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A worldwide survey of tomato yellow leaf curl viruses

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Summary. The name tomato yellow leaf curl virus (TYLCV) has been given to several whitefly-transmitted geminiviruses affecting tomato cultures in many tropical and subtropical regions. Hybridization tests with two DNA probes derived from a cloned isolate of TYLCV from Israel (TYLCV-ISR) were used to assess the affinities of viruses in naturally infected tomato plants with yellow leaf curl or leaf curl symptoms from 25 countries. Probe A which included most of the intergenic region was expected to detect only isolates closely related to TYLCV-ISR, especially after high stringency washes. In contrast probe B, which included the full-length genome, was expected to detect a wide range of whitefly-transmitted geminiviruses. Tomato samples from six countries in the Middle East, from Cuba or the Dominican Republic proved to be closely related to TYLCV-ISR and probably were infected by strains of the same virus. Samples from Senegal and Cape Verde Islands were also related to the Middle Eastern virus. Samples from nine other countries in the western Mediterranean area, Africa, or South-East Asia were more distantly related and probably represent one or more additional geminivirus species. Samples from five countries in Africa, Central or South America gave hybridization signals with the full-length viral genome, only after low stringency wash, indicating that these samples were infected by remote viruses. These results were supported by DNA and protein sequence comparison, which indicate that tomato geminiviruses fall into three main clusters representing viruses from 1) the Mediterranean/Middle East/African region, 2) India, the Far East and Australia, and 3) the Americas. Within the first cluster, two sub-clusters of viruses from the western Mediterranean or from the Middle East/Caribbean Islands were distinguished. The incidence of tomato yellow leaf curl diseases has increased considerably between 1990 and 1996.

Introduction

The name tomato yellow leaf curl virus (TYLCV) was coined in the early sixties to describe a virus transmitted by the whitefly *Bemisia tabaci* that affected tomato cultures in the Middle East [12, 13]. TYLCV outbreaks that were sporadic in the sixties [12] have become a serious economic problem, starting in the early seventies, when losses often reached 100% of the yield. By the end of the seventies, all regions of tomato culture in the Middle East were affected by the virus [1, 12, 31, 42, 45, 67]. TYLCV-like diseases were identified in Southeast Asia (Taiwan [24], Thailand [63]) in the beginning of the eighties. Both Eastern (Tanzania [16]) and Western Africa (Burkina-Faso [36], Mali [18], Nigeria [37], Senegal [17]) have not been spared by these diseases. TYLCV has spread recently to the Western Mediterranean basin (Italy [14, 40], Spain [48]) and has conquered new areas in Iran [3], in Asian Republics of the former USSR (Azerbaidjan, Turkmenistan, Uzbekistan [3]) and in China (P. Tien, pers. comm.). Three years ago, TYLCV was identified in the Caribbean Islands (Cuba [22], Dominican Republic [51, 59], Jamaica [66]), for the first time in the New World. The spread of TYLCV-like diseases and of many other whitefly-transmitted geminiviruses, has paralleled the worldwide expansion of the B biotype of *B. tabaci* [6].

Additional whitefly-transmitted geminiviruses affect tomato. In India and in Australia, the prevalent virus was named tomato leaf curl virus (TLCV). In the new World, the geminiviruses were termed as tomato mottle virus in Florida (TMoV), chino del tomato virus in Mexico (CdTV), tomato golden mosaic virus in Central and South America (TGMV), tomato yellow mosaic virus in South America (TYMV). These viruses clearly differ from the various TYLCVs in the symptoms they induce on tomato, in their host range [23], in their nucleotide sequence [57, 62] and in their reaction with panels on monoclonal antibodies [26].

Early diagnosis of TYLCV was essentially based on symptom observation, although symptoms vary greatly as function of soil, growth conditions and climate. Serology has been of limited use because whitefly-transmitted geminiviruses share many epitopes [64]. The analysis of DNA sequences has become the tool of choice, allowing one to accurately identify the virus and to evaluate its relationship with other TYLCV isolates [15, 28, 35, 56, 61].

