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Genetic Diversity of Tomato (*Solanum lycopersicum* L.) Begomovirus in Togo

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Abstract

Geminiviruses, in particular the members of the genus *Begomovirus*, are considered to be a major phytosanitary problem for tomato crops production in the world. They are responsible for yield losses of up to 20% to 100%. Regrettably, Togo is not spared from this situation. This work aims to show the genetic diversity of the begomoviruses affecting tomato crops production in Togo and their relationship with other begomoviruses. To achieve these objectives, 307 samples of tomato leaves and wild plant species with typical virus symptoms were collected in the Maritime, Plateaus, Central, Kara and Savannah regions and submitted to PCR analysis. The results revealed the presence of begomovirus in 25.40% of the analyzed samples. The PCR products obtained were submitted to direct sequencing. Phylogenetic analysis of sequences of DNA-A different regions of begomovirus identified in this work with that of other begomoviruses showed a nucleotide identity of 96% respectively for *Tomato leaf curl Togo virus*-Fontem, *Tomato Leaf Curl Togo Virus*, *Ageratum leaf curl Cameroon Alphasatellite*; 98% respectively to *Tomato leaf curl Nigeria virus*, *Ageratum leaf curl Cameroon virus*, *Tomato leaf curl Cameroon virus*-Fontem, *Ageratum leaf curl Cameroon virus* and 99% respectively to *Tomato leaf curl Kumasi virus*, *Pepper yellow vein Mali virus* Bazegahot and *Pepper yellow vein Mali virus*-Ouaga. These results suggest a high degree of genetic diversity of tomato begomoviruses identified in Togo.

Keywords

Begomoviruses, Direct Sequencing, Phylogenetic Relationships, Tomato, Wild Plants

1. Introduction

Begomoviruses, transmitted in persistent, circulative manner by *Bemisia tabaci* Gennadius (Homoptera: *Aleyrodidae*), are a major limiting factor for the production of many agricultural species in tropical and subtropical regions of the world [1]. In particular, approximately 70 species of the genus *Begomovirus* (family *Geminiviridae*) have been identified as naturally infecting tomato (*Solanum lycopersicum* L.) in different parts of the world [2] [3] and even in some places, these diseases have eliminated the tomato as an economically viable crop [4] [5].

The diversity of geminiviruses is reflected in seven genera (*Becurtovirus*, *Begomovirus, Curtovirus*, *Eragrovirus, Mastrevirus, Topocuvirus* and *Turncurtovirus*), which are defined on the basis of genome structure, host range and insect vector [6] [7]. With 288 species currently recognized by the International Committee of Taxonomy of Viruses (ICTV), the genus *Begomovirus* is the largest genus of plant viruses in relation to the number of members it includes (http://www.ictvonline.org/virusTaxonomy.asp). Their genome is a single-stranded circular DNA that can be monopartite (containing DNA-A) or bipartite (containing both genomic DNA-A and B) [8].

Based on genome organization, phylogenetic relationship and geographic distribution, begomoviruses have generally been divided into two groups: the begomoviruses of the Old World (Europe, Africa, Asia and Australia) and the begomoviruses of the New World (Americas) [9]. Most bipartite begomoviruses are found in the New World, while most monopartite species are found in the Old World. However, there are exceptions. Bipartite begomoviruses such as *Tomato leaf curl New Delhi virus* (ToLCNDV) [10] and *Tomato yellow leaf curl Thailand virus* (TYLCTHV) [11] are distributed in the Old World, while the monopartite *Tomato yellow leaf curl virus* (TYLCV) was introduced in the New World in the early 1990s and it is now widespread in the Caribbean basin and in the South America (Hawaii, Mexico and Guatemala) [12].

Contrary to the considerable genetic diversity that exists among begomoviruses infecting tomato crops, the symptoms induced by these viruses are relatively similar and include varying degrees of stunting, leaf curling, mottling and yellowing. In addition, symptoms vary depending on the cultivar, host plant species, age of the plant at the infection time, environmental factors and mixed infections with other viruses or pathogens. Thus it is difficult, if not impossible, to identify the species involved in an outbreak based solely on symptoms, hence the importance of a molecular analysis for the identification of these viruses.

In Western Africa begomoviruses have emerged recently and are caused by genetically distinct species that have evolved locally, hence these regions could be an important area for new evolutionary linkages of the species within the genus [4] [13] [14] [15].

The work aimed at studying the genetic diversity of begomoviruses infecting tomato and associated wild plants in tomato fields in Togo.

