Geminiviruses Infecting Tomato Crops in Nicaragua

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ABSTRACT

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Geminiviruses transmitted by whiteflies are believed to be responsible for the devastating epidemic in tomato crops in Nicaragua, as well as in other Central American countries. Polymerase chain reaction with degenerate primers was used to amplify partial sequences of the geminivirus coat protein gene from samples of diseased tomato plants collected from the major tomato-growing areas of Nicaragua. The data indicated the presence of geminiviruses in all tested regions of the country. DNA sequence analysis and phylogenetic analysis of the amplified sequences showed that they corresponded to four different geminiviruses related to the other begomoviruses native to the Americas. One of the viruses, which was detected in three regions of Nicaragua, is probably *Sinaloa tomato leaf curl virus*. The sequences of two of the other detected viruses showed close relationships with several geminiviruses, including *Tomato mottle virus*, *Tomato leaf crumple virus*, and *Sida golden mosaic virus*, all of which previously have been reported from Central America. The fourth virus is closely related at sequence level to a tomato-infecting geminivirus from Honduras, putatively designated *Tomato mild mottle virus*. This virus seems to be different from the other known American begomoviruses because it groups separately in the phylogenetic analysis.

Additional keywords: *Begomovirus*, *Bemisia tabaci*, *Lycopersicon esculentum*

Geminiviruses (family *Geminiviridae*) have emerged as important plant pathogens worldwide (2). They are characterized by twinned isometric particles and circular, single-stranded DNA genomes (10) and can be subdivided into three genera on the basis of host range, insect vector, and genome organization (12). Geminiviruses belonging to the genus *Begomovirus* (subgroup III) have monopartite or bipartite genomes, are transmitted by whiteflies (*Bemisia tabaci* Genn.), and infect dicotyledonous plants. The genomic component DNA-A encodes functions necessary for viral replication and encapsidation, while genes on DNA-B are required for viral movement in plants (10).

Since the mid-1980s, several important food crops, including beans (*Phaseolus vulgaris* L.) and tomatoes (*Lycopersicon esculentum* Mill.), in Central America and

the Caribbean Basin have been severely affected by whitefly-transmitted geminiviruses (3,14). Losses in many vegetable crops have been significant; therefore, the common response has often been massive overuse of insecticides at considerable cost but without significant benefit (6). The situation is complex, with a number of indigenous geminiviruses found to be infecting tomato in the Americas (14), including *Sinaloa tomato leaf curl virus* (STLCV) in Mexico (8) and Costa Rica (9), *Tomato mottle virus* (ToMoV) in Florida (1), *Tomato leaf curl virus* (ToLCV) in Panama (4), *Tomato leaf crumple virus* (TLCrV) in Mexico (13), *Havana tomato virus* (HTV) and *Taino mottle virus* (TTMoV) in Cuba (15,20), *Tomato dwarf leaf curl virus* (TDL CV) in Jamaica (16), and *Potato yellow mosaic virus* (PYMV) in Trinidad and Tobago (18). In addition, the monopartite geminivirus *Tomato yellow leaf curl virus* (TYLCV) from Israel has recently been detected in the western hemisphere (14).

In Nicaragua, a tomato disease believed to be transmitted by whiteflies was observed in the tomato-producing areas of Tisma in 1970s, but with very low incidence. In the early 1980s, the disease also appeared in the Sebaco Valley and was associated with a rapid increase in whitefly populations. In 1988, the epidemic affected 100% of the tomato crop in the Sebaco

Valley and other regions of the country, with drastic yield reductions. At the end of 1990, the problem had become nationwide and tomato production virtually vanished. Since then, the problem has persisted. After the occurrence of hurricane Mitch in October 1998, the epidemic was momentarily suppressed, probably due to a large reduction in whitefly populations. However, in February 1999, the whitefly population level increased again and 100% of the tomato fields were infected, showing different types of symptoms.

Geminiviruses transmitted by whiteflies are believed to be responsible for the disease of tomatoes in Nicaragua, but very little is known about the identity of the disease agent. In a preliminary survey of Central America, degenerate primers were used to amplify geminivirus DNA-A and DNA-B genomic components from tomato plants displaying virus symptoms (11). This led to the identification of two geminiviruses: one similar to the *Squash leaf curl virus* cluster in Guatemala, Honduras and Nicaragua, and another unknown virus in Costa Rica. The objective of this study was to determine the possible occurrence

Fig. 1. Locations where tomato plants were sampled for detection of geminiviruses. 1, Condega (samples PL, C, and ST); 2, La Almaguera (samples AL); 3, Sebaco (samples H and EE); 4, San Ramon (samples SR); 5, Muy Muy (samples Z); 6, Santa Lucia (samples SL); 7, Juigalpa (samples T); 8, Santo Tomas (samples TB); and 9, Tisma (samples Ti).