A field isolate of TYLCV from Israel was the first whitefly-transmitted geminivirus that was shown to possess a single genomic component [52, 54], in contrast to the whitefly-transmitted geminiviruses characterized at that time, that possess two circular single-stranded DNA genomic molecules of about 2.8 kb each (denominated DNA A and DNA B, reviewed in [39]). TYLCV isolates from Italy (Sardinia [35] and Sicily [15]) and from Spain [56] have also a single genomic molecule. However, TYLCV isolates from Thailand possess two genomic molecules [61], as do tomato geminiviruses from India (TLCV-IND [58]) and from the New World (TGMV [25] and TMoV [2]). The TYLCV genome strand (or the TYLCV-THA DNA-A) encodes two genes (V1 and V2),

including the capsid protein (V1) [34]. The virus genome complementary DNA codes for four genes (C1 to C4), including the Rep protein (C1) necessary for virus replication [20, 38]. The DNA-B of TYLCV-THA encodes two genes, BR1 and BL1, homologous to the genes of DNA-B from whitefly-transmitted geminiviruses of the New World [61]. A $\sim$ 300 nucleotide-long intergenic region (IR) (almost identical in DNA-A and B) contains a conserved stem-loop structure of $\sim$ 30 nucleotides bearing the nonnucleotide TAATATTAC believed to be the origin of replication [27].

Comparisons of the sequences of geminiviral genomes, genes, intergenic region, and gene products have been used to construct phylogenetic trees [28, 30, 57, 62]. These analyses have shown that New World and Old World whitefly-transmitted geminiviruses form two distinct groups. Among them, the TYLCVs were grouped according to a geographical gradient comprising isolates from the Eastern Mediterranean basin, the Middle East and South East Asia. Sequence comparisons have also suggested that the name TYLCV may cover different virus species (e.g. TYLCV-ISR, TYLCV-ITA (Sar), TYLCV-THA [28]).

We established the first geographical map of the extent of the TYLC-like diseases in 1989 [16], based on molecular hybridization on squashes from tomato leaves with cloned DNA probes derived from TYLCV-ISR [54]. Since this survey, TYLC disease has extended considerably and the genomes of several TYLCV isolates from different countries have been cloned and sequenced. In this paper we present an updated map of the incidence of TYLCV-like diseases worldwide and we review the molecular characteristics of TYLCV isolates available in the literature and in databases.

Materials and methods

Hybridization of tomato leaves squashed on membranes with probes derived from a TYLCV isolate from Israel (TYLCV-ISR)

A field isolate of TYLCV (clone pTYH 19) was collected in 1988, cloned and sequenced (TYLCV-ISR [54]). TYLCV-ISR was maintained in tomato plants (*Lycopersicon esculentum,* cv. FA 144) and transmitted by the whitefly *B. tabaci*, B type [10]. Leaves from uninfected tomato as well as from plants infected with the Israeli isolate of TYLCV were squashed onto nylon membranes (Hybond N, Amersham) [53]. Membranes were mailed for sampling and returned to Israel for TYLCV detection by hybridization with viral DNA probes. Two probes derived from TYLCV-ISR were used. Probe A consisted of a 347 bp *Alu*I/*Alu*I DNA fragment that encompasses 288 nucleotides of the virus intergenic region and 59 nucleotides of the V2 gene (pre-coat); probe B consisted of a cloned dimeric repeat of the full-length viral genome (plasmid pTYH 20.7 [53]) excised from the cloning plasmid pTZ18R using the restriction enzymes *Hind*III and *Bam*HI. Hybridization conditions were as described before [16, 53]. Membranes were washed first with 1 $\times$ SSC at 68 °C and subjected to autoradiography. The membranes were then washed with 0.1 $\times$ SSC at 70 °C (SSC is 0.015 M trisodium citrate, 0.15 M NaCl) and subjected again to autoradiography. Before hybridization with additional probes, the membranes were stripped from the radiolabeled probe with 0.5 M NaOH, 1 M NaCl and neutralized with 50 mM Tris pH 7.5, 1 M NaCl.