2. Material and Methods

2.1. Sample Collection

A total of 307 samples of tomato leaves and wild plants showing typical symptoms of begomoviruses; leaf curling, yellowing, stunting and leaf thickening (Figure 1) were collected in 2013, 2014 and 2015 from Maritime, Plateaus, Central, Kara and Savannah regions in Togo (Table 1). Samples were also collected on wild plants in tomato fields for the search of inoculum sources of begomoviruses. For molecular analyses, samples were dried in an oven at 40˚C. It is important to notice that the variability in the number of collected samples is due to the fact that the sampling protocol used, required that tomato fields should be separated from each other of 10 km in order to take geo-referenced positions.

2.2. DNA Extraction and PCR Running

Total nucleic acids were extracted from symptomatic tomato plants and wild plants through the methods adapted by [16]; 150 mg of desiccated tissue was ground in 500 µL of extraction buffer (2% de CTAB, 1, 4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, 1% PVP, pH 8, 0 and 0, 2% β-mercaptoéthanol) and incubated at 60˚C for 30 min. The mixture was kept on ice for 10 min followed by centrifugation at 13,000 rpm for 15 minutes at 4˚C. The supernatant was transferred to a new vial and diluted with distilled water before using in Polymerase Chain Reaction (PCR) reaction.

Begomoviruses identification was performed by PCR using six pairs of universal primers able to amplify the different regions of the component DNA-A

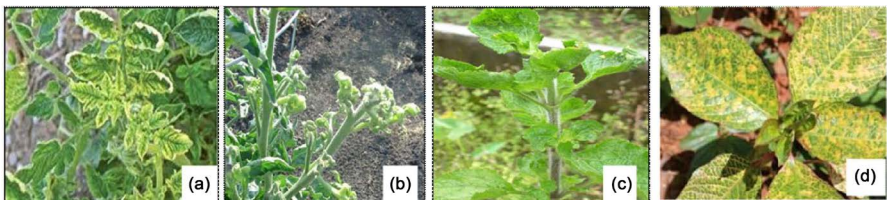

Figure 1. *Solanum lycopersicum* plants from Togo with leaf curl and yellowing symptoms (a), stunting symptoms (b), *Ageratum conyzoides* with leaf curl symptoms (c) and *Euphorbia heterophylla* with mosaic symptoms.

Table 1. Number of tomato and wild plants infected leaf samples in tomato production regions in Togo.

Regions	Samples from Tomato	Samples from wild plants
Maritime	41	54
Plateaus	8	5
Central	11	5
Kara	74	32
Savannah	46	31
Total	180	127

Table 2. Primers used in this study.

Primers	Sequences 5' ----------3'	Length (bp)	Amplified region (DNA-A)	PCR Conditions	References
AVcore ACcore	GCCHATRTAYAGRAAGCCNAGRAT GGRTTDGARGCATGHGTACANGCC	575	CP	94˚C /2min, 35 × (94˚C 1 mn/60˚C 2 mn/72˚C 2 mn) 72˚C /10min	[17]
Av494 Ac1048	GCCYATRTAYAGRAAGCCMAG GGRTTDGARGCATGHGTACATG	550	CP	94˚C /2 min, 35 × (94˚C 1 mn/55˚C 2 mn/72˚C 1 mn) 72˚C/10min	[18]
Deng A Deng B	TAATATTACCKGWKGVCCSC TGGACYTTRCAWGGBCCTTCACA	520	CP	94˚C /2 min, 30 × (94˚C 1mn/61˚C 1 mn/72˚C 2 mn) 72˚C/10min	[19]
PALIc 1960 PARIv 722	ACNGGNAARACNATGTGGGC GGNAARATHTGGATGGA	1200	AC1,AC2,AC3, CP	94˚C /2 min, 30 × (94˚C 1 mn/55˚C 2 mn/72˚C 2 mn) 72˚C/10min	[20]
PAR1c 496 PCRv 181	AATACTGCAGGGCTTYCTRTACATRGG TAATATTACCGGWTGGCC	500	CP		
TYv 2664 TYc138	ATTGACCAAGATTTTTACACTTATCCC AAGTGGGTCCCACATATTGCAAGAC	316	IR	94˚C/5 min, 30× (94˚C/1min, 62˚C/45 sec, 72˚C/1min.), 1× (94˚C/1 min, 56˚C/1 min, 72˚C/10 min).	[21]