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and magnitude of geminiviruses in tomato crops in Nicaragua.

MATERIALS AND METHODS

Collection of plant samples. Tomato leaf samples with disease symptoms were

collected in August 1998 and January 1999 from 11 fields at nine locations (at least one plant per field; Fig. 1 and Table 1), representing the major tomato-growing areas in Nicaragua. Samples from a field with symptomless tomato plants were col-

lected at La Almaciguera, Esteli. Healthy tomato plants grown in a greenhouse were used as negative controls.

Polymerase chain reaction. Direct polymerase chain reaction (PCR) detection of geminiviruses in leaf extracts was performed essentially as described by Wyatt and Brown (19). PCR was carried out in PCR tubes preincubated with tomato leaf extracts using the Expand High Fidelity PCR system (Boehringer Mannheim, Mannheim, Germany). As negative controls, PCR was run in tubes not preincubated with tomato leaf extracts or in tubes preincubated with tomato leaf extracts from healthy plants. Degenerate primers AV494 and AC1048 (19) were used for amplification by PCR of the core region of begomovirus coat protein sequences. The samples were heated to 94°C for 2 min, then subjected to 34 cycles of amplification using a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA). Each cycle consisted of 30 s at 94°C, 1 min at 55°C, and 2 min at 72°C. The last cycle was followed by 10 min of elongation at 72°C.

Reaction products were analyzed by electrophoresis on 1.0% agarose gels. The PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany).

Cloning and sequencing. The PCR fragments were cloned using TOPO TA Cloning (Invitrogen, Carlsbad, CA). The clones were sequenced using the Thermo Sequenase dye terminator cycle sequencing kit, (version 2.0; Amersham Life Science, Inc., Cleveland, OH) and an ABI Prism 377 DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA). The complete sequences were determined on both strands using synthetic oligonucleotides.

Sequence analysis. The nucleotide sequences of 533 bp (excluding primers) of the cloned PCR fragments, corresponding to amino acids 40 to 216 of the coat protein of *Bean golden mosaic virus* from Puerto Rico (7), were aligned with geminivirus sequences available in the GenBank database (Table 2). Parsimony analysis was accomplished using PAUP 3.1s (17).

RESULTS AND DISCUSSION

Detection and isolation of geminivirus DNA. The symptoms observed on tomato plants included yellow mottling, crinkling, curling, and severe stunting. PCR products of the expected size (550 bp, as estimated from EtBr-stained agarose gels following electrophoresis), were obtained for samples of symptomatic tomato plants from all fields with diseased plants (Table 1), whereas the samples from one location, La Almaciguera, where no symptoms were observed in the tomato plants, were negative in the PCR tests. The results, therefore, indicated that the severely diseased tomato plants were geminivirus infected

Table 1. Sites from which tomato plants were sampled in the field in Nicaragua in 1998 and 1999

Region	Location, field ^a	Sample ^b	Date	Tomato variety
Esteli	Condega			
	Piedra Larga	PL	August 1998	UC-82
	Los Cerritos	C	January 1999	UC-82
	Santa Teresa	ST	January 1999	Peto-98
Matagalpa	La Almaciguera	AL	January 1999	UC-82
	Sebaco			
	El Horno	H	August 1998	UC-82
	Estacion Exp.	EE	January 1999	Rio Grande
	San Ramon	SR	January 1999	Caribe
Boaco	Muy Muy			
	Zapotal	Z	January 1999	UC-82
	Santa Lucia	SL	January 1999	UC-82
	Juigalpa			
Chontales	Tecolostote	T	January 1999	UC-82
	Santo Tomas			
	Tierra Blanca	TB	January 1999	UC-82
Masaya	Tisma	Ti	January 1999	UC-82

^a Samples were collected from two to three fields at Condega and Sebaco, and from single fields at the other seven locations.

^b Also used for naming of cloned amplification products. All were positive for geminiviruses except sample AL.