Comparison of sequences of TYLCV genes and proteins

The nucleotide sequences of the virus genomes and the amino acid sequences derived from the genes encoding the Rep and the capsid proteins were retrieved from the geminivirus database (Internet address: http://geminivirus.scrips.edu/GeminiWebPage). Sequences were aligned using the Genetics Computer Group, University of Wisconsin-Madison (UWGCG) program Pileup. Phylogenetic trees were obtained from the alignments of the amino acids of the coat protein (CP) and of the replication-associated protein (Rep), and of the nucleotides of the intergenic region (IR), using the program Phylogenetic Analysis Using Parsimony (PAUP), version 3.1. One hundred bootstrap replications were performed.

The sequences of the following TYLCV isolates were used: from Cuba (TYLCV-CUB [44] and Zabalgogeazcoa, pers. comm.) Egypt (TYLCV-EGY [50]), Israel (virulent TYLCV-ISR [54]; and mild TYLCV-ISR (Mild) [4]), from Italy (Sardinia, TYLCV-ITA (Sar) [35]; and Sicily, TYLCV-ITA (Sic) [15]), from Nigeria (TYLCV-NIG [28]), Saudi Arabia (North TYLCV-NSA [28], and South TYLCV-SSA [28]), Spain (TYLCV-SPA [56]), Thailand (TYLCV-THA [61]), and Yemen (TYLCV-YEM [7]). The sequences of the following tomato geminiviruses were used: chino del tomato virus (CdTV [65], tomato golden mosaic virus (TGMV [25]), tomato leaf curl virus from India (TLCV-IND [58]) and from Australia (TLCV-AUS [19]), and tomato mottle virus (TMoV [2]). The sequences of cassava mosaic virus isolates from India (ICMV [29]) and Nigeria (ACMV-NIG [49]), were also used. Unpublished sequences of TYLCV from China (TYLCV-CHI, Tien, pers. comm.) and from the Dominican Republic (TYLCV-DOM [51] and Gilbertson and Maxwell, pers. comm.) were included.

Results

Identification of TYLCV by hybridizing squashes of tomato leaves with DNA probes derived from the Israeli strain of the virus TYLCV-ISR

Membranes containing leaf squashes of non-inoculated and of TYLCV-inoculated tomato from Israel were sent to our correspondents in the various countries. Leaves from plants presenting yellow leaf curl symptoms were squashed on the membrane (in most cases the plants were also photographed). The membranes were returned to Israel and were hybridized with the TYLCV-specific DNA probes. Two probes were used: A. a clone representing mainly the virus intergenic region (IR) was used to identify isolates close to TYLCV-ISR; B. a full-length clone was used to detect isolates distantly related to TYLCV-ISR. For each probe, autoradiograms were obtained after low and after high stringency washes. The TYLCV-ISR probes gave weak or no hybridization signal with squashes of tomato plants infected with any of TLCV-IND, TGMV, TMoV or CdTV.

The results obtained with samples collected during the last eight years are summarized in Table 1. Only the samples giving a positive signal with the TYLCV-ISR probes are listed. The date of the earliest sampling is indicated; all the results listed were confirmed by at least one additional sampling.

Tomato samples originating from five large geographical areas hybridized with the broad-range TYLCV DNA probe, after low stringency wash: the Eastern Mediterranean basin and Middle East (countries on the Mediterranean coast, from Turkey to Egypt, Sudan and the Arabian peninsula), the Western

Table 1. Identification of TYLCV by hybridizing virus-specific probes with squashes of tomato leaves

Eastern Mediterranean Basin and Middle East

Country	Locality	Date of sampling	Culture	Relatedness to TYLCV-ISR
Cyprus	South Coast	2/89	F	4
Egypt	Qalyub	1/89	F	4
	Ismailia	1–2/89	F	4
	Fayoum	2/89	F	4
Israel	Coastal Plain	9/88	F, G	4
	Jordan Valley	9/88	F, G	4
Jordan	Jordan Valley	12/89	F	4
Lebanon	Beyrouth	5/89	G	4
	Coast	1/93	G	4
Soudan	Wad Medani	1/91	F	2
	Gezira	11/92	F	2
Syria	Latakia	11/92	G	4
Turkey	Antalya	1/88	G	4
	Mersin	3/88	G	4
	South Coast	1/94	G	4

