

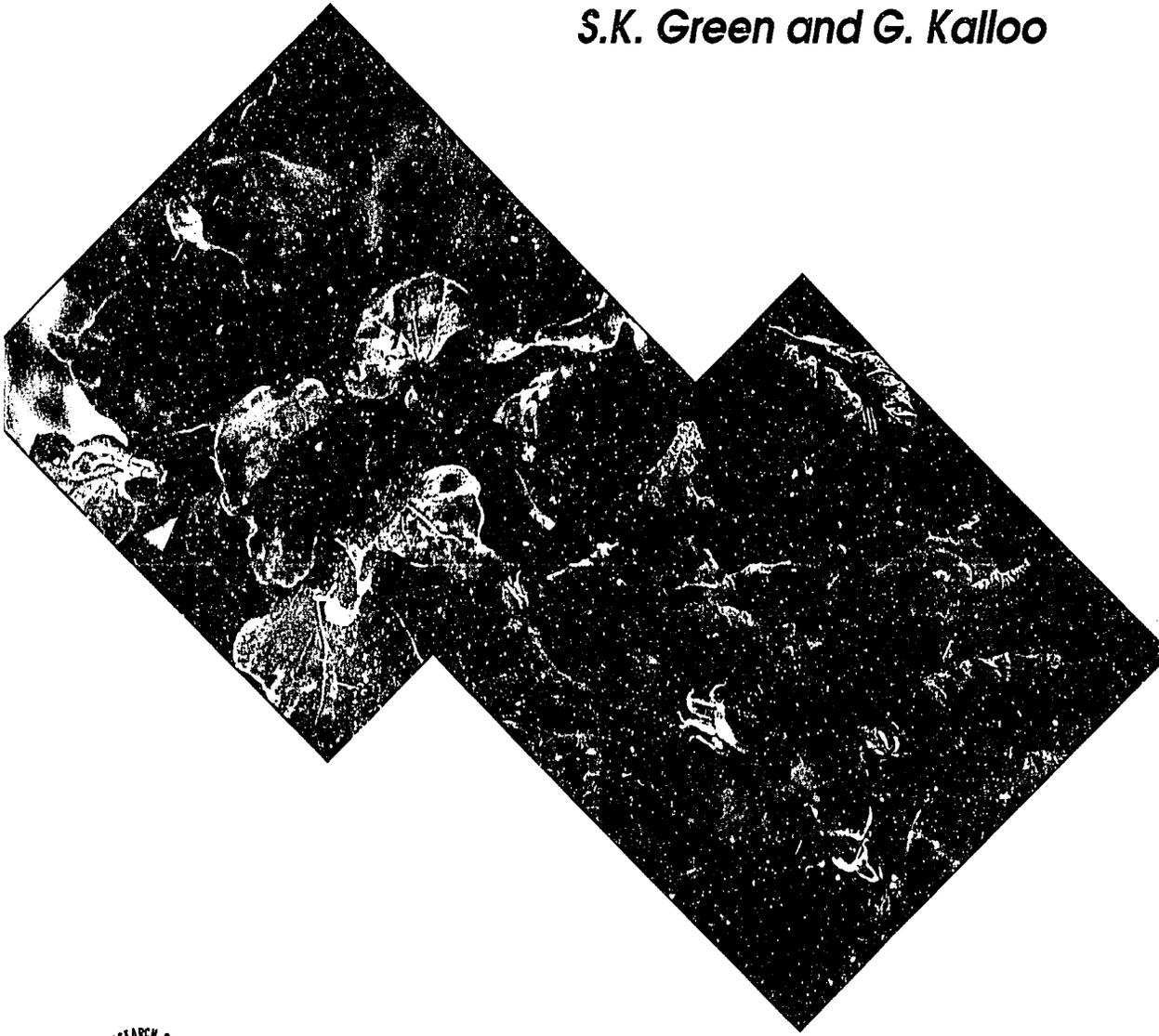
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# Leaf curl and yellowing viruses of pepper and tomato: an overview

*S.K. Green and G. Kalloo*



Asian Vegetable Research and Development Center

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Technical Bulletin No. 21

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**Leaf curl and yellowing viruses**

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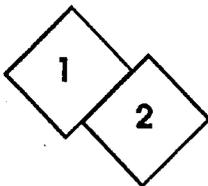


**Asian Vegetable Research and Development Center**

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*About the cover:* 1 — Tigré disease-affected pepper in Tamaulipas, Mexico (*courtesy J.K. Brown*)  
2 — Tomato leaf curl virus-affected tomato (*courtesy S.K. Green*)

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## Foreword

Many virus diseases affect tomatoes and peppers. Among these the leaf curl and yellowing diseases caused by whitefly-transmitted geminiviruses have become increasingly important in recent years. They cause considerable yield losses, chemical and cultural control measures are ineffective, and high levels of host plant resistance in commercial cultivars are not yet available. Furthermore, a number of different geminiviruses, some of which have still not been well characterized, appear to be involved in the leaf curl and yellowing disease syndrome.

Joint discussions with AVRDC's partners have identified the leaf curl virus complex of tomato as one of the center's major targets. It is being addressed in the crop improvement program at AVRDC. It is also a priority research topic for three of four AVRDC networks: the South Asia Vegetable Research Network (SAVERNET), the Collaborative Network for Vegetable Research and Development in Southern Africa (CONVERDS), and the Collaborative Network for Vegetable Research and Development in Central America (CONVERDCA).

At AVRDC the focus is on resistance breeding, the development of diagnostic probes, and virus diagnosis; the networks address epidemiological studies and multilocational resistance screening.

So far, the importance of whitefly-transmitted geminiviruses causing leaf curl and yellowing diseases of peppers has not been established since their symptoms are easily confused with similar symptoms caused by other viruses, insects, or mites. AVRDC is, therefore, assisting the national agricultural research systems (NARS) in the different regions to clarify this issue.

This review on leaf curl and yellowing viruses of tomatoes and peppers has been compiled by AVRDC virologist Dr. S.K. Green in collaboration with our SAVERNET partner Dr. G. Kalloo from India to assist breeders, virologists, and extension workers in the NARS in addressing various aspects of the leaf curl and yellowing virus syndrome on these two crops.

**S. Shanmugasundaram**  
*Director*  
*International Cooperation Program*

## Acknowledgments

The authors appreciate the generous assistance of several persons. Special credit goes to D.P. Maxwell, University of Wisconsin, Madison, USA for providing current information on geminiviruses and for reviewing the manuscript.

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## Introduction

Whitefly-transmitted geminiviruses cause epidemics in vegetable, staple, and fiber crops. The diseases are generally associated with local or regional whitefly (*Bemisia tabaci*) infestations and are characterized by one or more of the following symptoms: severe yellowing, yellow mosaic, leaf curl, and stunting. The prevalence and distribution of these viruses and their vector in subtropical, tropical, and fringe temperate regions has escalated in recent years for reasons not yet understood. Changing agricultural practices (most notably the continuous cultivation of crops such as cotton, beans, soybean, tomato, pepper, and melon which are susceptible to the viruses and are attractive hosts for the whiteflies) certainly account for some of the increase in the severity and the vast spread of diseases caused by geminiviruses (Brown 1991). This has led to an increased awareness throughout the world of the importance of these diseases and has spurred intensive research on the viruses involved.

The epidemiology of whitefly-transmitted geminiviruses is characterized by a close correlation between disease incidence and whitefly populations which often show strong seasonal fluctuations. This factor has been important in the development of certain control methods that are based on the timing of transplanting. There is, so far, no evidence for seed transmission of whitefly-transmitted geminiviruses, a factor of major significance in the international exchange of seeds.

In the past, exact diagnosis of the viruses involved in these diseases was not possible. Traditionally, serological methods have been a primary means of virus detection and virus diagnosis. This approach has met with limited success with whitefly-transmitted geminiviruses because they are extremely difficult to purify. The few polyclonal antisera produced showed high cross reactivity with heterologous antigens (Stein et al. 1983; Chiemsombat et al. 1991; Muniyappa et al. 1991). Cross reactivity with closely or distantly related geminiviruses was also observed with monoclonal antibodies (MacIntosh et al. 1992; Swanson et al. 1992). For many years, disease diagnosis has, therefore, relied mainly on the observation of typical disease symptoms and whitefly transmissibility. In some cases, visualization of nuclear inclusion bodies by light microscopy (Christie et al. 1986) and/or ultrastructural localization of virions by transmission electron microscopy (Osaki and Inouye 1978; Thongmeearkom et al. 1981; Harrison 1985; Saikia and Muniyappa 1989; Channarayappa et al. 1992) were used to confirm initial diagnosis. None of these features are, however, virus-specific and they could not be used to differentiate among the whitefly-transmitted geminiviruses. However, with the emergence of new technologies it has become possible to develop nucleic acid probes that accurately detect and identify these viruses. These techniques have provided some insights on the epidemiology and the genetic diversity of these viruses (Pouiston et al. 1989; Czosnek et al. 1990; Gilbertson et al. 1991).

Whitefly-transmitted geminiviruses infecting dicotyledonous plants are now known to have genomes of either one or two circular single-stranded DNA molecules. The genome of monopartite geminiviruses is about 2,800 nucleotides (nt) (Kheyr-Pour et al. 1991; Navot et al. 1991). The bipartite geminiviruses have two nearly equal-size DNA molecules of 2,600 – 2,800 nt, designated DNA-A and DNA-B. The nucleotide sequences of the two components of a given geminivirus are different, except for the sequence of an intergenic region of about 200 nucleotides called the common region. The nucleotide sequence of the common region, however, is divergent among different geminiviruses except for a highly conserved putative stem-loop motif (Lazarowitz et al. 1992) (fig. 1). Most bipartite geminiviruses require both DNA components for infection.

DNA-A encodes for all viral functions necessary for virus replication and encapsidation of viral DNA (Rogers et al. 1986; Sunter et al. 1987; Elmer et al. 1988; Hanley-Bowdoin et al. 1989; Sunter and Bisaro 1992; Lazarowitz et al. 1992; Lazarowitz 1992). The DNA-B encodes for functions associated with virus movement within the plant (Sunter et al. 1987; Revington et al. 1989; Sunter and Bisaro 1992; Lazarowitz 1992) and symptom expression (Revington et al. 1989; von Arnim and Stanley 1992).

The major whitefly-transmitted geminivirus diseases of peppers and tomato throughout the world are reviewed with emphasis on virus detection and characterization, epidemiology, chemical and cultural control methods, and resistance.

Color plates showing the symptoms of some of the leaf curl and yellowing diseases of peppers and tomato are shown on pages 14–20.

