Chapter B9

Resistance to *Tomato yellow leaf curl virus* in Tomato

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Introduction

Tomato yellow leaf curl virus (TYLCV) is one of the most devastating viruses of cultivated tomatoes in tropical and subtropical regions. TYLCV is a monopartite begomovirus, first described in Is[1]rael (Cohen and Nitzany, 1966). Although originally found only in the eastern Mediterranean, it is now a problem in the western Mediterranean, the Caribbean, Japan, and the southern U.S. (Polston and Anderson, 1997; Polston et al. 1999). Infection of susceptible tomato plants results in cupping of leaves, chlorosis, prominent stunting of the growing point, and flower abscission. Depending on the timing of infection, yield losses can reach 100%. In many tomato-growing areas, TYLCV has become the limiting factor for production of tomatoes in both open field and protected cultivation systems (Lapidot and Friedmann, 2002).

TYLCV is a monopartite begomovirus transmitted by the tobacco whitefly, *Bemisia tabaci* (Gennadius). The only known vectors of TYLCV are in the *B. tabaci* species complex (Brown et al. 1995), which includes *B. tabaci* and *B. argentifolii* (Bellows et al. 1994). TYLCV transmission by

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whiteflies has been characterized as being persistent and circulative in nature, that is the virus is retained through the life of the adult insect after acquisition, and moves through the insect body to the salivary glands where it can leave the body of the whitefly in the saliva (Cohen and Harpaz, 1964; Cohen and Nitzany, 1966; Nault, 1997). However, it has been shown for TYLCV that transmission efficiency declines with time (Cohen and Harpaz, 1964)

TYLCV has a small genome (2.8 kb) with 6 open reading frames that are organized bidirectionally (Fig. 1) (Gutierrez, 1999). The *Rep* (replication associated protein) gene is a multi-functional gene, essential for viral DNA replication, and is involved in transcriptional regulation (Fontes et al. 1994; Lazarowitz, 1992). In other begomoviruses, *TrAP* (transactivation of transcription) gene has been shown to play an important role in the systemic viral infection of *Nicotiana benthamiana*, enhance the expression of the coat protein and play a role in the suppression of host defense responses (Bisaro et al. 1999; Brough et al. 1992; Etessami et al. 1991). The *REn* (replication enhancer protein) gene of other begomoviruses has been shown to enhance replication and mutations in this gene were shown to attenuate plant disease symptoms (Etessami et al. 1991; Sunter et al. 1990). *REn* is not virus specific and is able to interact with the *Rep* of other geminiviruses (Sunter et al. 1994). C4 has been implicated to play a role in pathogenicity (Krake, 1998) and *V1* has been shown to play a role in virus movement (Wartig et al. 1997). The TYLCV coat protein gene is the most abundant protein produced by TYLCV (Timmermans et al. 1994). This protein is required for whitefly transmission, binds to viral single stranded DNA (ssDNA), may play a role in systemic movement, and contains a nuclear targeting signal, which mediates movement of viral nucleic acid into the host cell nucleus (Azzam et al. 1994; Briddon et al. 1990; Kunik et al. 1998; Palanichelvam et al. 1998). Therefore, its DNA replication cycle, like other begomoviruses, relies largely on the use of host cellular DNA replication proteins. Only the *Rep* gene is essential for begomoviral DNA replication. The replication strategy used by TYLCV consists of a first stage, the conversion of ssDNA into double-stranded DNA (dsDNA) intermediate, followed by the second stage in which the dsDNA is used as a template to produce ssDNA genomes by a rolling-circle replication mechanism (Gutierrez, 1999; Hanley-Bowdoin et al. 1999).