Western Mediterranean Basin

Country	Locality	Date of sampling	Culture	Relatedness to TYLCV-ISR
Italy	Sardinia	11/88	G	2
	Sicily	1/89	G	2
	Calabria	11/91	G	2
Spain	Almeria	11/92	G	2
	Murcia	11/92	G	2
	Aguilas	2/94	G	2
Tunisia	Tunis	11/88	G	2
	Sahel de Sousse	12/92	G	2
	Southern Oasis	1/94	G	2

Tropical Africa

Country	Locality	Date of sampling	Culture	Relatedness to TYLCV-ISR
Burkina Fasso	Bobo Dioulasso	1/92	F	1
	Ouagadougou	1/92	F	1
Cameroun	Foumbot	12/93	F	1
Cape Verde	Santiago Island	1/87	F	3
Ivory Coast	Bouake	1/91	F	2
Mali	Bamako	5/88	F	2
Nigeria	Zaria	5/88	F	2
Senegal	St Louis	7/87	F	3
	Kaolack	7/87	F	3
	Dakar	1/91	F	3
	Senegal River	1/92	F	3
Tanzania	Morogoro	5/90	F	1
	Arusha	3/94	F	1

Table 1 (*continued*)

Central and Southeast Asia

Country	Locality	Date of sampling	Culture	Relatedness to TYLCV-ISR
Taiwan	Tainan	3/88	F	2
Thailand	Khon Kaen	2/88	F	2
	Nong Kai	2/88	F	2
	Udon Thani	1/92	F	2
Turkmenistan	Goktepe	3/93	F	1

The Americas

Country	Locality	Date of sampling	Culture	Relatedness to TYLCV-ISR
Cuba	La Havana	1/93	F	4
Dominican Rep.	Bani	1/93	F	4
Argentina	Cordoba	8/92	F	1

The probes used were a full-length clone of the virus and the virus genome intergenic region (IR). Membranes were washed, starting with 1 × SSC at 68 °C (low stringency), submitted to autoradiography and then washed again with 0.1 × SSC at 70 ° (high stringency). The date of sampling is month/year. *F* Open field; *G* under cover (plastic or net). Relatedness to TYLCV-ISR was scored from 1 to 4 according to the hybridization signals obtained with the two probes

1 Signal with full-length probe after low stringency wash only
2 Signal with both probes after low stringency wash only
3 Signal with full-length probe after high stringency wash, and with IR after low stringency wash
4 Signal with both probes after high stringency wash

Mediterranean basin (Spain, Italy and Tunisia), Equatorial Africa (countries on the Atlantic coast and inland, and on the coast of the Indian Ocean), Central and Southeast Asia (Turkmenistan, Thailand and Taiwan), and the Caribbean Islands (Cuba and the Dominican Republic). Samples obtained from the region of Cordoba in Argentina (tomato presented typical symptoms and whiteflies were overwhelmingly present) also reacted with this probe. The latter case was the only time a TYLCV-like disease was identified on the American continent. The origin of this isolate is still unknown. When the virus IR was used as probe, only tomato samples from the Middle East and the Caribbean Islands were detected after high stringency wash, indicating that these plants were infected by strains of the same virus. When the membranes were washed at low stringency, the IR probe detected also samples from the Western Mediterranean basin, Western Africa and Southeast Asia, indicating that these plants were infected by more distantly related viruses and probably represent one or more additional geminivirus species.

Comparisons of the intergenic region (IR) and of capsid (CP) and replication-associated (Rep) proteins from TYLCV isolates

Up to date the complete nucleotide sequences of seven isolates of TYLCV have been published, including a virulent (TYLCV-ISR [54]) and a mild isolate from Israel (TYLCV-ISR (Mild) [4]). In addition, sequences of genes and gene fragments from many other isolates have been published in the literature and in databases, or have been made available to us. We have compared TYLCV isolates, according to geographical regions, based on three genetic elements of the virus: 1. The IR, the least conserved region of the geminiviruses [57, 62], except for the $\sim$ 30 nucleotide stretch forming a stem-loop structure; 2. the CP, necessary for recognition by the insect vector [5, 9]; 3. the Rep, necessary for replication in the infected plant [20, 38].