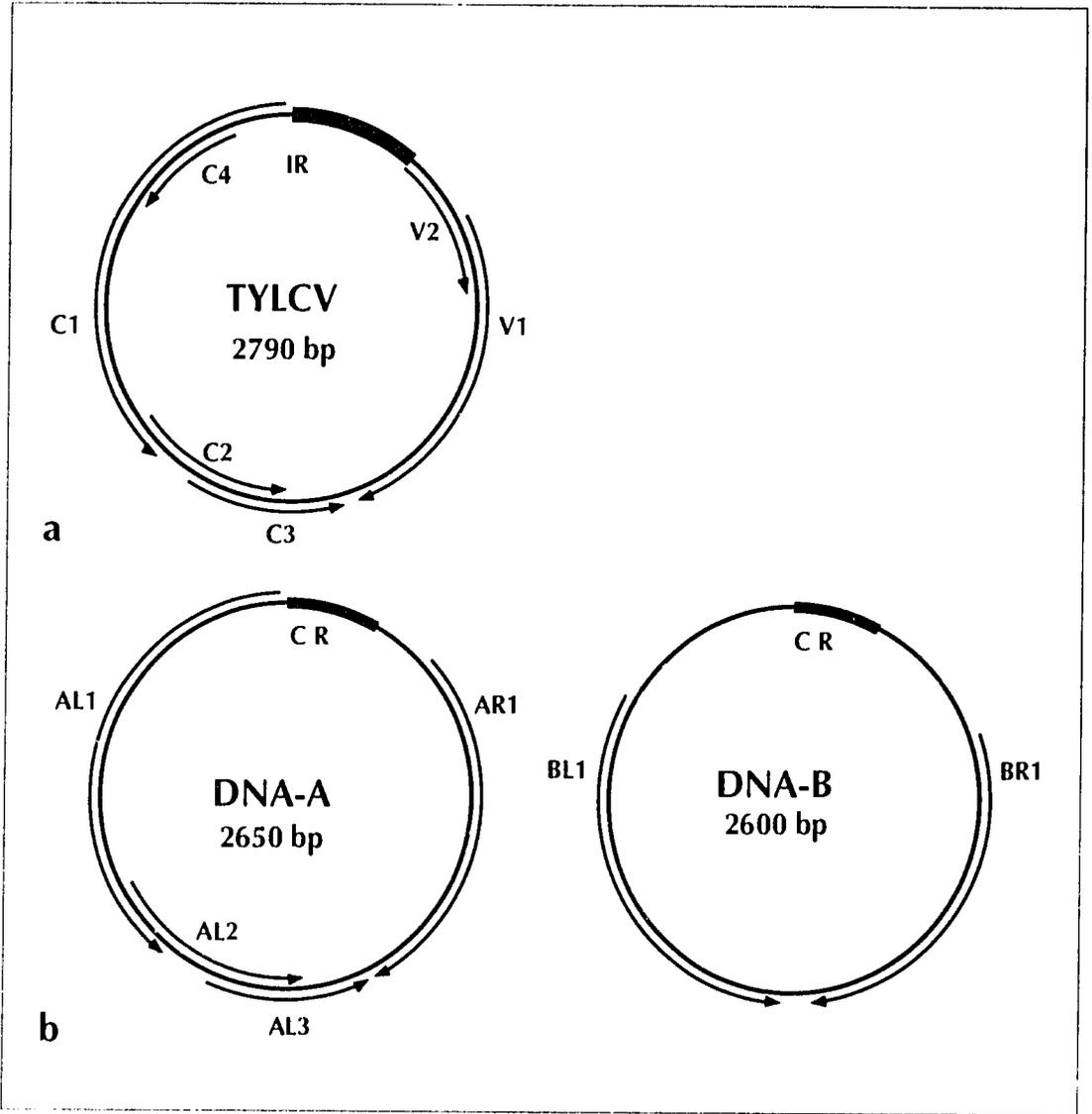


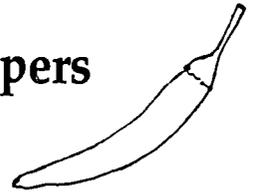
Fig. 1. Genome organization for the two types of whitefly-transmitted geminiviruses (Nakhla et al. 1992)

a) monopartite: TYLCV from Israel (Navot et al. 1991)

b) bipartite: bean golden mosaic geminivirus type II (Faria et al. personal communication)

(open reading frames [ORFs] are V or R [viral sense polarity] or C or L (complementary sense polarity). DNA-A or DNA-B are designated A or B. 1, 2, 3, or 4 represent the ORF number. The intergenic [IR] or common region [CR] is presented as a wide bar, with nucleotide number 1 at the beginning of this region.); bp = base pairs

# Leaf curl and yellowing diseases of peppers



## Southeast and East Asia

There are various reports of leaf curl diseases affecting peppers in Southeast and East Asia, particularly India (Vasudeva and Samraj 1948; Gattani and Mathur 1951; Vasudeva 1954; Mishra et al. 1963; Muniyappa and Veeresh 1984) and Sri Lanka (Sugiura et al. 1975; Shivanathan 1982). Losses of up to 80% have been reported in many parts of northern India (Singh et al. 1979). Symptoms usually are stunting of the plants and heavy crinkling and rolling of the leaves with chlorosis or yellowing. Older leaves may become leathery and brittle. Mites, thrips, and viruses are often associated with these leaf curl complexes.

Early reports indicate that the diseases are caused by tobacco leaf curl virus (TLCV) based both on their transmission by the whitefly *B. tabaci* to tobacco and tomato and on the typical symptoms produced on these hosts (Pal and Tandon 1937). Recent whitefly-transmission studies have indeed shown that an isolate of tobacco could be experimentally transmitted to *Capsicum annuum* and 34 other plant species, including *Lycopersicon esculentum*, *Beta vulgaris*, *Carica papaya*, *Cyamopsis tetragonoloba*, *Sesamum indicum*, *Phaseolus vulgaris*, and *Petunia hybrida* (Valand and Muniyappa 1992). The involvement of different virus strains has been implied (Singh and Lal 1964; Nariani 1968; Dhanraj et al. 1968). In India, leaf curl disease of peppers is prevalent throughout the country, although severity is less in southern India.

Control of the disease relies on vector control by various chemicals applied as foliar sprays or directly into the soil. These chemicals include nicotine sulphate, methylparathion, carbofuran, and others.

Screening for resistance started in the late sixties (Dhanraj et al. 1968). Most screening has been under field conditions, assessing disease incidence and disease severity (Sharma and Singh 1985; Memane et al. 1987; Tewari and Viswanath 1988). For disease rating, a coefficient of infection has been used, for which the percentage disease incidence was multiplied with a response value assigned to each observed disease severity grade. A good correspondence was usually obtained between field and greenhouse assessments (Mayee et al. 1975). Since then, a number of lines with varying degrees of resistance have been identified (table 1). Some of these lines also exhibit resistance or tolerance to other viruses (Tewari and Viswanath 1986). Many multiple virus-resistant varieties have been developed at Punjab Agricultural University, Ludhiana. Important multiple resistant lines are Perennial, BG-1, Lorai, and Punjab Lal (Thakur et al. 1987; Singh and Kaur 1990). The variety Pant C-1, resistant to mosaic and leaf curl disease, has been released by Pantnagar Agricultural University. However, some of these multiple disease-resistant lines have undesirable agronomic characteristics such as small fruit size, late maturity, and low yield. Recently, lines such as LS-

III, LS-IV, and LS-1 with good horticultural attributes have been developed (Hundel, J.S. personal communication 1993). However, no systematic multilocation testing has been conducted with these lines to determine the stability of resistance.

**Table 1. Pepper germplasm and breeding lines reported to be resistant or tolerant to leaf curl viruses in India**

Line	Reference
Jwala	Tewari and Ramanujam 1974
Karanja, Pant C-1, S 46-1, IC 18253, IC 18885, JCA 196, Cross 218, EC 121490	Bhalla et al. 1983
Pant C-1, Pant C-2, <i>Capsicum angulosum</i>	Konai and Nariani 1980
Jwala, G-4, CA-960	Dhanju 1983
Pant C-1, Lorai, Loungi, Perennial, S 118-2	Sharma and Singh 1985
Ci 1, LIC-45, N-146	Memane et al. 1987
Pant C-1, Pusa Jwala, NP 46A, JCA 19	Sangar et al. 1988; Brar et al. 1989
Perennial, Surjamani	Sooch et al. 1976
LS-VIII, LS-IV, LS-I	Hundel, J.S., personal communication <sup>1</sup> 1993
Pusa Jwala x Delhi Local, Sel 38-2-1, Sel 94-4-9-3, Sel 101-2-33	Tewari and Viswanath 1986

<sup>1</sup> J.S. Hundel, Department of Vegetable Crops, Punjab Agricultural University, Ludhiana, India.

## Americas

In the Americas, geminiviruses have only recently become an economic problem in pepper production. Following severe infestations of *B. tabaci* in the northeastern part of Mexico and the Rio Grande Valley of Texas during 1986–88, several previously unrecognized diseases occurred in hot and sweet peppers in that region (Campodonico and Montelongo 1988; Campodonico and Quintero 1988).

Pepper mild tigré virus (PMTV) is a member of a virus complex affecting peppers in the eastern and western coastal pepper production areas of Mexico, which the local growers refer to as 'tigré' disease (tiger disease). Tigré disease is usually characterized by leaf curling, small and puckered leaves, distinct marginal and interveinal chlorosis, and various degrees of stunting (Brown et al. 1989; Brown and Nelson 1989; Black et al. 1991). PMTV alone causes mild symptoms on peppers, which include veinal distortion, extremely mild mottle, interveinal chlorosis, and slight stunting. Symptoms develop about 12–14 days after whitefly inoculation (Brown et al. 1989; Brown and Nelson 1989). The virus cannot be transmitted mechanically. Experimentally, PMTV is transmissible by whiteflies from pepper to pepper and to tomato, from tomato to tomato, but not from tomato to pepper (Brown unpublished 1994). The virus can also be transmitted to

*Datura stramonium* using the whitefly vector. It is not yet known whether the disease caused by PMTV is economically important. Yield loss studies in the field have not been feasible, because the virus usually occurs in conjunction with other whitefly-transmitted geminiviruses, most of which have not been characterized.

Chino del tomate virus (CdTV) is another nonmechanically transmissible geminivirus isolated from tigré-affected peppers in Mexico (Brown and Hine 1984). The virus causes a mild mosaic and slight leaf disformation on peppers (Brown and Nelson 1988, 1989; Gallegos 1978). Yellow striping has occasionally been observed on green fruited bell peppers. The virus commonly infects tomato causing severe stunting, downward curling of the leaves, and interveinal chlorosis. *Malva parviflora* is another natural host besides tomato and peppers. Experimentally, this virus can be transmitted by whiteflies to *Datura stramonium* and *Phaseolus vulgaris* (Brown and Poulos 1990).

CdTV and PMTV most likely belong to the bipartite group of geminiviruses. The B component of CdTV has been cloned and sequenced (Brown, J.K. personal communication 1994).

In Northeastern Mexico (Tamaulipas), a similar disease complex called 'rizado amarillo del chile' (pepper yellow curl) affects peppers. Two different geminiviruses have been identified in the complex — pepper huasteco virus (PHV) (Garzon-Tiznado et al. 1993) and recently pepper Jalapeño virus (PJV) (Rivera-Bustamante, R. personal communication 1994). Pepper Jalapeño virus, presently undergoing molecular characterization, belongs to the squash leaf curl phylogenetic cluster (Rivera-Bustamante, R. personal communication 1994). The two viruses are not mechanically transmissible. When inoculated independently by means of whiteflies, the two viruses produce only mild symptoms. However, when inoculated in combination, symptoms very similar to those occurring in the field are produced. Pepper huasteco virus, a bipartite virus, has been cloned and has undergone molecular characterization (Garzon-Tiznado et al. 1993; Torres-Pacheco et al. 1993). Cloned viral DNAs were infectious when inoculated by a biolistic procedure. This procedure involves helium pressure-based bombardment with tungsten particles coated with full-length viral DNAs excised from plasmid vectors. Both the A and the B component were necessary for infectivity (Garzon-Tiznado et al. 1993). It has been suggested that PHV is either a very close variant of or the same virus as PV-WB (Rivera-Bustamante, R. personal communication 1994). PV-WB was first described from peppers in Weslaco, Texas affected by bright yellow splotches (Brown 1991). The common regions of both viruses have been cloned and sequenced and were found to be almost identical (Rivera-Bustamante, R. personal communication 1994).