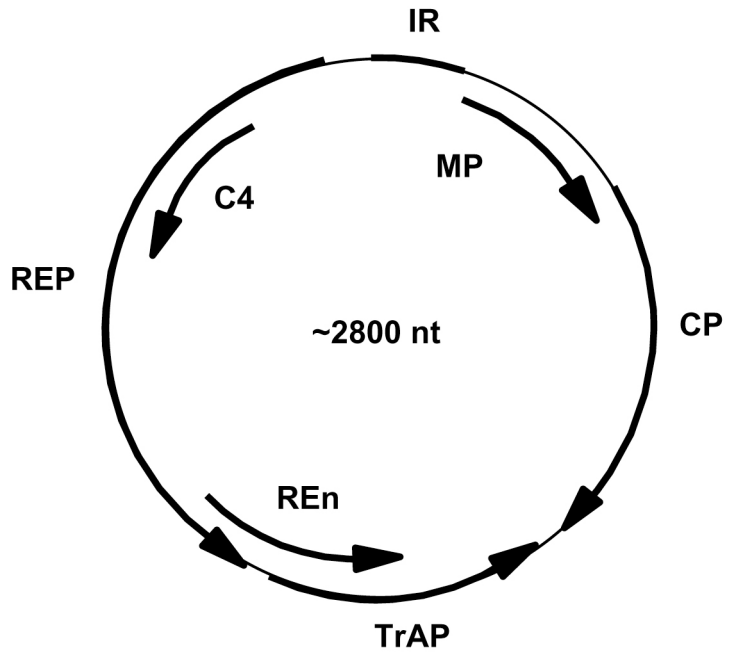

Figure 1. Genome organization of *Tomato yellow leaf curl virus*. The non-coding region is the IR (intergenic region). The encoded proteins are: MP (movement protein), CP (coat protein), Rep (replication initiation protein), TrAP (transcriptional activator protein), REn (replication enhancer protein), and C4 (a determinant of symptom expression). The arrows refer to the direction of transcription. The MP and CP are encoded on the virion (genomic) strand, while Rep, TrAP, REn and C4 are encoded on the complimentary DNA strand.

Taxonomy

In the past there was some confusion regarding the taxonomy of TYLCV. Several begomoviruses that induce symptoms in tomato similar to those elicited by TYLCV were initially named TYLCV. Later analyses of the sequences of these viruses showed them to be unique begomoviruses and not closely related to TYLCV. This confusion was addressed by a committee of the ICTV and a clarification published (Fauquet et al. 2003). A brief summary of changes with respect to viruses called TYLCV is presented

(Table 1). The problems that can arise due to an ambiguous viral nomenclature is manifested in a work that was published only 4 years ago regarding the mapping of TYLCV resistance originated from the wild tomato *Lycopersicon hirsutum* (Hanson et al. 2000). The authors screened resistant plants using three different isolates of TYLCV – or so they thought. Today we know that these viral isolates were in fact three isolates of *Tomato leaf curl virus* (ToLCV) and not TYLCV.

Two strains of TYLCV have been reported in Israel, TYLCV and TYLCV-Mld (Antignus and Cohen, 1994; Navot et al. 1991). TYLCV-Mld produces symptoms in tomato indistinguishable from those of TYLCV. TYLCV-Mld was recognized due to its ability to infect and induce disease symptoms milder than those of TYLCV on TY-20, a tomato cultivar resistant to TYLCV (Antignus and Cohen, 1994; Antignus, pers. comm.). TYLCV occurs much more commonly in tomato fields in Israel than TYLCV-Mld (M. Lapidot, unpub.). TYLCV was reported to be a recombinant virus between TYLCV-Mld and an ancestor of a second begomovirus, ToLCV, as described from India (Harrison and Robinson, 1999; Navas-Castillo et al. 2000). TYLCV possesses a portion (N-terminal region) of the *Rep* and intergenic region (IR) of ToLCV and the rest of the genome is very similar to that of TYLCV-Mld.

Geographic distribution

Until about 1990, TYLCV was recognized as a pathogen of tomato in the eastern Mediterranean, and was reported from Cyprus, Egypt, Jordan, Israel, Lebanon, Syria, and Turkey (Czosnek and Laterrot, 1997). However, over the last decade or so the geographic range of TYLCV has greatly expanded to include Japan, the western Mediterranean, the Caribbean, and the south-eastern U.S. (reviewed by Moriones and Navas-Castillo, 2000). TYLCV appeared in the eastern Caribbean in the late 1980's, and was found for the first time in tomato in Cuba in 1989, the Dominican Republic in 1992, Jamaica in 1993, The Bahamas in 1996, and Puerto Rico in 2001 (Bird et al. 2001; Martinez-Zubiaur et al. 1996; McGlashan et al. 1994; Nakhla et al. 1994; Polston et al. 1994; Ramos et al. 1996; Sinisterra et al. 2000). In the western Caribbean it has been found in Yucatan, Mexico in 1997 (Ascecio-Ibanez et al. 1999). TYLCV was detected for the first time in the United States in Florida in 1997, followed by Georgia in 1998, Louisiana in 2000, and Mississippi and North Carolina in 2001 (Ingram and Henn, 2001; Momol et al. 1999; Pappu et al. 2000; Polston et al. 1999; Polston et al. 2002; Valverde et al. 2001).