Table 2. Abbreviations and GenBank/EMBL/DBJ accession numbers for geminiviruses in phylogenetic analysis

Virus	Abbreviation	Accession number
Determined in this study		
<i>Sinaloa tomato leaf curl virus</i> clone EE69	EE69	AJ277052
<i>Sinaloa tomato leaf curl virus</i> clone TB86	TB86	AJ277053
<i>Sinaloa tomato leaf curl virus</i> clone SR115	SR115	AJ277054
<i>Sinaloa tomato leaf curl virus</i> clone ST28	ST28	AJ277055
<i>Sinaloa tomato leaf curl virus</i> clone Z31	Z31	AJ277056
Tomato geminivirus clone SL153	SL153	AJ277057
Tomato geminivirus clone T112	T112	AJ277058
Tomato geminivirus clone C32	C32	AJ277059
Tomato geminivirus clone Ti21	Ti21	AJ277060
Tomato geminivirus clone H11	H11	AJ277061
From database		
<i>Abutilon mosaic virus</i> —West Indies	AbMV-WI	X15983
<i>Abutilon mosaic virus</i> —Hawaii	AbMV-HI	U51137
<i>Bean calico mosaic virus</i>	BCMv	AF110189
<i>Bean dwarf mosaic virus</i>	BDMV	M88179
<i>Bean golden mosaic virus</i> —Brazil	BGMV-BR	M88686
<i>Bean golden mosaic virus</i> —Dominican Republic	BGMV-DR	L01635
<i>Bean golden mosaic virus</i> —Guatemala	BGMV-GA	M91604
<i>Bean golden mosaic virus</i> —Puerto Rico	BGMV-PR	M10070
<i>Cabbage leaf curl virus</i>	CaLCV	U65529
<i>Havana tomato virus</i>	HTV	Y14874
<i>Pepper Huasteco virus</i>	PHV	X70418
<i>Potato yellow mosaic virus</i> —Trinidad and Tobago	PYMV-TT	AF039031
<i>Potato yellow mosaic virus</i> —Venezuela	PYMV-VE	D00940
<i>Sida golden mosaic virus</i> —Costa Rica	SiGMV-CR	X99550
<i>Sida golden mosaic virus</i> —Honduras	SiGMV-HN	Y11097
<i>Sida golden mosaic virus</i> —Honduras (yellow vein)	SiGMV-HN/Yv	Y11099
<i>Sinaloa tomato leaf curl virus</i>	STLCV	AF040635
<i>Sinaloa tomato leaf curl virus</i> —Costa Rica	STLCV-CR	AF110515
<i>Squash leaf curl virus</i>	SLCV	M38183
<i>Taino tomato mottle virus</i>	TTMoV	AF012300
<i>Texas pepper virus</i> —Chiapas	TPV-CPS	AF077025
<i>Tobacco apical stunt virus</i>	TbASV	AF076855
<i>Tomato golden mosaic virus</i>	TGMV	K02029
<i>Tomato leaf crumple virus</i>	TLCrV	L34747
<i>Tomato leaf curl virus</i> —Panama	ToLCV-PA	Y15034
<i>Tomato mottle virus</i>	ToMoV	L14460
<i>Tomato yellow leaf curl virus</i> —Dominican Republic	TYLCV-DR	AF024715

and suggested that geminiviruses are widespread in tomato crops in Nicaragua.

Sequence analysis of PCR-amplified fragments. To partially characterize the geminiviruses, PCR-amplified products were cloned and sequenced. One PCR clone from each field was completely sequenced in both directions (except for Piedra Larga), with additional clones partially sequenced or analyzed using restriction enzyme digests. Identical or almost identical sequences or restriction digest patterns were obtained in the samples examined within a field. Thus, no evidence of mixed infections with different geminiviruses was obtained. Sequence analysis of the cloned PCR fragments (533 bp) revealed that they corresponded to four different groups, all similar to published sequences of bipartite begomoviruses (Fig. 2).

One sequence group, comprising fragments from samples in the regions of Matagalpa (EE, Z, and SR), Esteli (ST), and Chontales (TB), showed 97 to 99% identity at the nucleotide level to the coat protein gene of STLCV from Costa Rica (9). The high sequence identity suggested that these fragments were derived from Nicaraguan isolates of STLCV. Two other sequence groups, represented by fragments from Boaco (SL) and Chontales (T), showed highest sequence identities to *Sida golden mosaic virus* (SiGMV) from Honduras (92%) (5) and to TLcRV from Mexico (94%) (13), respectively. The sequences of a fourth group, from Sebaco (H), Condega (PL and C), and Masaya (Ti), displayed 98% nucleotide identity to a 199-bp sequence from a geminivirus designated as *Tomato mild mottle virus*

(TMMoV; GenBank accession number AF131071) from Honduras. Otherwise, the fourth group of sequences showed only limited similarity to published sequences; that is, approximately 80% nucleotide identity to *Clitoria geminivirus* (AF070924) and *Malva geminivirus* (AF070925) from Puerto Rico.