Four other whitefly-transmitted geminiviruses, apparently distinct from PMTV and CdTV, PHV, and PJV were identified in peppers in Mexico and Texas. The first, isolated from Weslaco, Texas, and designated as PV-WA, causes vein etching and a bright, splotchy, but mild chlorosis (Brown 1990). The virus is mechanically transmissible. The second, designated Texas pepper geminivirus (TPGV), was also associated with peppers in Texas (Stenger and Duffus 1989; Stenger et al. 1990). Leaves of affected plants tend to be distorted, curl upward at the margins, exhibit bright yellow spots, and at times show yellow margins that extend into the interveinal tissues (Black et al. 1991). This virus is mechanically transmitted and also infects tomato. This bipartite geminivirus (Stenger et al. 1990) belongs to the squash leaf curl virus phylogenetic cluster (Loniello, A.O. and Maxwell, D.P. personal communication 1993). The third virus, designated as Serrano golden mosaic virus (SGMV), infects peppers as well as tomato in Mexico and Arizona (Brown and Poulos 1990). The virus causes a bright golden mosaic without leaf curling on the leaves of infected pepper plants. Fruits may be smaller and misshapen. *Datura stramonium* and *Nicotiana benthamiana* can be experimentally infected by using whiteflies. The virus can be mechanically transmitted — although with great difficulty — from pepper to pepper, but not from tomato to pepper or pepper to tomato. The fourth

virus, tentatively named Sinaloa tomato leaf curl virus (STLCV) was recently found to infect peppers and tomato in Mexico (Brown et al. 1993). On pepper the virus causes a yellow mosaic mottle. The virus is experimentally transmissible by whiteflies from tomato to tomato, pepper, *Phaseolus vulgaris*, *Malva parviflora* and also to eggplant on which it causes symptomless infection. It can also be transmitted — with difficulty — by mechanical means from tomato to tobacco. Nucleotide sequence analysis of the coat protein gene (AR 1) indicates that STLCV and SGMV are very similar (Brown, J.K. personal communication 1994). Both viruses apparently also belong to the bipartite group of geminiviruses (Brown, J.K. personal communication 1994).

Whitefly-transmitted geminiviruses have also been detected in pepper from Antigua, Belize, Costa Rica, the Dominican Republic, Guatemala, Puerto Rico, and Trinidad by DNA hybridization using A-component probes for New World whitefly-transmitted geminiviruses. These viruses have not yet been further characterized (Brown, J.K. personal communication 1994).

## **Mediterranean**

In Turkey and Syria, peppers were found infected with tomato yellow leaf curl virus (TYLCV) (Czosnek, H. personal communication 1993) using a specific DNA probe.



*Tigré disease-affected pepper in Tamaulipas, Mexico (courtesy J.K. Brown)*



*Tigré disease-affected pepper in Tamaulipas, Mexico (courtesy J.K. Brown)*



*Tigré disease-affected pepper in Mexico (courtesy R. Rivera-Bustamante)*



*Pepper mild tigré virus (PMTV) in chili pepper cv. Anaheim (courtesy J.K. Brown)*

### *Leaf curl and yellowing diseases of pepper*



*Chino del tomate virus (CdTV) in chili pepper cv. Anaheim (courtesy J.K. Brown)*



*Serrano golden mosaic virus (SGMV) (courtesy J.K. Brown)*



*Serrano golden mosaic virus (SGMV) (courtesy J.K. Brown)*



*Serrano golden mosaic virus (SGMV) in chili pepper cv. Anaheim (courtesy J.K. Brown)*



*Sinaloa tomato leaf curl virus (STLCV) in chili pepper cv. Anaheim (courtesy J.K. Brown)*



*PV-WA virus in chili pepper cv. Anaheim*  
(courtesy J.K. Brown)



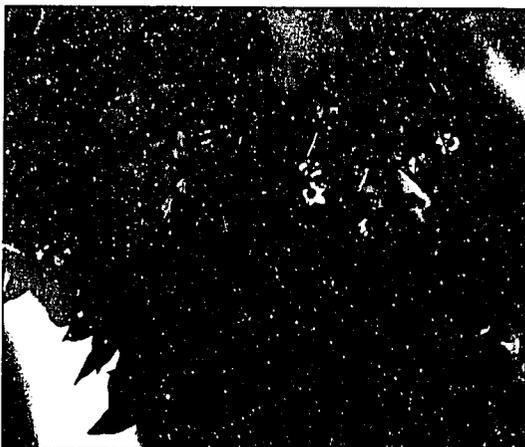
*Texas pepper geminivirus*  
(courtesy B. Villalon)



*Texas pepper geminivirus*  
(courtesy B. Villalon)



*Thailand tomato yellow leaf curl virus (TYLCV-Thai)* (courtesy K. Kruapan)



*Chili leaf curl syndrome in Sri Lanka.*  
The causal agent(s) have not yet been identified  
(courtesy L.L. Black)



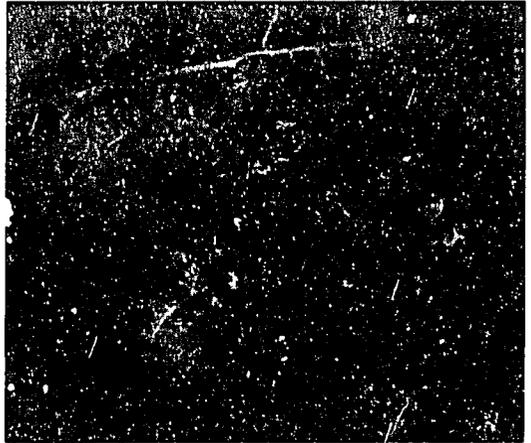
*Tomato yellow mosaic (mosaico amarillo del tomate) disease in Costa Rica (courtesy R. Lastra through J.K. Brown)*



*Tomato yellow mosaic (mosaico amarillo del tomate) disease in Venezuela (courtesy R. Lastra through J.K. Brown)*



*Pepper mild tigré virus in tomato cv. Pole Boy (courtesy J.K. Brown)*



*Chino del tomate virus in tomato cv. Pole Boy (courtesy J.K. Brown)*



*Eastern Mediterranean strain of TYLCV in San Juan de la Maguana, the Dominican Republic (courtesy D.P. Maxwell)*



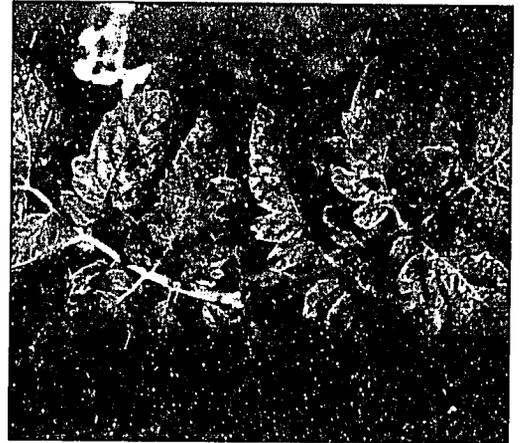
*Serrano golden mosaic virus (SGMV)*  
(courtesy J.K. Brown)



*Serrano golden mosaic virus (SGMV)*  
(courtesy J.K. Brown)



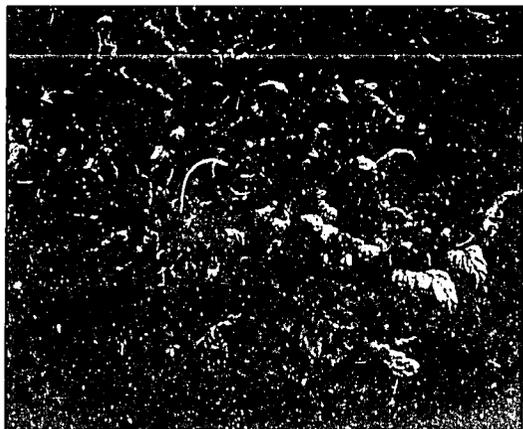
*Sinaloa tomato leaf curl virus (STLCV)* on  
tomato cv. Pole Boy (courtesy J.K. Brown)



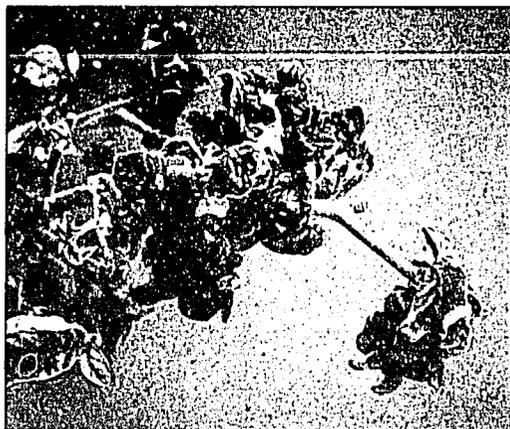
*Tomato mottle geminivirus (ToMoV)* in Florida  
(courtesy J.K. Brown)



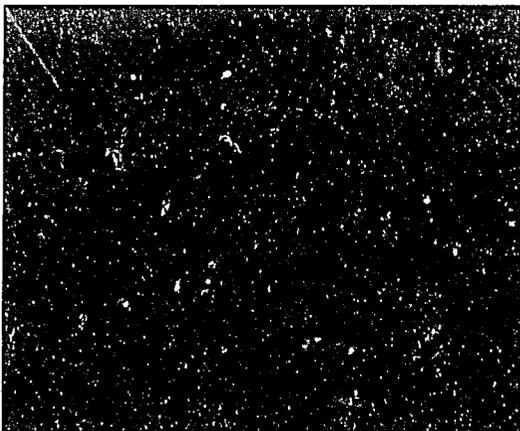
*Thailand tomato yellow leaf curl virus (TYLCV-Thai)*: tomato plant artificially inoculated with the A-component using a particle gun  
(courtesy J.K. Brown)



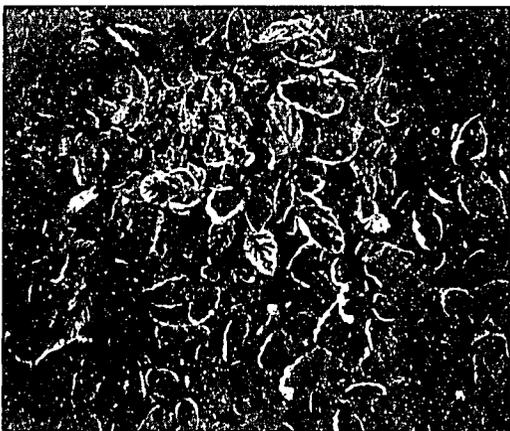
*Tomato yellow leaf curl virus (TYLCV) in Israel (courtesy H. Laterrot)*



*Tomato yellow leaf curl virus (TYLCV) in Egypt (courtesy D.P. Maxwell)*

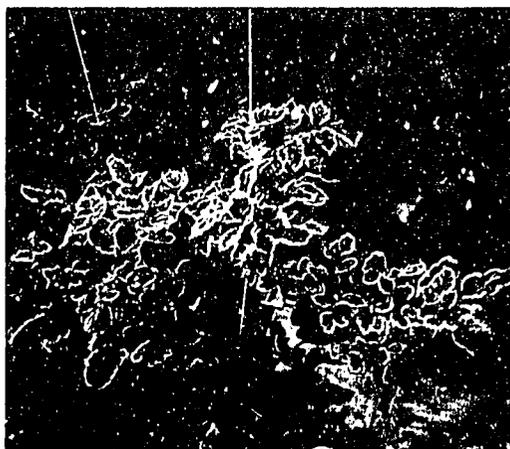


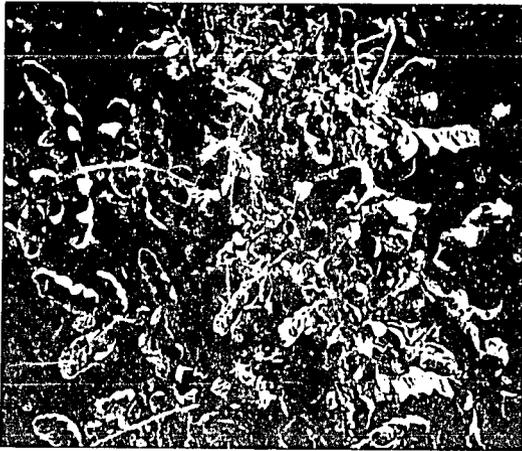
*Tomato yellow leaf curl virus (TYLCV) in Tunisia (courtesy H. Laterrot)*