Table 1. List of begomoviruses with names similar to *Tomato yellow leaf curl virus*

Currently Accepted virus Nomenclature	Acronym	Previous Nomenclature
Tomato yellow leaf curl virus	TYLCV	TYLCV, TYLCV-Il
Tomato yellow leaf curl China virus	TYLCCV	TYLCV-China, TYLCV-CN
Tomato yellow leaf curl Gezira virus	TYLCGV	
Tomato yellow leaf curl Malaga virus	TYLCMalV	
Tomato yellow leaf curl Sardinia virus	TYLCSV	TYLCV-Sardinia, TYLCV-Sar
Tomato yellow leaf curl Thailand virus	TYLCTHV	TYLCV-Thailand, TYLCV-TH
Tomato yellow leaf curl – Mild	TYLCV-Mld	TYLCV-IL[Mld]

The need for resistance

The management of TYLCV is difficult, expensive, and with limited options. In many regions traditional control measures for TYLCV emphasize vector control (Cohen and Antignus, 1994; Hilje et al. 2001; Palumbo et al. 2001; Polston and Anderson, 1997), mainly through multiple applications of insecticides or physical barriers. Chemical control methods have been only partially effective, since whitefly populations can reach very high numbers, leading to intensive pesticide use (sometimes twice daily) in attempts to eliminate the vector before it transmits the virus. Furthermore, there are concerns that the vector may develop pesticide resistance and the intense application of pesticides may have deleterious effects on the environment (Palumbo et al. 2001; Pico et al. 1996). Physical barriers such as fine-mesh screens have been used in the Mediterranean Basin to protect crops (Cohen and Antignus, 1994; Hilje et al. 2001; Palumbo et al. 2001; Polston and Anderson, 1997). Recently, UV-absorbing plastic sheets and screens have been shown to inhibit penetration of whiteflies into greenhouses (Antignus et al. 2001; Antignus et al. 1996). Furthermore, filtration of UV light was shown to hinder the whiteflies' dispersal activity, and consequently reduce virus spread (Antignus et al. 2001). However, adoption of physical barriers adds to production costs and these screens create problems of shading, overheating, and high relative humidity. Therefore, the best way to reduce yield losses due to TYLCV is by breeding crops resistant or tolerant to the virus (Lapidot and Friedmann, 2002; Morales, 2001; Pico et al. 1996).

Definition of resistance

A common problem for researchers interested in resistance is the lack of a standard terminology used by both plant breeders and plant pathologists. Breeders are mainly interested in improving the overall performance of a plant variety under field conditions. Thus, yield and fruit quality (as well as fruit color and shape) are paramount. In contrast, plant pathologists place an emphasis on the fate of the virus in the plant. A similar cause of confusion lies in whether or not a researcher makes the distinction between resistance to the pathogen *versus* resistance to the effects of the pathogen (i.e. symptoms of the disease). Another frequent source of confusion occurs when the resistance level in question is mediocre or unsatisfactory and is described with the terms "tolerance" or "field resistance" in an undefined manner. The definitions of resistance proposed by Cooper and Jones (1983) are used in this manuscript and are summarized below.

Resistance - A host plant is resistant if it can suppress the multiplication of a virus, and consequently suppress the development of disease symptoms.

Regardless of the mechanism of resistance (the host may be resistant to establishment of infection, viral replication or viral spread within the plant), the final outcome is the same – fewer virions accumulate in the resistant host. Resistance can range from very high (up to immunity – no virus accumulates in the host and the plant is, in fact, a non-host), to moderate, or low. However, even for low resistance, the resistant plant will accumulate fewer virions than the susceptible host, and may express milder disease symptoms and/or a delay in the onset of symptoms.

