first commercial tolerant cultivar, TY20, carrying tolerance from *L. peruvianum* (PILOWSKY & COHEN 1990), and the later, more advanced lines, showed delayed symptoms and lower accumulation of viral DNA (FRIEDMANN *et al.* 1998). An established breeding line, with resistance derived from *L. hirsutum*, showed total immunity to whitefly mediated inoculation (VIDAVSKY & CZOSNEK 1998). Nevertheless, the breeding of tomatoes resistant to TYLCV has been slow because of the complicated inheritance of the resistance/tolerance trait. Depending on the source, resistance has been reported to be controlled by one to five genes that are either recessive or dominant (ZAKAI *et al.* 1990). Thus a screening procedure for TYLCV resistance is necessary for all those breeding programs aimed at producing tomato cultivars resistant to TYLCV. Selecting plants solely on the basis of the presence or absence of symptoms in infected fields, without taking into account the time of inoculation and levels of inoculum, leads to a considerable number of escapees. Therefore, some very efficient screening methods have been established to develop lines which are highly tolerant to the virus and which do not exhibit any symptoms of the disease upon infection. However, in commercial fields in most regions of the world, tomato plants are still largely susceptible to various begomoviruses. In addition, there is concern that some asymptomatic, tolerant cultivars support replication of the virus, and can act as a source of begomovirus for susceptible crops (LAPIDOT *et al.* 2001).

8.2. Genetically engineered resistance

Research on transgenic, TYLCV resistant tomatoes began in the early 1990s. A range of different strategies have been applied, including the use of antisense RNA, CP genes, an intact replication-associated protein gene (*Rep*) and truncated versions of the latter. BENDAHMANE and GROENBORN (1997) demonstrated that use of the full-length antisense *Rep* confers moderate resistance to TYLCSV in *N. benthamiana*, and that this resistance is inherited in the R₂ generation as well. Interestingly, the level of homology between the antisense RNA and the challenging viral sequence specified the level of resistance obtained. FRANCO *et al.* (2001) showed that resistance of *N. benthamiana* to TYLCSV stems from a double mechanism involving antisense RNA of the TYLCSV *Rep* gene

and extrachromosomal molecules; however, the plants were not protected against TYLCV, which is a more severe virus. Recently, two more truncated *Rep* genes were shown to confer resistance. In the first (ANTIGNUS *et al.* 2004), 129 amino acids of the *Rep* protein conferred resistance to the mild strain of the virus while in the other (YANG *et al.* 2004), a construct consisting of two-fifths of the TYLCV *Rep* gene conferred high levels of resistance and often immunity to TYLCV in both tobacco and tomato. In the latter case, the authors suggested that the resistance may have been obtained through the mechanism of PTGS. However, it is important to note that silencing of the *Rep* gene can be overcome by the virus (LUCIOLI *et al.* 2003; NORIS *et al.* 2004b). The *ν1* gene (encoding the CP) of TYLCV was also used in transgenic tomato plants in an attempt to render them resistant to the virus (KUNIK *et al.* 1994). This approach was taken in accordance with many experiments which had shown that plants transformed with the *ν1* gene of a virus were more resistant when high levels of the viral CP were expressed. However, all of those experiments had been performed with RNA viruses and this was the first demonstrated case of CP-mediated resistance to a DNA virus. The resultant plants showed resistance to challenge by TYLCV which was associated with high levels of expressed CP. However, this resistance was expressed as a delay in symptoms, rather than total immunity to the virus.

With the advent of PTGS of target genes as a popular way of interfering with the viral life cycle, attempts were made to render plants resistant to the virus by transgenically producing dsRNA of the target gene, hence leading to destruction of its RNA. The first report describing silencing of the *Rep* protein came in 2004 (NORIS *et al.* 2004a), in which transgenic plants were challenged with TYLCSV: the virus overcame the silencing, though there was a delay in symptom appearance. A similar approach was taken by a Cuban team, which led to immunity of tomato plants to TYLCV infection (FUENTES *et al.* 2006). This was followed by another approach in which the non-coding conserved regions from three different strains of TYLCV were selected and used to design a construct that can trigger broad resistance against different viruses that cause TYLCD. This approach led to a high level of resistance to all three strains (ABHARY *et al.* 2006). Later, Gafni and colleagues obtained plants resistant to TYLCV by targeting the CP

gene with an inverted-repeat construct (ZRACHYA *et al.* 2007b).

8.3. Physical and chemical measures

In the last two decades, there has been a worldwide spread of the B biotype of *B. tabaci*, the only known vector of TYLCV. Among measures taken to minimise the damage, vector control by pesticides and physical barriers is commonly used, especially in countries for which the more resistant hybrid varieties are too expensive. Conventional chemical control of the whitefly is difficult to achieve because of the distribution of its immature forms, primarily on the underside of leaves, with older larvae and pupae located lower in the plant canopy. The diversity of cultivated and weed host plants attacked contributes to the source of infestation. A number of insecticides have effectively controlled this pest in the past but resistance develops rapidly. Several new materials, including insect-growth regulators and new pyrethroid insecticides, appear promising. However, the resistance phenomenon suggests that their efficacy will also be of limited duration. Thus, the current reliance on chemical control must be considered a temporary measure, pending the development of a satisfactory integrated pest management program.

At the moment, growers use a variety of chemicals to combat the whitefly: acephate, buprofezin, cyfluthrin, deltamethrin, imidacloprid, permethrin and pirimiphos methyl are the active substances in the most popular products. Chemicals are used in both protected and unprotected cultivation (ATTARD 2002). Physical control is usually achieved by using a very fine net to stop the adult whiteflies from reaching the tomato plants. This is used on protected cultivations, as well as in the open field. Yellow traps are also used in glasshouses, in the form of sticky yellow plastic cards hung at several intervals along the rows of tomato plants. The use of photoselective plastic covers that block ultraviolet (UV) light has been proposed as a good method of controlling TYLCD because it interferes with the whitefly's vision, resulting in an over 50% reduction in disease incidence.

9. Concluding remarks

Our understanding of the life cycle of TYLCV has increased considerably in recent years. Never-

theless, many unanswered questions remain with respect to its biology and epidemiology, and the disease it causes appears to be spreading on a global scale. Although much work has been invested in the development of TYLCV-resistant tomato lines, effective and durable resistance to TYLCV remains elusive. With the new "genetic engineering" technologies, the introduction of novel genetic traits, from the pathogen itself and/or from other sources, should be considered in the battle against the disease. Today's challenge, then, is to gain a better understanding of the functions of the viral genes and their gene products, as well as of their interactions among themselves and with the host cell. In the fight against TYLCV, such knowledge is rapidly becoming a necessity.

Acknowledgements. The authors apologise to colleagues whose original works were not cited due to lack of space. We would like to thank KATHARINA KITTELMANN for providing us the electron microscopy image of geminiviruses and for critical reading of the manuscript. We gratefully thank the Israeli Science Foundation (ISF), the US-Israel Binational Research and Development Fund (BARD) and the US-Israel Binational Science Foundation (BSF) for supporting the research described herein which was performed in our laboratory. This paper is contribution No. 140/2009 from the Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel.

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Received for publication June 21, 2009

Accepted after corrections September 18, 2009

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