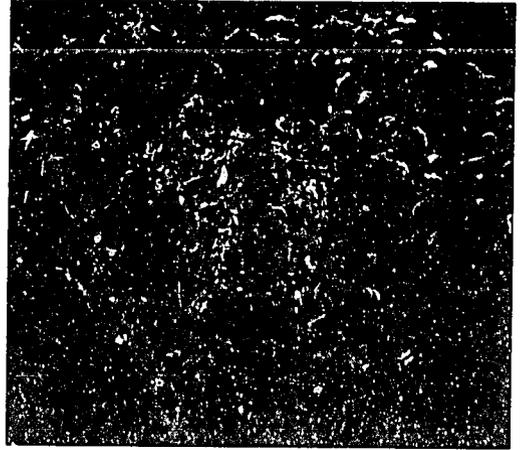
*Tomato yellow leaf curl virus (TYLCV) in Cyprus (courtesy H. Laterrot)*

*Tomato yellow leaf curl disease in Sicily. The virus involved is a whitefly-transmitted geminivirus which is distinct from the TYLCV of Egypt and Israel (courtesy H. Laterrot)*





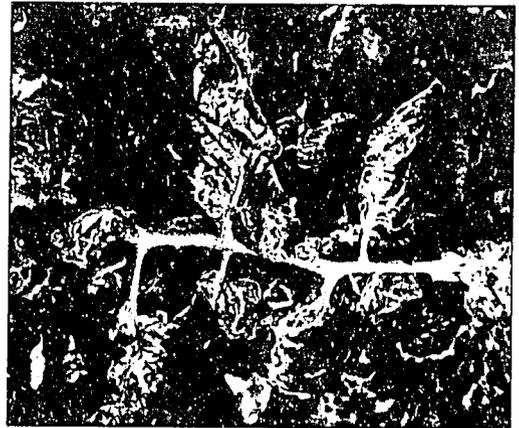
*Tomato yellow leaf curl virus (TYLCV) in Senegal (courtesy H. Laterrot)*



*Tomato yellow leaf curl virus (TYLCV) in Mali (courtesy H. Laterrot)*

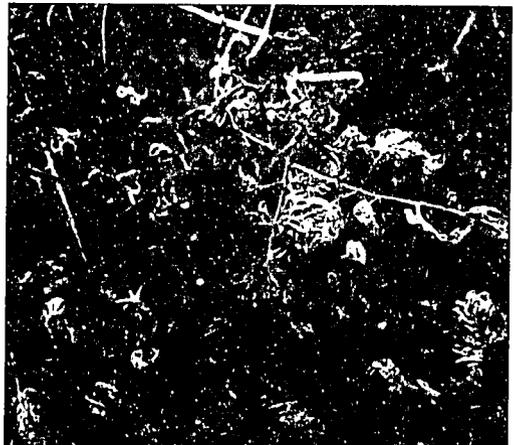


*Leaf curl virus-infected tomato in Guatemala. The virus involved is a whitefly-transmitted geminivirus which has not been exactly characterized (courtesy D.P. Maxwell)*

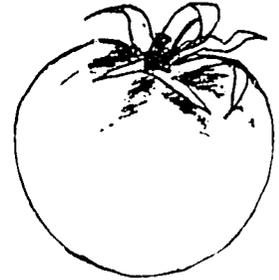


*Leaf curl virus-infected tomato in Guatemala. The virus involved is a whitefly-transmitted geminivirus which has not yet been exactly characterized (courtesy D.P. Maxwell)*

*Leaf curl virus-infected tomato in Costa Rica (courtesy D.P. Maxwell)*



## Leaf curl and yellowing diseases of tomato



### Mediterranean

#### *Tomato yellow leaf curl virus (TYLCV)*

Tomato yellow leaf curl disease has been a major constraint to tomato production in the Near East since 1966 (Cohen and Nitzany 1966). This virus is described here in detail because it is the best characterized virus of those causing yellowing and leaf curl diseases of tomatoes.

#### The disease

Affected tomato plants are stunted and their branches and petioles tend to assume an erect position. Leaflets are smaller than those of healthy plants, puckered, and often show an accentuated upward curling of their margins with or without yellowing. Growth of the plants is inhibited. Affected plants produce either no fruit or few small-sized fruits depending on the stage of development at which the viral attack occurs. Yield losses in tomato usually range from 50 to 75% (Yassin and Nour 1965; Makkouk et al. 1979; Al Musa 1982; Makkouk and Laterrot 1983) but may be as high as 100%, making tomato production unprofitable.

#### The virus

Particles of tomato yellow leaf curl virus are 20 x 30 nm in size. The genome of TYLCV from Israel has been cloned, sequenced, and found to be monopartite and to contain only one single-stranded DNA of approximately  $0.63 \times 10^6$  daltons, corresponding to 2,787 nucleotides (Czosnek et al. 1988; Navot et al. 1991, 1992).

Infectivity of the DNA was demonstrated. Single-stranded DNA served as a template for in vitro synthesis of its double-stranded form. Restriction analysis with various restriction enzymes yielded several DNA fragments. A head-to-tail dimer of the cloned TYLCV-DNA was inoculated into tomato plants via *Agrobacterium tumefaciens* (agroinoculation or agro-mediated inoculation) inducing symptoms indistinguishable from those produced by natural infection (Navot et al. 1991; Kheyr-Pour et al. 1991; Bendahmane et al. 1991). The virions produced were acquired and transmitted by whiteflies to test plants, inducing typical symptoms (Antignus and Cohen 1992). Therefore, the single component of TYLCV carries all the information necessary to induce the full disease cycle (Navot et al. 1991; Kheyr-Pour et al. 1991; Bendahmane et al. 1991).

## Virus transmission

The virus is transmitted in nature by the whitefly *B. tabaci* in a semipersistent (circulative) manner. Minimum acquisition and inoculation feeding periods are 15–30 minutes. The latent period in the vector is more than 20 hours. The virus is retained by the vector for up to 20 days but not throughout the life span of the whitefly (Cohen and Nitzany 1966). The virus can be acquired by larval as well as adult stages of the insect, but is not transmitted to the progeny. Whiteflies can carry a finite number of virions, in the range of 600 million, indicating that their acquisition is regulated (Zeidan and Czosnek 1991). TYLCV DNA replicates in the insect shortly after virus acquisition (Zeidan and Czosnek 1993). A single whitefly is able to transmit the virus and the rate of transmission increases with increased population density of the vector (Mansour and Al Musa 1992).

Although symptoms usually appear at about 15 days post-whitefly inoculation, viral DNA can be detected 7 days earlier. TYLCV-DNA concentration peaks at 4 days before symptom appearance. The highest concentrations of TYLCV-DNA were found in rapidly growing tissues such as shoot apices, young leaves, and roots. Young leaves and apices are best for inoculation by whiteflies (Ber et al. 1990).

Mechanical transmission has not been possible and there are no reported cases of transmission through seed.

## Geographic distribution

Tomato yellow leaf curl disease was first reported to be a major constraint to tomato production in the Near East in the mid-1960s (Cohen and Harpaz 1964; Cohen and Nitzany 1966). This disease has since also spread to Turkey (Abak et al. 1991), the Arabian Peninsula (Mazyad et al. 1979), Sudan (Yassin and Nour 1965), and West and East Africa (Defrancq d' Hondt and Russo 1985; Czosnek et al. 1991; Dembele 1993).

By using a TYLCV-Israel-specific DNA probe containing the intergenic region in squash blot hybridization tests, an exact assessment of the worldwide spread of TYLCV virus has been possible (Czosnek et al. 1989, 1990; Navot et al. 1989). The virus was found to affect tomato plants in three large geographical regions: the Mediterranean Basin (Cyprus, Egypt, Jordan, Israel, Lebanon, Turkey), Western Africa (Cape Verde, Senegal), and Eastern Africa (Sudan, Tanzania) (Czosnek et al. 1991).

Comparison of the AL1 (C1) genomic region of TYLCV from Egypt with the TYLCV from Israel showed 96% nucleotide sequence homology, confirming that these two viruses are nearly identical (Nakhla et al. 1992). However, tomato yellow leaf curl viruses from southern Italy, Sardinia, and Spain causing almost identical symptoms as TYLCV from Israel (Luisoni et al. 1989; Credi et al. 1989; Kheyr-Pour et al. 1993; Noris et al. 1994) were found to be quite different from TYLCV, showing only 70% nucleotide sequence homology (Czosnek et al. 1991). The AL1 (C1) region of the TYLCV from Sardinia was found to have only 78% nucleotide sequence homology with the corresponding region of TYLCV from Egypt. These two isolates should, therefore, tentatively be considered separate strains of TYLCV. This was confirmed by cloning and sequencing the isolate from Sardinia (Kheyr-Pour et al. 1991).

Several tomato-infecting geminiviruses are known to occur in Asia. These seem to be only remotely related to TYLCV from Israel or TYLCV from Sardinia. Leaf curl virus-infected tomato from India, Thailand, and Taiwan did not react with a narrow range TYLCV-Israel-specific probe consisting of a 347-bp DNA

fragment of the TYLCV intergenic region (Navot et al. 1991), which detects only the TYLCV from Israel and its closest relatives. However, they did react with a broad range nucleic acid probe which consisted of the full length DNA clone of the TYLCV from Israel, indicating that these tomatoes were infected with a distantly related geminivirus. These viruses will be described later.

Tomato plants from North America (Florida), the Caribbean (Guadeloupe), Central America (Costa Rica), and South America (Venezuela) showing symptoms typical of geminivirus infection did not react at all with any of the probes of the TYLCV from Israel (Czosnek et al. 1990) and, thus, are considered distinctly different geminiviruses. These viruses are discussed later.

## Host range

In nature, the virus mainly infects tomato. The experimental host range of TYLCV is narrow, mainly infecting some species of the Solanaceae, Compositae, and Caprifoliaceae. The virus was studied by artificial inoculation of 40 species and varieties of plants belonging to nine families using an isolate from Israel (Cohen and Nitzany 1966). Thirty to 50 whiteflies, previously given an acquisition access feeding period of 48 hours on infected plants, were caged for 48 hours on test plants. *Lycopersicon hirsutum* and *Datura stramonium* were found susceptible to the virus and had clear symptoms. Although systemic infection was established by positive whitefly transmission tests with the following species no symptoms were observed on *Lens culinaris*, *Lycopersicon peruvianum*, *L. pimpinellifolium*, *Malva nicaensis*, *Nicotiana glutinosa*, *N. tabacum* Samsun, and *Phaseolus vulgaris* Bulgarit.

The following species and varieties were apparently immune: *Ageratum houstonianum*, *Althaea rosea*, *A. setosa*, *Arachis hypogaea*, *Beta vulgaris*; *Chenopodium amaranticolor*; *Cucumis sativus* Beit Alpha; *Ecballium elaterium*, *Euphorbia cybirensis*, *E. paralias*, *Gomphrena globosa*, *Gossypium hirsutum* Acala 4-42, *Abelmoschus esculentus*, *Ipomoea batatas* Lam. 21 and Gokoku, *Malva parviflora*, *Medicago sativa* Peruvian, *Nicotiana repanda*, *N. rustica*, *N. tabacum* White Burley, *Pisum sativum* var. *arvense* Dunn, *Physalis floridana*, *Ricinus communis*, *Sida rhombifolia*, *Trifolium alexandrinum* Faheli, *Vicia faba* Cypriote, and *Zinnia elegans* Will Rogers.