Tolerance - This is a unique instance where in response to virus infection, the host expresses negligible or mild disease symptoms, but supports normal levels of virus multiplication. Thus, the plant, rather than being resistant to the virus, "tolerates" the pathogen and, despite its presence, expresses milder symptoms and produces a good yield (Cooper and Jones, 1983; Walkey, 1985). Hence, tolerance is not a code name for low-level forms of resistance but is, rather, a specific plant response: milder symptoms despite a normal level of virus accumulation.

Inheritance of TYLCV resistance

There have been focused and prolonged efforts to breed cultivars resistant to TYLCV. Since all cultivars of tomato (*Lycopersicon esculentum*) are extremely susceptible to TYLCV, wild *Lycopersicon* species were screened for their response to the virus in order to identify and introgress genes for resistance (reviewed in Laterrot, 1992; Nakhla and Maxwell, 1998; Pico et al. 1996; Pico et al. 1999; Pilowsky and Cohen, 2000). Thus, breeding programs have been based on the transfer of resistance genes from accessions of wild origin into the cultivated tomato. Progress in breeding for TYLCV resistance has been slow, primarily because of the complex genetics of the resistance, the interspecific crossability barriers between the wild and domesticated tomato species, and the need to set up a reliable screen for resistance to the virus, which is dependent on the availability of viruliferous whiteflies (Lapidot and Friedmann, 2002; Lapidot et al. 1997; Vidavsky et al. 1998).

This chapter will review some of the work done on different resistance sources to TYLCV with an emphasis on the inheritance of the resistance. For a list of resistant wild *Lycopersicon* species see previous reviews by (Laterrot, 1992; Nakhla and Maxwell, 1998; Pico et al. 1996; Pico et al. 1999; Pilowsky and Cohen, 2000). The inheritance of resistance to TYLCV from a number of the different resistant sources has been identified and a summary is presented in Table 2.

Table 2. Inheritance TYLCV resistance derived from different wild *Lycopersicon* sources.

Source of resistance	Species	Accession No.	Inheritance
L. pimpinellifolium	LA 121	Monogenic, partial dominance	Pilowsky and Cohen, 1974
	Hirsute-INRA; LA 1478	Monogenic, dominant	Kasrawi, 1989
L. peruvianum	PI 126935	Recessive, controlled by five genes	Pilowsky and Cohen, 1990
	PI 126926 & PI 126930 & PI 390681 & LA 441	Three interacting genes, one with partial dominance the others recessive	Friedmann *et al*., 1998
L. chilense	LA 1969	A major gene (*TY-1*) with partial dominance and two modifier genes	Zamir *et al*., 1994
L. hirsutum	LA 386	Dominant polygenic	Hassan *et al*., 1984
	LA 1777 & LA 386	Two mechanisms: (1) Resistance controlled by two to three additive recessive genes (2) Tolerance controlled by a dominant major gene	Vidavsky and Czosnek, 1998
L. cheesmanii	LA 1401	Recessive	Hassan *et al*., 1984

Breeding for TYLCV resistance was initiated in Israel in the late 1960's using accessions of the wild tomato *L. pimpinellifolium* (Pilowsky and Cohen, 1974). It was found that the TYLCV resistance derived from accession LA121 was monogenic with partial dominance (Table 2). Other studies with different accessions of *L. pimpinellifolium* such as hirsute INRA found the resistance to be mediated by a single dominant gene (Table 2). In a later study, bulked segregant analysis was employed to identify random amplified polymorphic DNA (RAPD) markers that were linked to the TYLCV resistance derived from *L. pimpinellifolium* hirsute INRA (Chague et al. 1997). Four RAPD markers were identified which were linked to a quantitative trait locus (QTL) responsible for up to 27.7% of the resistance. This differs from earlier results in which the resistance was reported to be mediated by a single dominant gene (Kasrawi, 1989). Interestingly, this QTL was mapped to chromosome 6 (Chague et al. 1997) as was the TYLCV resistance gene, *TY-1* (see below). However, the level of resistance from accessions of *L. pimpinellifolium* was found to be insufficient – while resistant plants derived from LA121 showed moderate disease symptoms following infection, these plants suffered from markedly reduced growth and yield (Pilowsky and Cohen, 1990). Thus other sources of resistance were sought.