D = A, G, T; H = A, C, T; K = G, T; M = A, C; N = A, C, G, T; R = A, G; W = A, T; Y = C, T

according to protocols of [17] [18] [19] [20] and [21] (**Table 2**). The universal primer pair Avcore/ACcore [17] which amplify a 575 nucleotide fragment corresponding to the core region of coat protein (CP) gene of almost all begomoviruses. Two sets of Begomovirus group specific universal primers Deng A/Deng B primers [19] and Av494/Ac1048 [18] which are capable to amplify the core CP of many begomoviruses were used for viral detection. The primer pair PALIc1960/PARI722 produces a 1.2 Kb band upon PCR and had been designed to amplify the bottom half region of the A genome component of most WTGs [20]. Primers PAR1c496/PCRv181 [20] amplify ~300 bp of the A component that contains ~172 bp of region between the beginning of the loop and the beginning of the CP and~126 bp of the CP. Primers TYv2664/TYc138 were used to amplify the IR of TYLCV-Mld.

The parameters for the PCR reaction were optimized for 25 µl. The final concentrations of reaction components were: 200 µM deoxynucleotide triphosphate (dNTPs), 1x Taq DNA polymerase buffer, 2.5 mM $MgCl_2$, 0.8 units Taq DNA polymerase, 1 µM of each complementary and virus-sense primers and 2 µl of DNA. PCR cycle parameters were as described in **Table 2**. All PCR reactions were performed in a programmable thermocycler (Mastercycler ep gradient S, Eppendorf, Hamburg, Germany).

The amplified products, along with 10 kb DNA ladder, were resolved in 1% agarose gel in Tris-borate EDTA (TBE), pH 8.0 buffer with 10 µl/100ml GelGreenTM. Gel electrophoresis was carried out at 70 V until tracking dye has reached the bottom of the gel. The DNA bands were viewed and photographed using a gel documentation system (BioRad, Hercules, CA, USA).

2.3. Sequencing and Phylogenetic Analysis

PCR products from amplifications were purified using the Agentcourt AMPure XP magnetic beads (Beckman Coulter, Inc. 250 S. Kraemer Blvd. Brea, CA 92821 USA) according to the manufacturer's protocol. The Illumina Nextera XT Index kit (Illumina Inc., San Diego, CA, USA) was used according to the manufacturer's instructions to assign a code to each sample prior to sequencing. The purity of the PCR products was verified at the Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA, USA) and the sequencing was performed at the UPJV Molecular Biology platform via the high throughput technique using the kit V2 of the Illumina Miseq (Illumina Inc., San Diego, CA, USA).

The partial sequences were assembled using the FROG software (http://bioinfo.genotoul.fr/fileadmin/user_upload/FROGS_poster_Jobim_2015.pdf) and aligned with the sequences available in the GenBank database using the BLASTn algorithm (http://www.ncbi.nlm.nih.gov). The multiple alignment of the sequences was obtained with the ClustalX software [22] with default parameters. Phylogenetic analyze was performed by the Neighbor-Joining method using Darwin5. A thousand Bootstrap replicas were used to evaluate the robustness of the topology of the final tree. The nucleotide sequences of isolates *Tomato geminiviruses* Lebanon (TOGV-LB), *Tomato geminiviruses* Gezira (TOGV-SD), *Tomato yellow leaf curl virus*-Puerto Rico (TYLCV-PR), *Tomato geminiviruses Kuwait* (TYLCV-KU), *Tomato yellow leaf curl virus*-Reunion (TYLCV-RU), *Tomato yellow leaf curl virus*—Egypt (TYLCV-EG), *Tomato yellow leaf curl virus*—Israel (TYLCV-IS), *Tomato yellow leaf curl virus-Mild* [Spain7297] (TYLCV-SP), *Tomato yellow leaf curl virus—Mild* [Shizuokua] (TYLCV-JP), *Tomato yellow leaf curl virus*—Cuba (TYLCV-CU), *Tomato yellow leaf curl Mali virus* (TYLCV-ML), *Tomato leaf curl Nigeria virus* (ToLCNGV), *Tomato leaf curl Cameroon virus*—Fontem (ToLCCMV-Fontem) and *Pepper yellow vein Mali virus*-Ouagadougou (PepYVMLV-OUAGA) were included in the analysis.

3. Results

3.1. Begomoviruses Identification by PCR

A total of 307 samples among with, 180 samples from tomatoes and 127 from wild plants were collected and based on PCR analysis, 66 tomatoes and 12 wild plants were revealed to be positive for the presence of begomoviruses. In the Maritime Region, 10.52% of the samples analyzed were positive, 53.84% in the Plateaus region, 31.25% in the Central Region, 44.33% in the Kara region and 11.68% in the Savannah region (Table 3). Surprisingly, no infection by begomoviruses was reported in Kpendjal Prefecture. These results could suggest that the insect vector *B. tabaci* is not present in the environment. The results obtained from PCR showed that the isolate TYLCV-Mild was not detected in all the collected samples. Different fragment sizes were obtained from five geminiviruses

Table 3. Percentage of samples infected by begomoviruses in the five economic regions of Togo.