Unlike the Israel isolate, a TYLCV isolate from Jordan did not infect *P. vulgaris*. Additional immune hosts of the Jordanian isolate are: *Amaranthus caudatus*, *A. retroflexus*, *A. tricolor*, *Chenopodium quinoa*, *C. album*, *Brassica chinensis*, *B. nigra*, *B. oleracea* var. *botrytis*, *B. oleracea* var. *capitata*, *Capsella bursa-pastoris*, *Citrullus vulgaris* Crimson Sweet, *Cucumis melo* Amco Sweet, *C. melo* var. *flexuosus*, *C. sativus* Zios, *Cucurbita pepo* Victoria, *Luffa acutangula*, *L. cylindrica*, *Glycine max*, *Phaseolus vulgaris* Black Turtle, Bountiful, Gold Crop, Pinto and Top Crop, *Pisum sativum* Local, *Vigna unguiculata* California Blackeye, *Hibiscus esculentus*, *Malva sylvestris*, *Capsicum annuum*, *C. frutescens*, *Datura tatula*, *Nicandra physaloides*, *Nicotiana benthamiana*, *N. clevelandii*, *Solanum melongena*, and *S. nigrum* (Mansour and Al Musa 1992).

## Disease control

Several options are available to reduce TYLCV incidence in the field. These aim to control the vector and include the use of chemicals, reflective mulches, mixed cropping with plants more attractive to the vector, elimination of weeds and other crops which may serve as virus reservoirs, production of pathogen-free tomato transplants, and adjusting the date of planting so that it does not coincide with high vector population. The most effective and environmentally safe method is the planting of tolerant lines which have recently become available from national programs and private seed companies.

## 1) Chemical control of the vector

In Israel, best results were obtained with endrin, methidathion, and cutnion (Nitzany 1975). In trials in Lebanon, a slight delay in infection was observed and a slight increase in yield obtained when the insecticides azinophos methyl, methidathion, and methomyl were used twice weekly for 8–10 weeks after transplanting. However, the small yield increase did not justify, either economically or ecologically, the use of 16–20 pesticide applications (Makkouk and Laterrot 1983).

Yassin (1975) reported significant decrease in TYLCV incidence and significant increase in yield after weekly sprays of omethoate, malathion, or mevinphos when used either in the seedbed or after transplanting. In Jordan, Sharaf and Allawy (1981) reported two- to threefold increases in tomato yield and a reduction in virus incidence when the insecticides permethrin, methidathion, and pyrimiphos-methyl were applied together with mineral oils (HiPar or SUNOCO). However, these insecticides were unsuccessful at certain times of the year when large populations of whiteflies exist. Spraying the plants in the field with only mineral oils or sesame oil was also found to considerably reduce the incidence of the disease (Yassin et al. 1982). Best control was attained by physically protecting both seedbeds and young plants and by applying chemicals at regular intervals before and after transplanting.

However, since whiteflies rapidly develop resistance to insecticides, the benefits of insecticides usually decrease in successive years (Cohen and Melamed-Madjar 1974). Moreover, experience suggests that chemical measures can at best provide only partial control of TYLCV. Additional means are needed to contain outbreaks effectively.

## 2) Control by crop management

*Adjustment of planting date and avoidance of vector.* In southern Turkey, TYLCV incidence could be considerably reduced by planting the fall crop a few weeks later, at the beginning of October, instead of the usual planting in August/September when whitefly populations are highest (Abak et al. 1991). Similarly, in the Jordan valley, disease incidence is lower when the fall crop is planted later (Kasrawi 1991).

*Crop mulching.* Covering the soil with fresh wheat straw decreases TYLCV incidence (Cohen and Melamed-Madjar 1974). The mulch is most effective in seedbeds and at early stages of growth giving a 2-week delay in virus spread. With yellow polyethylene mulch, virus spread can be delayed by 4 weeks. The whiteflies are attracted to the yellow plastic where they are then killed by the heat (Cohen 1981; Keren et al. 1991; Zamir 1991).

*Mixed cropping.* Crops known to be good hosts for the vector, but not for the virus, attract the vector and, hence, reduce virus incidence when grown in mixed stands. TYLCV infection was delayed in Jordan when tomato was interplanted with cucumber (Al Musa 1981) or with pigeon pea (*Cajanus cajan*) (Yassin and Abu Salih 1976). In Egypt, whitefly trap crops such as melon are planted as borders around the tomato fields. Whiteflies colonize these plants which are then sprayed with an insecticide (Mazyad, H. personal communication via D.P. Maxwell 1993).

*Elimination of virus sources.* Elimination of volunteer tomatoes and common weed hosts such as *Datura stramonium* and *Malva nicaensis* within or near the tomato crop has also been suggested to reduce disease incidence (Makkouk and Laterrot 1983; Kasrawi 1991).

*Direct crop cover.* Lightweight nonwoven fabrics (17 g/m<sup>2</sup>) were shown to reduce and delay disease incidence (Reyd 1993).

### 3) Host plant resistance to the virus

Programs to develop TYLCV-resistant/tolerant cultivars have existed since 1974 in Israel<sup>1</sup> and later in Egypt<sup>2</sup> and France<sup>3</sup> using several wild species, including *L. pimpinellifolium*, *L. peruvianum*, *L. hirsutum*, and *L. cheesmanii* (Laterrot 1986, 1991; Zamir et al. 1991; Pilowsky and Cohen 1974, 1990).

Although the genetic basis for most of the apparent resistances in the wild species has not been fully defined, it appears to range from a single incomplete dominant gene in the case of *L. pimpinellifolium* (Kasrawi 1989; Pilowsky and Cohen 1974) to a polygenic pattern that is recessive in *L. cheesmanii* and polygenic dominant in *L. hirsutum* (Hassan et al. 1984). A very high level of resistance was recently found in one accession of *L. hirsutum*, LA 1777 (Moustafa 1991; Fargette 1991; Laterrot, H. personal communication 1993).

The breeding strategy of the European Economic Community (EEC) group has been to gradually develop improved populations derived from four resistance sources: *L. pimpinellifolium* (LA 121, LA 1478, LA 1582, Hirsute), *L. hirsutum* (PI 129157 H2, LA 1777), *L. peruvianum* (CMV sel. INRA), and *L. cheesmanii* (LA 1401) (Laterrot 1991, 1992; Laterrot and Makkouk 1983). These four species are used in recurrent selection to combine the resistance elements of the various progenitors to obtain improved plant populations for breeding purposes. This breeding approach depends heavily upon multilocational selection by members of the TYLCV resistance breeding network group which includes 34 cooperators in 14 countries (Cyprus, Egypt, India, Israel, Italy, Jordan, Lebanon, Mali, Senegal, Sudan, Taiwan, Thailand, Tunisia, and Turkey) where TYLCV and related leaf curl viruses of tomato are endemic. Screening for resistance is done under natural infection at a time of the year and in an area where crops of susceptible varieties are almost all attacked. Using seeds of the selected plants, INRA carries out backcrosses or intercrosses with improved varieties. The latter are either varieties which present advantages from the growers' point of view and/or those which were observed as being less affected by the virus in countries where TYLCV is endemic. Among the varieties found<sup>4</sup> to be less affected by the virus during severe epidemics are Columbian, Roza, Progress No. 1 (United Arab Emirates, Senegal), Lignon C8-6, Lignon C20-5 (Mali, Senegal, Cuba), Rowpack (Cape Verde), VF 145 B 7879 (Egypt) (Laterrot 1992a, b), Anahu (Egypt, Sudan) (Yassin and Abu Salih 1972), and EC 104 395 (India, Sudan, United Arab Emirates) (Fadl and Burgstaller 1984; Hassan et al. 1991; Varma et al. 1980).

Recently, a new source of resistance in *L. chilense* LA 1969 has been identified by the group in Israel. This accession reveals a level of resistance which is higher than that of other resistance sources regardless of whether transmission is by *Bemisia tabaci* or by grafting. When grown in the Jordan Valley under natural epidemic conditions, *L. chilense* did not show any symptoms (fig. 2). Furthermore, using TYLCV-specific probes, the virus was found to be present in only two out of 58 plants (Zakay et al. 1991; Zamir et al. 1991). When LA 1969 was grafted to virus-infected *L. esculentum*, the virus content in LA 1969 was on the borderline for detection by serology. It was, however, detected by back grafting LA 1969 to a sensitive variety (Fargette 1991) (table 2).

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<sup>2</sup> Department of Horticulture, Cairo University, Giza, Cairo (research leader: A.A. Hassan).

<sup>3</sup> Institut National de la Recherche Agronomique (INRA), Montfavet with an international network supported by the EEC (European Economic Community) (research leader: H. Laterrot).

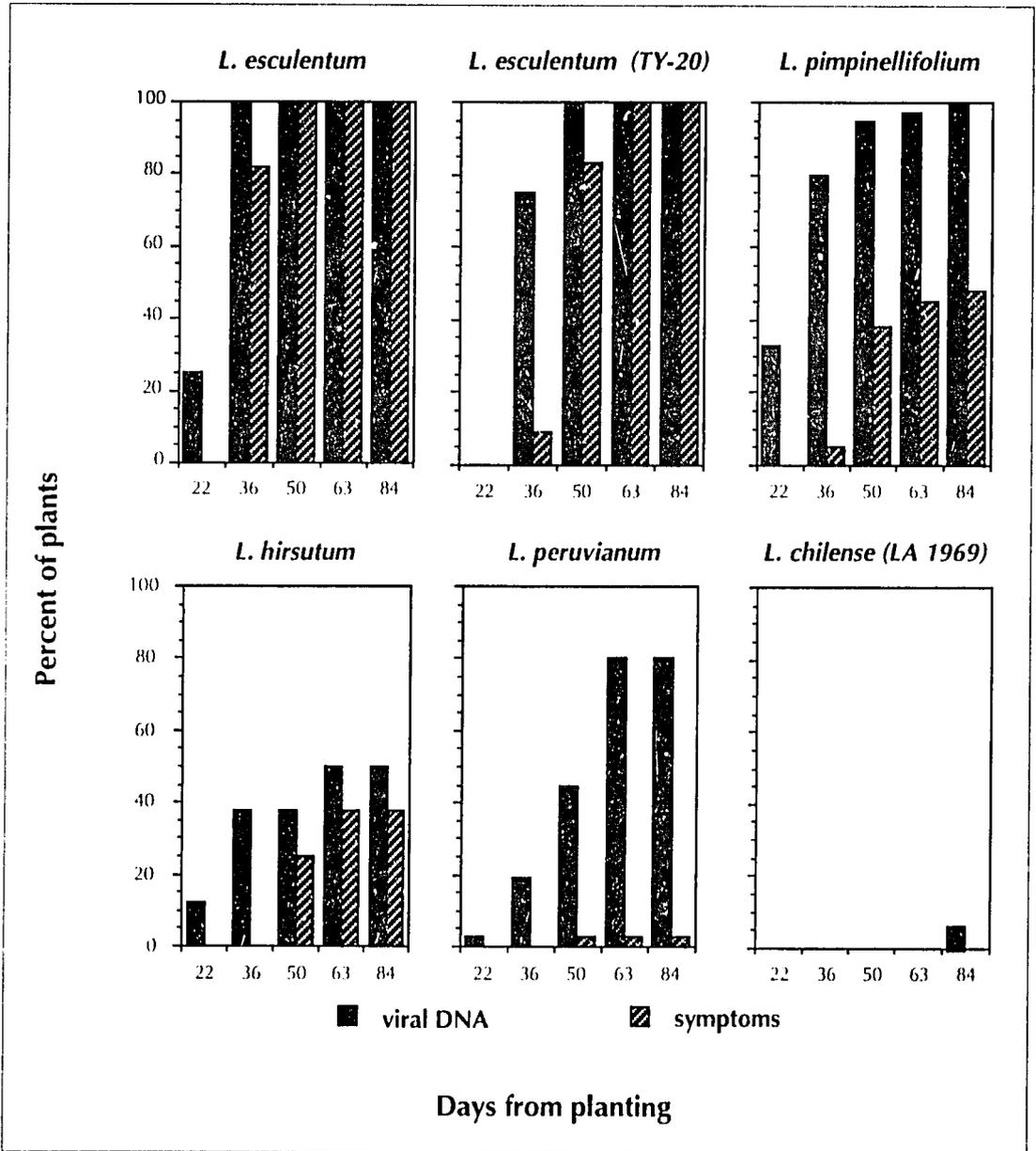


Fig. 2. TYLCV DNA accumulation and symptom development in *Lycopersicon* accessions grown in the Jordan Valley (Zamir et al. 1991)

**Table 2. Symptom intensity, percentage of plants infected, and virus concentration of plants grafted with African and Indian isolates of leaf curl virus (after Fargette 1991)**

<i>Lycopersicon</i> sp.	African isolate			Indian isolate		
	Symptoms <sup>1</sup>	Infected plants %	Virus concentration <sup>2</sup>	Symptoms	Infected plants %	Virus concentration
<i>L. esculentum</i> Marmande	+++	100	+++	+++	92	+++
<i>L. esculentum</i> TY 20	++	100	+++ ++*	++	92	+++ ++*
<i>L. pimpinellifolium</i> LA 1478	?	100	+++	?	90	+++
<i>L. peruvianum</i> CMV/INRA	-	42	++	-	17	+
<i>L. hirsutum</i> LA 1777	-	29	++	-	8	+
<i>L. chilense</i> LA 1969	-	13	+	-	0	-

<sup>1</sup> +++ = strong; ++ = intermediate; ? = weak or doubtful; - no symptoms.