The first commercial resistant hybrid, 'TY-20', was released in 1988 (Pilowsky et al. 1989). 'TY-20' carried resistance derived from *L. peruvianum* (accession PI 126935) that was later determined to be mediated by five recessive genes (Pilowsky and Cohen, 1990). The resistance in 'TY-20' induced a delay in the development of disease symptoms upon infection but, despite this, infected 'TY-20' plants were still able to produce an acceptable yield. The resistance in *L. chilense* (accession LA 1969) is controlled by a major partially dominant gene termed *TY-1* and at least two more modifier genes (Zamir et al. 1994). *TY-1* was mapped to chromosome 6 using restriction fragment length polymorphism (RFLP), while the two modifier genes were mapped to chromosomes 3 and 7 (Zamir et al. 1994). Since it is relatively easy to introgress a single dominant gene, a number of commercial hybrids have been released carrying *TY-1* resistance. Currently, all the commercial TYLCV-resistant tomato hybrids, including those carrying *TY-1,* out-yield susceptible hybrids in the presence of TYLCV. However, since all these hybrids display disease symptoms after infection, higher levels of TYLCV resistance were sought.

One approach being used to increase levels of resistance is to combine different resistance genes into a single cultivars (i.e., pyramiding resistances) (Kelly et al. 1995). An example of this is line TY-172, which exhibited the highest level of resistance during a field trial, in which the yield components of various resistant cultivars and lines, which had been inoculated with TYLCV, were evaluated and compared (Lapidot et al. 1997). TY-172 had

been derived from four different accessions of *L. peruvianum* (Friedmann et al. 1998). These four accessions were crossed with *L. esculentum*, and the resulting F_1 interspecific hybrids were backcrossed to the susceptible parent until a BC_3F_3 generation was secured. At this stage crosses were made between the four different lines, and F_2 and F_3 generations were produced and screened for resistance. A highly resistant F_3 line was selected, and its F_4 offspring were bulked and designated TY-172 (Friedmann et al. 1998). TY-172 is a symptomless host of TYLCV, which contains very low levels of viral DNA. Either when infected in the greenhouse with viruliferous whiteflies, or when grown in the field under conditions of natural infection, TY-172 shows no symptoms of TYLCV infection. Attempts to produce disease symptoms on TY-172 plants by grafting with a susceptible infected donor were unsuccessful. Thus, even when exposed continuously to very high levels of viral inoculum, line TY-172 did not develop disease symptoms (Friedmann et al. 1998). When TY-172 was crossed with susceptible lines, the resulting hybrids exhibited milder symptoms and although they had a lower viral content than the susceptible parent, it was nevertheless much higher than that of TY-172, suggesting partial dominance of the resistance. Analysis of F_2 populations, suggested that the resistance in line TY-172 is controlled by at least three interacting genes (Friedmann et al. 1998).

Two other examples of improved resistance through the combination of different resistance sources are lines 902 and 908, which express high levels of resistance to TYLCV. The resistance in these lines was derived from the cross between *L. hirsutum* accessions LA 1777 and LA 386. The resulting F_1 plants were crossed with *L. esculentum* followed by selfing of resistant, symptomless individuals, which resulted in two stable BC_1F_4 lines, designated 902 and 908 (Vidavsky and Czosnek, 1998). Line 902 does not produce disease symptoms and does not support viral accumulation following whitefly-mediated inoculation with TYLCV. However, virus accumulation was detected in line 902 following grafting with an infected susceptible donor. Segregation analysis indicated that two to three additive recessive genes control the resistance to TYLCV in line 902 (Vidavsky and Czosnek, 1998). Also, line 908 does not show any disease symptoms following whitefly-mediated inoculation but, unlike line 902, TYLCV does accumulate in the plants. Segregation analysis indicated that a single dominant major gene controls the resistance in 908 (Vidavsky and Czosnek, 1998).