Eastern Mediterranean Basin and Middle East

The complete sequence of TYLCV isolates from Israel (TYLCV-ISR) and from Egypt (TYLCV-EGY) have been published. In addition, the CP sequence of two isolates from Saudi Arabia (from the North- TYLCV-NSA, and from the South- TYLCV-SSA) and the Rep 131 N-terminal amino acids of an isolate from South Yemen are available (TYLCV-YEM).

The CP of TYLCV from Israel, from Egypt and from North Saudi Arabia share 95–96% homology, indicating that these isolates are closely related (IR and Rep of TYLCV from israel and Egypt are almost identical). In contrast, the CP of TYLCV from South Saudi Arabia is distinct from that in the North (78% homology only). TYLCV isolates from the Middle East differ from the TYLCVs of both the Western Mediterranean and Southeast Asia (40–50% homology in the IR, 70–85% in the CP, and 75–77% in the Rep). TYLCV-YEM groups with the Middle Eastern TYLCV isolates. The two Israeli isolates (virulent and mild) share high homologies in the CP and Rep (98 and 87%, respectively), but only 78% in the IR.

Western Mediterranean Basin

Three TYLCV isolates have been cloned and sequenced, two from Italy (Sicily - TYLCV-ITA (Sic), and Sardinia - TYLCV-ITA (Sar), and one from Spain (Murcia, TYLCV-SPA). Another isolate from Spain (Malaga), has been sequenced entirely (Bejarano, pers. comm.) and is almost identical to the isolate from Murcia.

The CP and Rep of these isolates are highly homologous (90–96%) indicating that they constitute a separate geographically-related group. They are different from the TYLCVs from Israel (85–88% homology in CP and 75–76% in Rep) and from Thailand (71–72% in CP and 72–75% in Rep).

Tropical Africa

The complete nucleotide sequence of a TYLCV isolate from tropical Africa is not yet available, even though TYLCV has been identified in many African