<sup>2</sup> +++ = strong; ++ = intermediate; + = weak; \* = reaction obtained with old plants.

LA 1969 was also shown to be highly resistant to geminiviruses present in Florida (Scott and Schuster 1991) and Taiwan (Green, S. K. unpublished results 1993). Efforts are presently under way by the EEC, Israel, and Florida groups, and by AVRDC to introgress this resistance into cultivated tomato. Because this accession is self-sterile and crosses with *L. esculentum* usually result in defective seed, the use of embryo rescue is necessary, at least in the initial breeding steps. The breeding program in Israel aims to utilize molecular markers to map the TYLCV resistance genes which originate in *L. chilense* (Zamir et al. 1991). The main TYLCV resistance gene (*Ty-1*) has been mapped in *L. chilense* LA 1969, using RFLPs on the tomato genome and has been introgressed into the cultivated tomato (Zamir et al. 1993). Recently, TYLCV-resistant tomato plants have been developed using the TYLCV capsid protein gene (Kunik et al. 1994).

Several TYLCV-tolerant cultivars have been released by the private sector. The first one available was the F<sub>1</sub> hybrid TY-20 which was released in 1988 in Israel (Hazera Seed Co.) for open field cultivation. *L. peruvianum* PI 126935 was the source of TYLCV tolerance which was polygenic and recessive (Zamir et al. 1991; Pilowsky and Cohen 1990). When infected with TYLCV, the leaves of young TY-20 plants exhibit only a mild interveinal chlorosis. In mature plants, the leaflets usually become slightly cupped. The plants give an acceptable yield in spite of TYLCV infection, however, only when early infection is prevented. Serological tests have shown that in young plants of TY-20, the virus multiplies and reaches concentrations similar to those in susceptible lines. It is now known that resistance is expressed only in old plants (Fargette 1991). It is, therefore, recommended that TY-20 seedlings be grown in an insect-proof greenhouse or screenhouse and treated against whiteflies every 2 days with a synthetic pyrethroid. During the first month after transplanting to the field, plants should continue to be sprayed against whiteflies every 2–3 days. Thereafter, it is recommended that insecticidal sprays be applied every 7–10 days to avoid whitefly population build-up and direct damage to the plants. Recently, a new generation of TYLCV-tolerant lines, TY-7170 and TY-7171 with improved fruit quality, has been developed from the same tolerance source by the same seed company.

The response of the hybrid TY-20 and some of the TYLCV-tolerant wild *Lycopersicon* species with respect to symptom severity, percentage of plants infected, and virus content has recently been demonstrated (fig. 2) (Fargette 1991; Zakay et al. 1991).

Other commercial TYLCV-tolerant cultivars are Fiona F<sub>1</sub> (=E437), Jackal F<sub>1</sub> (=E438) (Sluis and Groot), Big Strike F<sub>1</sub> (GSN Semences), Top 21 F<sub>1</sub> (Clause), Saria (Peto Seed), Tyking F<sub>1</sub>, Tydal F<sub>1</sub>, Tyger F<sub>1</sub>, Tygold F<sub>1</sub>, Tycoon F<sub>1</sub>, and Tymoor F<sub>1</sub> (Royal Sluis) (Laterrot 1993). The sources of resistance in these hybrids have not been revealed.

#### 4) Host plant resistance to the vector

Several wild *Lycopersicon* spp. such as *L. hirsutum*, *L. hirsutum* f. *glabratum*, and *L. pennellii* have been used to introduce whitefly resistance into tomato (Berlinger and Dahan 1987; Kisha 1984). This resistance is based on either the density of the leaf trichomes (Georgiev and Sotirova 1978; Snyder and Carter 1985) or on the tacky exudation of the glandular leaf trichomes (Dahan 1985; Plage 1975).

## Southeast and East Asia

The first report in Asia of a disease on tomato that causes yellowing and leaf curl and is transmitted by whiteflies is from India (Vasudeva and Samraj 1948). Diseases with similar symptoms have also been described on tomato from Cambodia (Rowell et al. 1989; Marom 1993), Indonesia (AVRDC 1985), Japan (Kobatake et al. 1981; Osaki and Inouye 1978, 1981), Malaysia (Abu Kassim 1986), the Philippines (Retuerma et al. 1971; AVRDC 1985), Taiwan (AVRDC 1987, 1984; Green et al. 1987), and Thailand (Giatgong 1980; Thongrit et al. 1986; Chandrasikul and Patrakosol 1986).

A whitefly-transmitted geminivirus was isolated in samples from Thailand (Thanapas et al. 1983; Attathom et al. 1990). A DNA probe with sensitivity at the picogram level was constructed that could detect viral DNA from squash-blotted samples of infected tissues and from individual whiteflies (Chiemsombat et al. 1990). This virus did not react with the specific intergenetic probe of the Israel TYLCV, indicating it is different from that virus (Czosnek et al. 1991). This was confirmed when the Thailand tomato yellow leaf curl virus (TYLCV-Thai) was cloned and sequenced. The virus is bipartite (Rochester et al. 1990) and the common region comparisons confirm that it is not closely related to TYLCV from Israel or Italy. The DNA-A of this virus was ligated into a bluescript plasmid and cloned into *E. coli*. The gene construct was then transformed into *Agrobacterium tumefaciens*. Characteristic symptoms indistinguishable from those caused by natural infection were produced in young tomato plants at 14–20 days after agroinoculation. Viral DNA was detected in those plants by nucleic acid hybridization. Although this is a bipartite geminivirus, the A component was able to cause systemic infection in plants following agroinoculation in the absence of the B component. However, symptoms were delayed and attenuated, and whiteflies fed on these plants were not able to transmit the virus to healthy plants (Rochester et al. 1990). The virus has been partially purified and antiserum has been produced (Chiemsombat et al. 1991). This antiserum reacted with one continuous precipitin line (without spur) with the homologous virus and also with tobacco leaf curl virus. The virus was also found to react (with spur) with antiserum produced against a Thai isolate of mungbean yellow mosaic virus (MYMV). This demonstrates the low specificity of polyclonal antisera generally observed against geminiviruses.

In Taiwan, symptoms of yellowing, leaf curling, and stunting of tomato were first observed in 1981 (Green et al. 1987). Electronmicroscopic examination of leaf dip preparations revealed geminate virus particles. The virus was transmitted by grafting and by the whitefly *B. tabaci*, but not by sap inoculation. One single viruliferous whitefly was able to transmit the virus after 48-hour feeding on an infected plant. The latent period of the virus in the vector was found to be 3 hours, considerably shorter than the 20 hours reported for TYLCV from Israel. The host range, determined by grafting and whitefly transmission, includes *D. stramonium*, *Lonicera japonica*, *Nicotiana benthamiana*, *Petunia hybrida*, *Physalis floridana*, and *Solanum melongena* (AVRDC 1984; Green et al. 1987). By nucleic acid hybridization using a TYLCV broad range DNA probe, the virus was identified as only very distantly related to TYLCV from Israel (Czosnek et al. 1990, 1991; Nakhla et al. 1992; Chiang, B.T., Maxwell, D.P., and Green, S.K. personal communication 1993). DNA-A clones of the Taiwan tomato leaf curl virus (TLCV Tai) have been obtained and partially sequenced (Chiang, B.T., Maxwell, D.P., and Green, S.K. personal communication 1993). The nucleotide sequence analysis and nucleotide comparisons of the AL1 (C1) and AL2 (C2) regions show that the TLCV-Taiwan is distinct from TYLCV isolates and other whitefly-transmitted geminiviruses from the Eastern Hemisphere (Maxwell, D.P. et al. unpublished 1992). Contrary to the situation of whitefly-transmitted geminiviruses of tomato in other geographic regions, the virus and the disease have in the past only occurred sporadically in Taiwan and are not yet considered of economic importance. The reasons for this are not fully understood yet. It is possible that the climatic conditions in Taiwan have in the past not favored a year-round high vector population which usually contributes to TYLCV epidemics. Also, chemical weed control which would eliminate many of the weed hosts that serve as natural reservoirs for TYLCV is widely practiced in Taiwan. However, in 1993, following a severe dry spring and summer, abundant whitefly populations were observed on tomato in southern and central Taiwan with a concomitant epidemic of yellow leaf curl disease.

From Japan a disease called yellow dwarf, caused by tobacco leaf curl virus, has been reported from tomato (Kobatake et al. 1981; Osaki and Inouye 1978, 1981). Honeysuckle and *Eupatorium chinense* were reported as the major weed hosts of this virus, whereas, eggplant and soybean harbored high populations of whiteflies (Kobatake et al. 1981). The disease now occurs mainly in southwestern Japan as a result of a surge of whitefly populations in large-scale soybean plantings following efforts to reduce the rice crop (Ikogami, M. personal communication 1993). Presently the disease is not of economic importance in Japan because of appropriate control measures.

The Indian tomato leaf curl virus (ITmLCV) is discussed in detail next because it is one of the most intensively studied tomato geminiviruses in Asia.

## *Indian tomato leaf curl virus (ITmLCV)*

### **The disease**

Leaf curl disease on tomato in India was first reported by Vasudeva and Samraj (1948). The virus, which they named tomato leaf curl virus, causes mosaic, interveinal yellowing, veinclearing, and crinkling and puckering of the leaves accompanied sometimes by inward rolling of the leaf margins. The older leaves become leathery and brittle. The disease induces severe stunting, bushy growth, and partial or complete sterility depending on the stage at which infection has taken place. Infected plants bear few or no fruit. The pathogen was shown to be transmitted by whiteflies but not by sap inoculation (Vasudeva and Samraj 1948; Nariani and Vasudeva 1963; Verma et al. 1975; Muniyappa et al. 1991). The disease is serious throughout India and yield losses may be as high as 100% (Lal and Singh 1961; Sastry and Singh 1973; Datar 1984; Kallou

1988). It appears to be caused by a complex of several virus strains based on symptom variations on different indicator hosts (Singh and Lal 1964; Nariani 1968; Reddy et al. 1981). Reddy et al. (1981) observed various symptoms on tomato. Isolates were divided into five groups: isolate 1 — severe leaf curl with thickening of veins; isolate 2 — severe symptoms with enation; isolate 3 — screw pattern of leaf arrangement; isolate 4 — vein purpling and leaf curl; and isolate 5 — exclusively downward curling of the leaves. Cross protection studies indicated that the five isolates were all related and were consequently designated tomato leaf curl virus strain 1. Nariani (1968) reported another strain which was designated as tomato enation leaf curl virus caused by *Nicotiana virus 10A*.