Mechanism of resistance

Despite the considerable efforts devoted to the development of TYLCV-resistant cultivars, very little is known about the mechanisms of the

introgressed viral resistances. The levels of TYLCV DNA accumulation in TY-20 and four other TYLCV-resistant lines were compared with that in a susceptible line (Rom et al. 1993). Following whitefly-mediated inoculation and for a period of approximately 40 days, samples were taken from the plant apex of inoculated plants and analyzed using dot-blot hybridization. It was found that at all time points, the resistant cultivars accumulated significantly less viral DNA compared to the susceptible line. The authors concluded that viral DNA accumulation was positively correlated with symptom severity, and suggested the monitoring of viral DNA level as a tool for the selection of TYLCV-resistant genotypes (Rom et al. 1993). These results were consistent with those of another study in which different *Lycopersicon* accessions were screened for resistance to TYLCV using the amount of viral DNA present in inoculated plants as an indicator of resistance (Zakay et al. 1991). Another study used serological assays to rank the level of resistance of tomato lines to three different tomato begomoviruses (including *Tomato yellow leaf curl Sardinia virus*) and found a positive correlation between the level of resistance and amount of virus detected in the plant (Fargette et al. 1996). The authors concluded by suggesting that viral resistance should be assessed using serological assays.

A more recent study indicated that there was not always a good correlation between severity of disease symptoms and levels of TYLCV DNA accumulation with the effects on yield (Lapidot et al. 1997). The effects of TYLCV on total yield and yield components of four resistant F_1 tomato cultivars and two breeding lines were evaluated in the field. Plants of resistant and susceptible cultivars were infected with TYLCV at the first-leaf stage by whitefly-mediated inoculation. After a short recovery period, the plants were transplanted to the field. Inoculated plants of each cultivar or line were compared with their respective control, non-inoculated plants, in terms of total yield, average fruit weight and number, and plant fresh weight. Disease symptom severity and level of viral DNA accumulation in the inoculated plants were monitored throughout the growing season (approximately 90 days). There were substantial differences among the entries with respect to the amount of yield loss caused by TYLCV as well as the amounts of viral DNA accumulated. All the resistant cultivars showed milder symptoms, expressed lower yield losses, and accumulated lower amounts of viral DNA when compared to the susceptible variety. Hence, a positive correlation was observed between disease resistance and amounts of viral DNA when resistant plants were compared to susceptible controls. However, there was not a strong correlation between lower amounts of viral DNA with higher crop yields. Plants of the highly resistant breeding lines TY-172 and TY-197 suffered the least relative yield loss and showed the lowest level of viral DNA. However, while TY-172 and TY-197 plants accumulated viral DNA to the same level, TY-172 plants expressed a higher level of resistance to the virus than TY-197 plants as determined by the

effects on yield, suggesting that reduction in virus titer is not the only factor that determines resistance level. Thus, although the accumulation of TYLCV DNA can serve as an indicator for resistance level, it is best that this is not used as the sole indicator (Lapidot et al. 1997).

The first attempt to understand the mechanism underlying a TYLCV resistance at the molecular level was using the resistance to TYLCV derived from *L. chilense*, which contains the resistance locus *TY-1* (Michelson et al. 1994). Two nearly isogenic tomato lines, which differed only in the presence or absence of the *L. chilense* chromosome segment associated with resistance to TYLCV, were developed by RFLP-assisted selection. Plants from line 50, which did not contain the *TY-1* allele from *L. chilense*, were susceptible and showed disease symptoms after whitefly-mediated inoculation with TYLCV under field conditions. In contrast, plants from line 52, due to the presence of the *TY-1* allele from *L. chilense*, were resistant to TYLCV and remained symptomless after whitefly-mediated inoculation with TYLCV under field conditions. The effect of the *TY-1* gene on TYLCV accumulation and translocation was studied by comparing viral DNA accumulation in lines 50 and 52. TYLCV DNA accumulation in plants of the line 52 was found to be a function of the amount of inoculum. When the inoculum titer was low (three whiteflies per plant), TYLCV DNA accumulated to a low level in the resistant line. When the inoculum was high (50 whiteflies per plant) similar amounts of viral DNA accumulated in both the resistant and susceptible lines 28 days after inoculation. However, the rate of DNA accumulation was slower in the resistant line than in the susceptible line. When the movement of viral DNA from the inoculated leaf (youngest leaf of each plant) was followed, it was found that in the susceptible plants viral DNA moved to the upper leaves and to the roots, the same route as followed by photoassimilates. In contrast, viral DNA movement was restricted to the second leaf and to the shoot apex in the resistant plants. The authors concluded that the *TY-1* gene is associated with inhibition of disease symptoms through two mechanisms: by reducing viral DNA accumulation in inoculated tissue exposed to low inoculum titers and at higher titres of virus inoculum by limiting viral long-distance movement (Michelson et al. 1994)