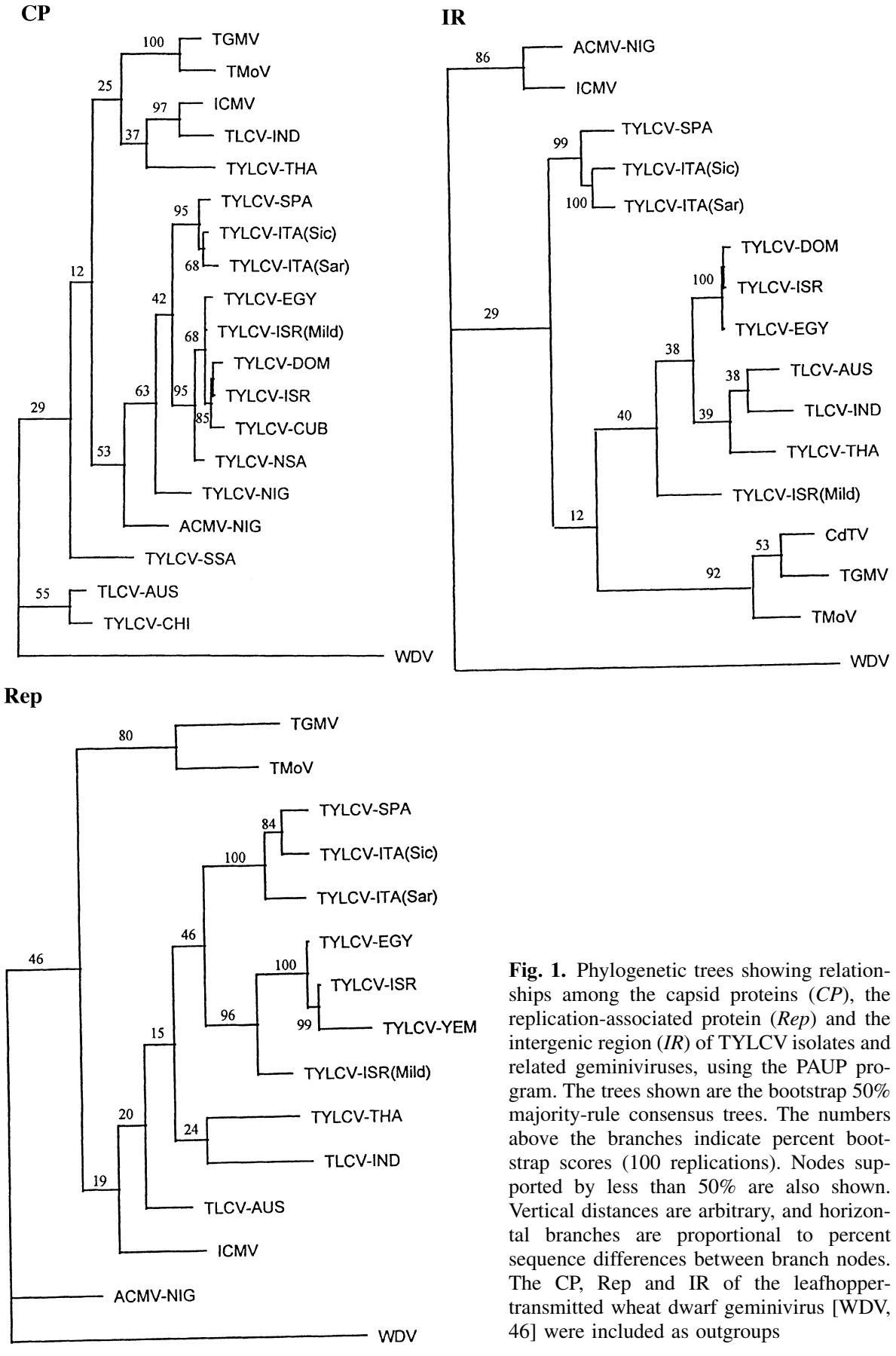

Fig. 1. Phylogenetic trees showing relationships among the capsid proteins (*CP*), the replication-associated protein (*Rep*) and the intergenic region (*IR*) of TYLCV isolates and related geminiviruses, using the PAUP program. The trees shown are the bootstrap 50% majority-rule consensus trees. The numbers above the branches indicate percent bootstrap scores (100 replications). Nodes supported by less than 50% are also shown. Vertical distances are arbitrary, and horizontal branches are proportional to percent sequence differences between branch nodes. The CP, Rep and IR of the leafhopper-transmitted wheat dwarf geminivirus [WDV, 46] were included as outgroups

countries (Table 1). The capsid protein gene of a TYLCV isolate from the region of Ibadan in Nigeria was cloned and sequenced (TYLCV-NIG). It has the closest homology with the capsid of TYLCV from Italy (90%) and from Israel (85%). It shares a higher degree of homology (80%) with the capsid of another whitefly-transmitted geminivirus from Nigeria, the African cassava mosaic virus (ACMV-NIG), than with the TYLCV from Thailand (76%).

Far East

The complete sequence of a TYLCV isolate from Thailand (TYLCV-THA) has been published. In addition, the sequence of the 76 N-terminal amino acids of the CP of a TYLCV isolate from China (TYLCV-CHI) (GuangXi province) is available (Tien and Liu, pers. comm.).

The TYLCVs from Thailand and China are different entities since their CP shares only 73% homology. Both isolates share about equal homologies with TLCV isolates from India (TLCV-IND) and from Australia (TLCV-AUS). The CP of TYLCV from Thailand is more homologous to the Indian cassava mosaic virus (ICMV) than to that of any other TYLCV and TLCV isolate of the region (79% v. 77–73%).

Caribbean Islands

A TYLCV isolate from the Dominican Republic (TYLCV-DOM) has been completely sequenced (Gilbertson and Maxwell, pers. comm.). In addition, the sequence of the 108 N-terminal amino acids of the CP of a TYLCV isolate from Cuba (TYLCV-CUB) is available.

The Caribbean isolates are almost identical to the Israeli virus (95–98% homologies in the IR, CP and Rep). They are different from the Western Mediterranean TYLCVs (79–84% homologies in the CP). Homologies with the other New World tomato geminiviruses CdTV, ToMV and TGMV, is only 55% in the IR and 56–69% in the CP.