Regions of Togo	Number of collected samples tomatoes (*Solanum lycopersicum*)	Number of samples reacted positively to PCR	Number of collected samples wild plants	Number of samples reacted positively to PCR	Percent disease incidence (%)
Maritime	41	8	54	2	10.52
Plateaus	8	5	5	2	53.84
Central	11	5	5	0	31.25
Kara	74	44	32	3	44.33
Savannah	46	4	31	5	11.68
Total of Togo	180	66	127	12	25.40

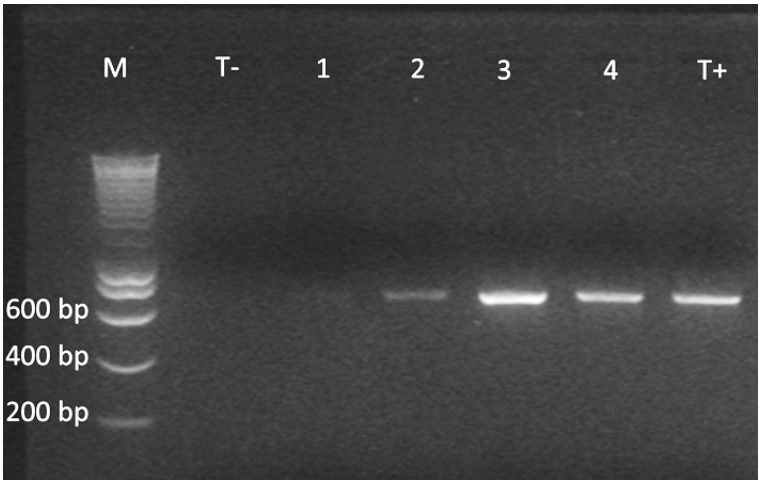

Figure 2. Electrophoretogram with the pair of primers AVcore/ACcore giving fragments of approximately 700 base pairs. M = marker, 1, 2, 3 and 4 = tomato samples, T+ = positive control, T− = negative control.

primer pairs (**Figure 2**).

3.2. Sequence Analysis and Identification of New Viruses

A total of 123 PCR products including 111 from tomato and 12 from wild plants were fully sequenced to identify the different species of the genus *Begomovirus*. Partial sequences up to 500 nucleotides were obtained from each reaction and processed with the FROG software (http://bioinfo.genotoul.fr/fileadmin/user_upload/FROGS_poster_Jobim_2015.pdf). These partial sequences were compared to sequences published in GenBank using the BLASTn algorithm (http://www.ncbi.nlm.nih.gov). This comparison showed that the degree of similarity was between 95% and 100%. Moreover, the alignment enabled to observe that 11 sequences were close to *Tomato leaf curl Nigeria virus* (FJ685621.1), 15 to *Ageratum leaf curl Cameroon virus* (FR873228.1; FR873230; FN675292.1), 7 to *Tomato leaf curl Kumasi virus* (EU847739.1; FM210063.1; FM210062.1), 2 to *Tomato leaf curl Togo virus* (FJ685620.1; HE659517.1), 1 from *Tomato leaf curl Cameroon virus*-Fontem (HE659516.1), 1 to *Pepper yellow vein Mali virus*—Bazegahot (FM876848.1) and 1 to *Pepper*

yellow vein Mali virus—Ouaga (FM876851.1). 123 PCR products were fully sequenced but only 49 sequences available were obtained among which 10 did not show any similarity with Genbank sequences (Table 4).

3.3. Phylogenetic Analysis

Phylogenetic analysis was performed with the partial sequences of Togo isolates and control sequences available in GenBank using ClustalX multiple alignment software. The phylogenetic tree was designed using the DARWin5 program and the Neighbor-Joining method. Of the positive PCR samples, 44 sequences from tomato and 4 from wild plants were selected for phylogenetic analysis. This analysis indicated that the Begomoviruses isolated in Togo form seven groups (Figure 3). Groups 1 and 2 contain begomovirus isolates from tomato and two from wild plants *Solanum macrocarpon* (L.) and *Euphorbia heterophylla* (L.). Groups 3, 4 and 5 contain only Begomovirus isolates from tomato. Most of GenBank isolates and Group 7 contain both GenBank and tomato isolates.

Table 4. Comparison of Begomovirus sequences isolated in Togo with those of Genbank.