A phylogenetic analysis was carried out, including the partial nucleotide sequences of 10 Nicaraguan virus isolates and available sequences of begomoviruses from the New World (Table 2). The STLCV sequences from Costa Rica and Mexico and the Nicaraguan STLCV-like sequences formed one well-supported group (Fig. 3). This result further supports the idea that the Nicaraguan STLCV-like sequences represent Nicaraguan isolates of STLCV. The presence of STLCV in three different regions of Nicaragua and in Costa Rica and Mexico shows that STLCV is widely distributed in Central America.

The sequences of the clones SL153 and T112 were found in the phylogenetic analysis to group with the American begomoviruses and to be clearly distinct from the other Nicaraguan sequences. A relationship for SL153 and T112 with ToMoV (1), TLcRV (13), and SiGMV-Honduras (5) is suggested but not well supported. Therefore, these two sequences cannot be assigned to any specific geminivirus species until the viruses from which they originated are more thoroughly characterized.

The sequences C32, H11, and Ti21 form a separate group outside of the other American begomoviruses, suggesting that they, together with TMMoV from Hondu-

ras, represent a new group of American begomoviruses.

Our data confirm that tomato plants in all major tomato-growing areas of Nicaragua are infected with geminiviruses and suggest that they represent at least four different strains or species. Thus, geminiviruses are an important causal agent of the severe disease epidemics in tomato crops in Nicaragua, as reported for other parts of Central America and the Caribbean region (14). Identification of the viruses involved is an important step toward the management of the disease. This study shows the complexity of geminivirus diseases of tomato in Nicaragua, which are widespread and caused by several distinct geminiviruses. In addition, it is possible that these geminiviruses infect other crops and weeds that can serve as bridging hosts (18). For example, SL153 displayed the closest rela-

Ti21	IYRTVRSADV	PRGCEGPCKI	QSFEQRHDIS	HTGKVMCISD	VTRGNGITHR	VGKRFVCVKS
C32	-----T---					
H11	-----T---					
EE69	--VM-GP--	-K-----V	--Y-----	-V-----		-L-----
SR115	--VM-GP--	-K-----V	--Y-----	-V-----		-L-----
TB86	--VM-GP--	-K-----V	--Y-----	-V-----		-L-----
SL153	--L-TP--		--Y-----	-V-----		
T112	--ML-TP--		--Y-----	-V-----		
BGMV-PR	--MYK-P--	-K-----V	--Y-----	-V-----	I-----	
Ti21	YILGKIWMDD	NIKLNHNTS	VMFWLVRDRR	PNGTAMDFGH	LFNMFDNEPS	TATVKNDLRD
C32	-----V---			-S-----		
H11	-----V---			-S-----		
EE69	-----V---			-Y-IP--Q	V-----	-I-----
SR115	-----V---			-Y-IP--Q	V-----	-I-----
TB86	-----V---			-Y-IP--Q	V-----	-I-----
SL153	-----E---			-Y-P--Q	V-----	
T112	-----E---			-Y-P--Q	V-----	
BGMV-PR	-----E---	-M-----	-I-----	-Y-P--Q	V-----	-F-----
Ti21	RFQVMHRFHA	KVTGGQYASN	EQTLVKRFWR	VNHRVTYNNQ	EAARYENHTE	NALMLYM
C32	-----L---			-Q-----		
H11	-----L---			-Q-----		
EE69	-----Y---		--AI-R--K	--NH-V--H	--GK-D---	--L---
SR115	-----Y---		--AI-R--K	--NH-V--H	--GK-D---	--L---
TB86	-----Y---		--AI-R--K	--NH-V--H	--GK-D---	--L---
SL153	-Y-----K-YG		--AI--K	--NH-V--H	--GK-----	--L---
T112	-Y-----K-YG		--AI--K	--NH-V--H	--GK-----	--L---
BGMV-PR	-Y-----N-	--S-----	D-AL-R--K	--NH-V--H	--GK-----	--L---

Fig. 2. Alignment of the deduced amino acid sequences for the 10 completely sequenced polymerase chain reaction-amplified fragments. The sequences correspond to amino acids 40-216 of the *Bean golden mosaic virus*-Puerto Rico coat protein (7). The amino acid sequences of Z31 and ST28 were identical to EE69.

Fig. 3. Phylogenetic analysis of tomato-infecting Nicaraguan geminiviruses and other American geminiviruses available from GenBank based on the alignment of partial nucleotide sequences of the coat proteins. The strict consensus tree obtained from parsimony analysis is shown. Numbers represent bootstrap values exceeding 50 out of 100 replicates. The sequences of the Nicaraguan geminiviruses from this study (C32, Ti21, H11, EE69, TB86, SR115, ST28, Z31, SL153, and T112) are in bold. For abbreviations of virus names and accession numbers, see Table 2.

tionship with SiGMV, a virus that is known to infect *Sida* spp., which are common weeds in tomato fields in Nicaragua.

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