Relationships among the IR, the CP and the Rep of TYLCV isolates and strains

Figure 1 presents phylogenetic trees obtained from the alignment of nucleotide and amino acid sequences of the IR, the CP and the Rep of TYLCV isolates and strains. The relationships among the TYLCV isolates reflects their geographical origin. The phylogenetic trees presents three main branches representing isolates from the Eastern Mediterranean basin (Italy, Spain), the Middle East (Egypt, Israel, Saudi Arabia) and the Far East (China, Thailand). TLCV isolates usually group with the TYLCV isolates (and with the cassava viruses) according to a geographical pattern. Tomato geminiviruses from the New World constitute a clear distinct group. The Caribbean TYLCV groups with the Middle Eastern isolates, indicating that they are exogenous to this region. The

phylogenetic tree based on the IR presents the clearest pattern, probably because the sequence of the IR is highly specific of a given virus.

Discussion

The name TYLC, or TLC, has been used to characterize viral diseases affecting tomato crops occurring within a broad tropical and subtropical belt, worldwide [23]. As a first approach, identification of the virus was based on symptom observation. The determination of the host range by whitefly transmission tests has been of help but sometimes results were surprising. For example, TYLCV from Jordan does not infect *Phaseolus vulgaris* unlike TYLCV from Israel that gives symptoms on *P. vulgaris*, even though both isolates are found just a few kilometers apart [13, 43]. Serological methods have met with limited success because antibodies raised against whitefly-transmitted geminiviruses cross-react with many viruses of this family [41, 64] even though heterologous monoclonal antibodies sometimes permit one to distinguish between TYLCV isolates from different regions (e.g. TYLCV from Senegal and from Nigeria, [47]). No means have been more powerful to identify and to classify TYLCV isolates than the analysis of their nucleotide sequences.

Several years ago, we started to use cloned DNA probes derived from a field isolate of TYLCV from Israel in squash-blot hybridization tests [53] to identify the virus in diseased tomato plants [16]. The results provided by the cloned probes usually confirmed the virus identification based upon symptom observation. In many cases, such as Italy, Spain, Tropical Africa, Thailand, and the Caribbean, squash-blot hybridization provided the first diagnosis of a TYLCV-like disease, sometimes several years before the local TYLCV isolate was cloned and sequenced. For example TYLCV was first identified in Italy (Sardinia and Sicily) in 1988–89, while the complete sequences of TYLCV-ITA (Sar) and of TYLCV-ITA (Sic) were published in 1991 and 1995, respectively. Similarly, TYLC-like diseases were identified in countries and regions where molecular data was either poor or not available yet, such as Central Asia and Eastern Africa. Recently the squash-blot technique has been instrumental in identifying a TYLCV outbreak in the Caribbean Islands as a recent import from the Middle East. Cloning and sequencing the local TYLCV isolate has always confirmed the diagnosis provided by the squash blot method. This method has been extremely powerful in its simplicity and is used routinely by us and by others as a screening and detection test for geminiviruses (e.g. [8, 50]).

Sequence comparison of many geminiviruses has allowed to propose rules to discriminate between different viruses and between strains of the same virus [57, 62]. Strains of any one virus have greater than 90% sequence identity. Isolates of the same strain have nucleotide sequence identities in their IR greater than 90% and distinct geminiviruses have nucleotide identities less than 85% [50]. The CP of viruses from different geographical regions have 65–80% sequence identity, whereas viruses from the same region have homologies

greater than 80%. These rules were applied to the identification and the classification of TYLCV isolates.

We have used three highly specific components of the viral genome to determine the relationships between the various TYLCV isolates: the IR, the capsid and the Rep protein. It seems that the IR has less constraints in its variation than the virus open reading frames, except for the conserved stem-loop sequence, some promoter elements and Rep binding sites [21]. The sequence of the IR is highly specific for a given virus. Replacement of the IR of TYLCV-ITA (Sar) by the IR of TYLCV-ISR prevented replication of TYLCV-ITA (Sar) DNA in tomato protoplasts [33]. The variability of the capsid protein is constrained to assure at least two functions: First, protein-protein interaction to assemble the capsid, and second, recognition by putative receptors of the insect vector [13]. Similarly, the Rep protein is variable within the limits of ensuring the binding to the virus origin of replication and the rolling circle mechanism of replication [33].