Weeds, including those belonging to the species *Euphorbia*, *Acanthospermum*, *Ageratum*, and *Parthenium*, are considered important reservoirs of ITmLCV in nature (Saikia and Muniyappa 1989, Muniyappa et al. 1991).

Extensive research on the host range, virus-vector relationship, epidemiology, chemical and nonchemical control, as well as on cultural management practices to reduce virus and/or vector incidence has been conducted in the last 15 years.

## The virus

The virus has only recently been purified and molecular characterization has been initiated. It was purified from chloroform-clarified extracts in 0.1 M citrate buffer pH 6.0 by precipitation with polyethylene glycol, followed by sucrose density gradient and cesium sulfate gradient centrifugation. Purified virus particles reacted in immunosorbent electron microscopy with antisera to four other whitefly-transmitted geminiviruses, i.e., African cassava mosaic, squash leaf curl, tomato golden mosaic, and bean golden mosaic. No reaction was observed with the Indian cassava mosaic virus and the tobacco leaf curl virus from Japan (Muniyappa et al. 1991; Harrison et al. 1991).

By using the polymerase chain reaction (Rojas et al. 1993) and two sets of primers designed to amplify total DNA-A components, it was established that ITmLCV is similar in size to the Old World geminiviruses, but is slightly larger than the DNA-A of the geminiviruses in the Americas. Furthermore, the common region of ITmLCV is more similar to that of the tomato leaf curl viruses from Taiwan and Australia and less similar to TYLCV from Israel, the Thailand tomato yellow leaf curl virus, tomato golden mosaic (TGMV) from Brazil, and tomato mottle virus (ToMV) of Florida. These findings and the fact that the AL1 and AR1 open reading frames showed less than 80% nucleotide sequence identity with seven other tomato-infecting geminiviruses indicate that ITmLCV is a distinct geminivirus (Chatchawankanphanich et al. 1993).

## Transmission

Basic studies on the virus/vector relationship conducted by Butter and Rataul (1977) showed that a minimum acquisition feeding period of 32 min is required by a viruliferous whitefly to cause infection on tomato. Preacquisition or preinoculation starving of the vector results in higher levels of transmission. Females are more efficient in transmission of the disease than males. The latent period of the virus in the vector is between 21–24 hours. Whitefly colonies raised on okra are more efficient in transmitting the virus than those raised on chili, cotton, cowpea, tobacco, or tomato. Virus transmission appears to be affected by temperature, with 33–39°C being optimal. The latent period of the virus in tomato plants was only 8 days in summer and 90 days in winter (Butter and Rataul 1977). The high incidence of leaf curl disease in the autumn is attributed to the effect of temperature on virus transmission (Mayee et al. 1974).

## Disease control

### 1) Chemical control of the vector

Insecticides, oils, antibiotics, and antiviral chemicals (Mukerjee and Raychaudhuri 1966; Raychaudhuri 1966; Sastry and Singh 1971; Butter and Rataul 1973; Thirumalachar et al. 1973; Varma and Poonam 1977; Kalloo et al. 1986) have been tried to reduce the insect vector and inhibit virus multiplication. Disease incidence was shown to be significantly reduced by application of cycocel (200–500 ppm) at the seedling stage (Arora et al. 1989a, b; Banerjee et al. 1991) and weekly sprays of 8-azaguanine (0.1%) (Varma and Poonam 1977).

### 2) Host plant resistance to the virus

Breeding for resistance to ITmLCV is conducted in several institutes throughout India. Various methods have been employed to systematically screen *Lycopersicon* germplasm for resistance such as subjecting plants to natural epidemic conditions in field locations where disease incidence is high, by artificial inoculation in the laboratory and greenhouse, by grafting, or by whiteflies.

Artificial inoculation is usually done with female whiteflies raised on eggplant. After 48-hour acquisition feeding on ITmLCV-infected leaves, the whiteflies are released on healthy seedlings at the 3–4 leaf stages where they are allowed a 48-hour inoculation feeding (Banerjee and Kalloo 1991). The infection percentage by this method is usually 100%, compared to 83–100% incidence commonly observed in field screenings. Symptoms appear between 17–31 days, compared to 23–40 days under field conditions. A scale for classifying disease reaction was developed by Banerjee and Kalloo (1987b).

Tolerance was found in *L. esculentum* Nova (Mayee et al. 1974) and EC 104395 (Varma et al. 1980). This tolerance was later confirmed in Sudan (Fadl and Burgstaller 1984; Dafalla 1993) and in the United Arab Emirates (Hassan et al. 1991) where TYLCV is known to prevail. Resistance was also revealed in various wild species (table 3) which have been used for incorporating resistance into the cultivated tomato. Among the wild species *L. hirsutum* f. *glabratum* and *L. peruvianum* were highly resistant (Banerjee and Kalloo 1987b).

Resistance in *L. pimpinellifolium* A 1921 was found to be monogenic and incompletely dominant (Banerjee and Kalloo 1987a) and resistance in *L. hirsutum* f. *glabratum* (B 6013) was governed by two epistatic genes (Banerjee and Kalloo 1987b). Two independent genes for resistance seem to be involved in these two wild species with that of *L. hirsutum* f. *glabratum* dominant over the other (Banerjee and Kalloo 1990).

Small fruit size and late maturity were found associated with ITmLCV resistance in *L. pimpinellifolium* LA 1921 and *L. hirsutum* f. *glabratum* B 6013 (Banerjee and Kalloo 1989a). Five TLCV-tolerant breeding lines, LCP-2, LCP-3, LCP-9, LCP-15, and LCP-22, have been developed by backcrossing to *L. esculentum* involving *L. pimpinellifolium* as resistant donor parent. Disease incidence was 28–35% in these lines, compared to 92–100% in the susceptible *L. esculentum* parent (Kalloo and Banerjee 1990b). Six other TLCV-tolerant lines, i.e., H-2, H-11, H-17, H-23, H-24, and H-36, were developed from *L. hirsutum* f. *glabratum* B 6013, also following a backcross pedigree method. Disease incidence at 120 days after inoculation in these lines ranged from 8.3 to 35%, compared to 61–89% in the susceptible cultivar (Kalloo and Banerjee 1990a).

**Table 3. *Lycopersicon* accessions with resistance/tolerance to tomato leaf curl virus in India**

Species and line	Reference
<i>L. peruvianum</i>	
E.C. 65980, E.C. 65968, E.C. 148898, E.C. 148897	Varma et al. 1980
B 6002/77	Banerjee and Kalloo 1987b
PI 127830, PI 127831	Saikia and Muniyappa 1989 Muniyappa et al. 1991
Line 996-22 x 996-24 SIB, LA 444, 63 L-Chincha, Peru	Joshi and Choudhury 1981
<i>L. peruvianum</i> f. <i>glandulosum</i>	
B 6005	Banerjee and Kalloo 1987b
<i>L. peruvianum</i> f. <i>typicum</i>	
B 6002	Banerjee and Kalloo 1987b
<i>L. peruvianum</i> var. <i>regulare</i>	
49 B - 295	Joshi and Choudhury 1981
<i>L. esculentum</i>	
EC 104395	Varma et al. 1980
Nova, Nematex, HS 110	Mayee et al. 1974
<i>L. chilense</i>	
Line 414-2 x 414-1 SIB, LA 267, 55 L-Antofagaster, Chile	Joshi and Choudhury 1981
Line 986-22 x 986-24 SIB, LA 458, 63 L-Tacna, Peru	
<i>L. glandulosum</i>	
E.C. 66003, E.C. 66002	Varma et al. 1980
<i>L. hirsutum</i>	
LA 386, LA 1777	Muniyappa et al. 1991
PI 390658, PI 390659, PI 390513	Saikia and Muniyappa 1989
PI 127826	Banerjee and Kalloo 1987a
<i>L. hirsutum</i> f. <i>glabratum</i>	
B 6013	Banerjee and Kalloo 1987a
<i>L. hirsutum</i> f. <i>typicum</i>	
A 1904	Banerjee and Kalloo 1987b
<i>L. pimpinellifolium</i>	
A 1921	Banerjee and Kalloo 1987a
XXXII-354-A- Silvestra, S1-496, PRS	Som 1973
P <sub>13</sub> (2247), XXXII-354-A-Silvestra	Joshi and Choudhury 1981

Two TLCV-resistant varieties (Hisar Anmol and Hisar Gaurov) derived from a backcross pedigree of *L. hirsutum* f. *glabratum* x *L. esculentum* have been identified by the variety evaluation committee of Haryana Agricultural University, Hisar. These determinate lines with medium-sized, red fleshy fruit with thick pericarp are presently undergoing multilocational testing in India to test the stability of resistance (Kalloo and Banerjee 1993).

Several biochemical attributes such as high levels of total crude protein and phenol (Banerjee and Kalloo 1989b) have been found associated with resistance. An additional protein band of high molecular weight was found to be present in susceptible *Lycopersicon* species (Varma and Poonam 1977) which was absent in resistant species. These characters may prove to be useful in the selection of resistant lines.

## Australia

A whitefly-transmitted geminivirus called tomato leaf curl virus (TLCV) causes severe crop damage to tomato in the northern parts of Australia. The virus has been cloned and the complete nucleotide sequence determined (Dry et al. 1993). Plants infected with TLCV show disease symptoms similar to those caused by tomato yellow leaf curl virus of Israel. The virus is monopartite with a DNA of 2,766 nucleotides. Agroinfection has been achieved with a full length TLCV clone.

## Americas

In the Americas, leaf curl and yellowing diseases of tomato have been reported since the early 1960s (Costa 1969; Debrot et al. 1963). These diseases have emerged as the major cause of serious losses in tomato production (Brown 1991; Brown and Bird 1992). Efforts to control the insect vector have resulted in insecticide applications on a 3–7 day schedule in most of Central America's tomato plantings. Identification of the viruses involved started in the mid-80s. Polymerase chain reaction methods have been developed to aid in the diagnosis of these geminiviruses (Rojas 1992; Rojas et al. 1993; Rivera-Bustamante, R. personal communication 1994; Brown, J.K. personal communication 1994). Several distinct whitefly-transmitted geminiviruses involved in these diseases have now been isolated; biological and molecular characterization are under way.

Tomato golden mosaic virus (TGMV) was the first whitefly-transmitted geminivirus reported from Brazil (Costa 1969). In contrast to most other whitefly-transmitted geminiviruses of tomato, the virus is mechanically transmissible, although with some difficulty. It has a restricted host range, infecting mainly solanaceous plants. The virus belongs to the bipartite group of geminiviruses (Hamilton et al. 1983, 1984). A similar disease causing 'mosaico amarillo del tomate' (tomato yellow mosaic) was described in Venezuela (Debrot et al. 1963; Lastra and Uzcátegui 1975). The virus involved in that disease, tomato yellow mosaic virus (TYMV), has biological properties similar to TGMV. The virus is mechanically transmissible to *L. esculentum*, *Datura stramonium*, *Nicandra physaloides*, *Nicotiana glutinosa*, *Nicotiana tabacum* Samsun, White Burley, Virginia, and *Petunia hybrida*. The virus is very labile. Its infectivity in sap extracted from infected plants is lost within 15 minutes. Antioxidants, such as 2-mercaptoethanol, sodium diethyldithiocarbamate (DIECA) and dithiothreitol added to the extraction buffer greatly increase transmission efficiency. Whiteflies transmit the virus after a minimum acquisition period of 2 hours and a latent period of 20 hours. Thereafter the insects remain infectious for a maximum of 7 days. Females are more efficient than males in transmitting the virus (Uzcátegui and Lastra 1978).