Recently, the first step was made to elucidate the resistance mechanism shown by TY-172 (derived from *L. peruvianum*) under conditions of high inoculum pressure (Segev et al. 2004). The resistance mechanism was addressed by inoculating selected leaves on intact TY-172 and susceptible tomato plants with TYLCV and comparing the amount of viral ssDNA and dsDNA produced at the inoculation site over time. The plants were inoculated with whiteflies using clip cages, thus, a clear inoculation site was created on the inoculated leaf. Moreover, the use of clip cages allowed control over the number of whiteflies used to inoculate each plant, thus

reducing variation due to varying amounts of inoculum and enabling comparisons between different inoculated plants.

When the amount of TYLCV DNA at the site of inoculation was evaluated over time, it was found that at each time point, the amount of new viral ssDNA in the resistant host was much lower than that of the susceptible host. However, the changes observed in viral ssDNA detected over time were not reflected by parallel changes in the amounts of TYLCV dsDNA detected in the same tissues. Viral dsDNA accumulated to the same level in both the resistant and susceptible hosts at all time points examined. Moreover, the amount of viral dsDNA detected was much lower than the amount of viral ssDNA detected in both resistant and susceptible hosts, which is consistent with the role of viral dsDNA as an intermediate form of DNA in begomovirus replication. It is well established, that upon begomovirus entry to the plant cell, the viral ssDNA serves as a template for the synthesis of a dsDNA intermediate replicating form. In the second stage of the replication cycle, the dsDNA replicating form serves as template for the production of new viral ssDNA, via a rolling circle mechanism (Gutierrez, 1999; Hanley-Bowdoin et al. 1999).

To test whether TY-172 resistance also affects long-distance movement of the virus, the appearance of viral DNA at the plant apex was monitored following inoculation of the third leaf from the top. Viral DNA was detected in the plant apex two days after inoculation in both the susceptible and resistant plants. Viral DNA accumulation at the plant apex was the same in both hosts until seven days after inoculation, after which a greater amount of viral DNA was found in the susceptible host. Overall, these results suggest that TY-172 interferes with the accumulation of viral ssDNA but not with viral long distance movement (Segev et al. 2004).

Concluding Remarks

Substantial progress has been made in the development of TYLCV-resistant tomatoes since efforts began nearly 40 years ago. Although no resistance was found in the cultivated tomato (*L. esculentum*), several sources of resistance have been found in various wild tomato species. These resistances vary in their mode of inheritance and, for the few that have been studied, are based on different resistance mechanisms. Since these individual sources provide only a limited level of resistance, improved resistance has been obtained by combining different resistances into single cultivars. However, for this approach to be successful, distinct virus resistance genes must be brought together, i.e. combining the same resistance genes (or alleles), even those originating from different resistant wild sources will probably not result in improved resistance. In order to do this, one must be able readily to distinguish different resistance genes. Resistance genes can be distinguished by developing linked molecular

markers, using these markers to map the different resistance genes, leading ultimately to the identification and isolation of the resistance genes. However, development of linked molecular markers could be very difficult when resistance is controlled by complex genetics, as seems to be the case with most of the resistances to TYLCV. Instead of following the genes that mediate the resistance, another approach would be to identify the mechanism by which the resistance interferes with viral infection. Combining different resistance genes which operate *via* different mechanisms and which are able to operate simultaneously, may potentially lead to the development of tomato plants with superior and long-lasting resistance to TYLCV.

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