TYLCV-ISR and TYLCV-EGY can be considered to be isolates of the same strain because their IRs share more than 95% homology. Similarily the IR of TYLCV from Israel and from the Dominican Republic share homologies greater than 95%, indicating that TYLCV-DOM originated from the Middle East and was recently introduced to the Caribbean. On the other hand, the IR of the two isolates from Italy (from Sicily and Sardinia) share approximately 60% homology, and each one shares about the same homology with the Spanish isolate, indicating that these three isolates cannot be considered as the same entity.

Comparison of the amino acid sequence of the Rep protein provided a classification less stringent that obtained with the DNA sequence of the IR. For example, the two TYLCV isolates from Italy share 90% homology in the Rep protein (versus 60% in the IR); the same level of homology is shared between the Rep of TYLCV-ITA (Sar) and TYLCV-SPA. The Rep protein of TYLCV isolates from the Western Mediterranean, the Middle East (together with the Caribbean) and the Far East share 72–77% homology, pointing to at least three distinct geographically related viruses. No data is available for TYLCV Rep from Equatorial Africa.

A similar geographically-associated variation has been proposed from the comparison of the CP of TYLCV isolates and of various whitefly-transmitted geminiviruses [28, 35, 50, 57]. Differences associated with geography are best illustrated when comparing the isolates from North and South Saudi Arabia. Their capsid proteins share only 78% homology. The Northern isolate is closely related to TYLCV-ISR (95% homology), while the Southern isolate is not (81% homology). The difference between the two Saudi TYLCV isolates was explained by their separation by a large expanse of desert [28]. On the other hand, the two Italian isolates of TYLCV are found in two islands (Sicily and Sardinia) separated by 300 km; nevertheless their capsid protein are highly (95%) homologous. In several cases, the CP of TYLCV was more homologous to the CP of other whitefly-transmitted geminiviruses occurring in the same

region than to TYLCV isolates from other regions. For example the CP of TYLCV from Nigeria is more related to the local ACMV (80%) than to TYLCV from Thailand (76%), a fact that may point to adaptation of the geminivirus to its vector. Moreover, viruses in different geographical regions have different epitope profiles, whereas those from the same region have similar profiles [26, 41]. Differences in the sequence of the capsid protein may lead to differences in virus transmissibility by *B. tabaci* [47].

The identification and the classification of whitefly-transmitted geminiviruses was done so far without taking into account the possible variation with time of a given virus in a given place due either to modifications of the resident virus or to the invasion of new strains. Although is not easy to appreciate changes in the nucleotide sequence of any geminivirus with time, the fact that TYLCV was studied in Israel for the last 35 years may provide some clues. Besides the field isolate cloned in 1988 (TYLCV-ISR), a clone inducing mild symptoms in tomato (TYLCV-ISR (Mild) was generated from the TYLCV culture characterized in the early sixties [12] and maintained in the greenhouse for the last 30 years. Sequence comparison between these two isolates show marked changes in the IR (78% identity, the stem-loop element being strictly conserved). These differences may indicate the virus that was present in the field has evolved or that it was replaced by a different, more virulent, strain. Indeed, TYLCV has acquired new hosts during the last three decades [13] such as bean [55], lisianthus [11] and petunia (unpubl. obs.). Alternatively, the isolate from the sixties may have mutated during its passage by whiteflies in the greenhouse. The fact that TYLCV variants were found to occur in plants following agroinoculation of cloned viral DNA [32] indicate that TYLCV sequence changes are not rare events. This is despite the general conclusion that mutational rate caused by copying errors during DNA replication is limited by the host proof-reading mechanism [60].

The fact that the recent outbreak of TYLCV in the Caribbean Islands was probably due to an import from the Middle east underlines the dangers of virus spread resulting from the circulation of plant materials and persons. From a limited source, it is likely that TYLCV has rapidly spread to the three major islands (Cuba, Jamaica, Dominican Republic – Haiti), vectored by the whitefly *B. tabaci*. Storms may have facilitated the insect, and virus, expansion.

The great variability of TYLCV worldwide should be considered when breeding programs for virus resistance are established. A tomato line tolerant/resistant to a particular TYLCV isolate may not be as effective against another distantly-related virus isolate, or even several years after the program started.

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