A geminivirus affecting tomato in Mexico (Gallegos 1978) was characterized and designated chino del tomate virus (CdTV) (Brown and Hine 1984). CdTV causes the most severe foliar symptoms and the greatest degree of stunting in tomato of any of the New World-type bipartite whitefly-transmitted geminiviruses (Brown, J.K. personal communication 1994). Symptoms are distinct from those of tomato yellow leaf curl virus and include small leaves, leaf curling, interveinal chlorosis, mild to no mosaic and vein distortion making the leaves slightly twisted and misshapen. Affected plants are stunted and have little to no fruit set. If fruit are produced, they are small and misshapen. Pepper and *Malva parviflora* are natural hosts of this virus besides tomato (Brown and Nelson 1988). Experimental hosts include *Datura stramonium* and *Phaseolus vulgaris*. The virus belongs to the bipartite group of geminiviruses. The B component has been cloned and sequenced recently (Brown, J.K. et al. personal communication 1993). The virus probably also occurs in Nicaragua (Brown 1991).

Serrano golden mosaic virus (SGMV) has been found to infect tomato in Mexico and Arizona. The virus, which also infects peppers, is mechanically transmitted — with difficulty — from peppers to peppers, but not from tomato to pepper or from tomato to tomato. The virus also belongs to the bipartite group of geminiviruses (Brown and Poulos 1990).

Pepper mild tigré virus (PMTV) is known to affect tomato, as well as peppers, in the Tamaulipas region of Mexico (Brown et al. 1989). Symptoms on tomato are leaf curling, interveinal chlorosis, and moderate stunting. Symptoms take 19–20 days to develop after whitefly feeding. The natural host range appears to be confined to tomato and peppers. *Datura stramonium* can be infected experimentally, by using whitefly transmission.

A previously undescribed virus disease has been reported from Sinaloa, Mexico. The virus, named Sinaloa tomato leaf curl virus (STLCV), affects tomatoes with foliar curling, chlorosis, purpling, and shortened internodes. It is experimentally transmitted by whiteflies from tomato to tomato, pepper, eggplant, *Datura stramonium*, *Nicotiana benthamiana*, *Malva parviflora*, and *Phaseolus vulgaris*. The virus can be mechanically transmitted — although with difficulty — from tomato to tobacco (Brown et al. 1993). PMTV and STLCV most likely also belong to the bipartite group of geminiviruses (Brown, J.K. personal communication 1994).

In the late 1980s, tomato production in Florida, USA, was threatened by a whitefly-transmitted geminivirus (Simone et al. 1990; Kring et al. 1991), which was subsequently cloned and sequenced (Abouzid et al. 1992; Gilbertson et al. 1991, 1993). This geminivirus, which causes downward leaf curling and mild leaf distortion, mottled interveinal chlorosis, and an overall reduction in plant height, has been designated tomato mottle geminivirus (ToMoV). It is bipartite and sap-transmissible and, along with its symptoms in tomato, is readily distinguishable from TYLCV. The host range of this virus is mainly confined to the *Solanaceae* family, infecting species in the genera *Lycopersicon*, *Nicotiana*, *Physalis* and *Solanum* (Polston et al. 1993). However, the virus also infects *Phaseolus vulgaris*. Whiteflies were unable to transmit the virus to potato and two species of pepper. One weed species, *Solanum viarum* (tropical soda apple) was found to be naturally infected but due to its distribution, low rates of natural infection, and unsuitability as an acquisition and transmission host, is not considered to play a significant role in the epidemiology of ToMoV at this time (McGovern et al. 1994). Infected tomato plants from older fields and abandoned fields are believed to be the most important sources of infection (Polston, J.E. personal communication 1994). Studies of the transmission of ToMoV have shown that the virus is readily acquired and transmitted by *B. argentifolii* from tomato to tomato (Polston and Webb unpublished 1994). Transmission could be achieved in as little as 2 hours, and individual adult whiteflies transmitted about 8% of the time. Twenty adult whiteflies were usually sufficient to infect 100% of tomato plants. Virus was not acquired by immature whiteflies. Research is in

progress to develop ToMoV-resistant fresh market tomato lines. Initial field screenings indicated that several TYLCV-tolerant accessions were partially resistant to ToMoV, and that many *L. chilense* accessions including LA 1960 had no disease symptoms. Preliminary genetic analysis of the F<sub>2</sub> crosses with LA 1960 indicated the involvement of two to three recessive genes (Scott and Barten 1992).

In the Dominican Republic, a Western Hemisphere-type geminivirus was found associated with tomatoes in 1990–1991 by DNA hybridization with a general nucleic acid probe (Brown, J.K. personal communication 1994; Rojas 1992). Subsequently, the presence of three different bipartite viruses was evidenced by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis (Polston, J.E. personal communication 1994). In the spring of 1992, however, tomatoes with symptoms similar to those of TYLCV were observed in the Northern Region of the Dominican Republic (Brown, J.K. and Bird, J. personal communication 1994). In most fields, the symptoms were observed at low frequency (about 1–3%); however, in one field nearly 100% of the plants had TYLCV-like symptoms. Samples of these plants hybridized strongly with a DNA-A probe of TYLCV-Thai but not with a DNA-A general probe of Western Hemisphere-type whitefly-transmitted geminiviruses. In 1992–93 and 1993–94, a whitefly-transmitted geminivirus caused extensive losses in tomato production. Infected plants were severely stunted, leaves were small, cupped, curled upward, and often had yellow margins. Flower abscission was also observed. In 1993, this geminivirus was characterized as an isolate of the Eastern Mediterranean strain of TYLCV based on PCR fragment size, and restriction fragment length polymorphisms of PCR fragment, DNA hybridization reactions with TYLC from Israel (Polston et al. 1994) and partial sequencing of a cloned full length PCR fragment (Nakhla et al. 1994). The partial nucleotide sequence of the intergenic region had 97% sequence identity with the homologous region of TYLCV from Israel and only 63% homology with the respective region of the tomato mottle geminivirus from Florida (Nakhla et al. 1994). This is the first reported occurrence of a monopartite whitefly-transmitted geminivirus in the Western Hemisphere.

TYLCV-like symptoms have also been observed in tomatoes in the spring of 1993 in Jamaica. The presence of the Eastern Mediterranean strain of TYLCV has been detected in fresh market and processing tomatoes collected in May 1994 from St. Catherine Parish, Jamaica. This identification was based on size of PCR fragments and restriction fragment length polymorphisms of these PCR fragments and by strong hybridization with DNA of the TYLCV from Israel (McGlashan, D., Polston, J.E., and Bois, D. personal communication 1994). Additionally, tomato samples collected in May 1994 from the major tomato growing regions in Jamaica gave strong hybridization at high stringency conditions with the TYLCV probe from the Dominican Republic (McLaughlin, W., Wernecke, M., Roye, M., and Nakhla, M.K. personal communication 1994). Potato yellow mosaic geminivirus was found in association with tomatoes collected in 1993 in St. Elizabeth Parish, Jamaica (Roye, M., McLaughlin, W., and Maxwell, D.P. personal communication 1993).

Still other tomato-infecting geminiviruses have been reported from the Caribbean Basin. One from Cuba is apparently different from TGMV of Brazil, TYMV of Venezuela, CdTV of Mexico, and TYLCV from Israel (Gomez and Gonzalez 1993).

Additionally, a distinct geminivirus based on sequence data of DNA-A was found in association with tomatoes in Trinidad (Hidayat, S.H. and Maxwell, D.P. personal communication 1993).

Recently, an outbreak of a whitefly-transmitted geminivirus causing yellowing and leaf curling was observed on the island of Martinique. The virus has, however, not yet been further characterized (Hostachy and Alley 1993).

## AVRDC and the leaf curl virus complex of tomato

Realizing the importance of the leaf curl virus complex on tomato and pepper in the tropics and subtropics, AVRDC has been actively cooperating with the TYLCV resistance breeding network group under INRA leadership.

AVRDC has screened INRA's improved populations for resistance to the Taiwan tomato leaf curl virus (TLCV-Tai) in the screenhouse by the double-sandwich grafting method (AVRDC 1984). This method involved grafting of stem tips of about 10-cm length to leaf curl-infected stock plants. After 7 weeks the stem tips were cut above the graft union and grafted to healthy *N. benthamiana* which reacts within a few days with pronounced leaf curling symptoms if the grafted stem tip contains TLCV-Tai. This method was adopted in the absence of diagnostic tools, and because the grafted stem tips which were mostly wild species usually did not exhibit any clear leaf curl symptoms. Recently, however, a nonradioactive DNA probe representing the DNA-A component of TLCV-Tai has been developed in cooperation with D.P. Maxwell of the University of Wisconsin which has made direct testing of the grafted stem tips possible. The same populations were also field-screened in Thailand by AVRDC's Asian Regional Center (ARC) under natural epidemics for resistance to the Thailand tomato yellow leaf curl virus. Seeds of resistant or of symptomless plants from these screenings have been returned to INRA for further population advance.

Several of AVRDC's breeding lines, particularly PT 3027 (F<sub>1</sub>), CL 5915-153D4-3-3, CL 5915-229D4-1-3, and CL 1131-0-0-43-8-1, were shown to possess a considerable level of field tolerance in Cambodia and in Bangladesh with low disease incidence and high yield, in spite of high incidence of leaf curl disease (Rowell et al. 1989).

AVRDC has recently also initiated a breeding program using *L. chilense* LA 1969 which was found to be also immune to TLCV-Tai (Green, S.K. unpublished 1992) to incorporate leaf curl virus resistance into its most promising heat-tolerant, bacterial wilt-resistant tropical tomato lines. F<sub>1</sub> plants have been produced via embryo rescue (Kuo, G. unpublished 1993). AVRDC will also introgress resistance from *L. chilense* into cultivars suitable for tropical and subtropical highland production.

Leaf curl virus of both tomato and pepper has been declared one of the priorities of AVRDC's South Asian Vegetable Research Network (SAVERNET). This network is funded by the Asian Development Bank and includes Bangladesh, Bhutan, India, Nepal, Pakistan, and Sri Lanka. Leaf curl virus of tomato is also being addressed in another vegetable network in Africa, the Collaborative Network for Vegetable Research and Development in Southern Africa (CONVERDS). Member countries include: Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, Swaziland, Tanzania, Zambia, and Zimbabwe. The emphasis is to develop

or test resistant/tolerant lines or cultivars adapted to the African highlands. Whitefly-transmitted geminiviruses are also an important part of a soon to be established network, the Collaborative Network for Vegetable Research and Development in Central America (CONVERDCA) which will cover the following countries: Costa Rica, El Salvador, Guatemala, Honduras, and Nicaragua.

AVRDC's strategy in support of these networks involves the following:

- AVRDC will assemble and multiply all materials with reported resistance/tolerance to leaf curl disease of tomato and pepper from cooperators in France, India, Israel, and elsewhere.
- Because several strong leaf curl virus resistance breeding programs are already in existence elsewhere, AVRDC will initially evaluate these selected tolerant/resistant materials in three selected geographic regions: South Asia (SAVERNET countries), Southern Africa (CONVERDS countries), and in Central America (CONVERDCA countries) where leaf curl and yellowing diseases are a problem. Specific populations will be developed for each region for selection by national program scientists jointly with AVRDC scientists. Further breeding and selection will be done directly by the national programs in the regions.
- Using the polymerase chain reaction technology, AVRDC will produce nonradioactive probes specific to the TYLCV from Israel, the TLCV-Tai, the TYLCV-Thai, the ITmLCV, and other distinct Asian tomato geminiviruses, and to the major geminiviruses infecting tomato and pepper in Central America and the Caribbean countries. These probes will be made available to researchers of the three AVRDC networks and should be useful for resistance screening/breeding as well as for epidemiological studies.
- AVRDC, in close cooperation with the University of Wisconsin (Madison), will provide technical assistance on the characterization of tomato-infecting geminiviruses in the countries of CONVERDS, SAVERNET, and CONVERDCA.
- As soon as resistance from *L. chilense* has been fixed into tropical lowland or highland tomato, AVRDC will distribute its lines to its cooperators and to any other interested persons or institutions for multilocational testing.

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