N°	Origin	Code of samples and host plants	Genbank virus	Degree of similarity
1	Maritime Region	16-83a/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	98%
2	Maritime Region	16-83b/2014 (Tomato)	Tomato leaf curl Togo virus-Fontem |HE659517.1|	96%
3	Maritime Region	16-83c/2014 (Tomato)	Tomato leaf curl Nigeria virus-[Nigeria:2006] |FJ685621.1	98%
4	Maritime Region	16-83f/2014 (Tomato)	No found	NS
5	Maritime Region	16-93/2014 (Tomato)	No found	NS
6	Maritime Region	35-71a/2013 (Tomato)	No found	NS
7	Maritime Region	35-71b/2013 (Tomato)	No found	NS
8	Maritime Region	35-76/2013 (Tomato)	Tomato leaf curl Togo virus-[Togo:2006] |FJ685620.1|	96%
9	Maritime Region	35-82/2013 (Tomato)	Tomato leaf curl Nigeria virus-[Nigeria:2006] |FJ685621.1|	99%
10	Central Region	30-233/2014 (Tomato)	Tomato leaf curl Nigeria virus-[Nigeria:2006] |FJ685621.1|	99%
11	Central Region	27-235a/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	99%
12	Central Region	27-235b/2014 (Tomato)	Tomato leaf curl Nigeria virus-[Nigeria:2006] |FJ685621.1|	99%
13	Central Region	27-235c/2014 (Tomato)	Tomato leaf curl Kumasi virus clone (GOTB2-2)|EU847739.1|	99%
14	Central Region	27-235d/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG23:2009] |FR873230.1|	98%
15	Central Region	27-235e/2014 (Tomato)	Ageratum leaf curl Cameroon alphasatellite [CM:ODL1D1:Ok:09] |FN675292.1|	96%
16	Plateaus Region	25-10a/2015 (Tomato)	No found	NS
17	Plateaus Region	25-11a/2015 (Tomato)	Tomato leaf curl Kumasi virus clone (GOTB2-2)|EU847739.1|	96%
18	Kara Region	5-272a/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG23:2009] |FR873230.1|	97%
19	Kara Region	5-272b/2014 (Tomato)	Tomato leaf curl Kumasi virus partial V1 AMJ11|FM210063.1|	98%

Continued

20	Kara Region	5-272c/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	97%
21	Kara Region	5-278/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	97%
22	Kara Region	5-279a/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	97%
23	Kara Region	5-279b/2014 (Tomato)	Tomato leaf curl Kumasi virus partial V1 LIONGO1 |FM210062.1	99%
24	Kara Region	5-279c/2014 (Tomato)	Tomato leaf curl Kumasi virus partial V1 AMJ11 |FM210063.1|	97%
25	Kara Region	5-281/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	98%
26	Kara Region	8-250/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	96%
27	Kara Region	8-252/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	97%
28	Kara Region	8-260a/2014 (*Euphorbia heterophylla)*	Tomato leaf curl Nigeria virus-[Nigeria:2006] |FJ685621.1|	99%
29	Kara Region	8-260b/2014 (*Euphorbia heterophylla)*	No found	NS
30	Kara Region	8-260c/2014 (*Euphorbia heterophylla)*	No found	NS
31	Kara Region	14-226a/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	98%
32	Kara Region	14-226b/2014 (Tomato)	Tomato leaf curl Cameroon virus - Fontem |HE659516.1|	98%
33	Kara Region	14-226c/2014 (Tomato)	Tomato leaf curl Kumasi virus partial V1 LIONGO1 |FM210062.1|	98%
34	Kara Region	14-226d/2014 (Tomato)	Tomato leaf curl Nigeria virus-[Nigeria:2006] |FJ685621.1|	98%
35	Kara Region	14-227/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	96%
36	Kara Region	20-182a/2014 (Tomato)	Tomato leaf curl Kumasi virus clone (GOTB2-2)|EU847739.1|	97%
37	Kara Region	20-182b/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	98%
38	Kara Region	20-185/2014 (Tomato)	Tomato leaf curl Nigeria virus-[Nigeria:2006] |FJ685621.1|	98%
39	Kara Region	20-187/2014 (Tomato)	Tomato leaf curl Kumasi virus clone (GOTB2-2)|EU847739.1|	99%
40	Kara Region	20-194/2014 (Tomato)	Ageratum leaf curl Cameroon virus [CM:AGFG24:2009] |FR873228.1|	98%
41	Kara Region	20-215a/2014 (Tomato)	Tomato leaf curl Nigeria virus-[Nigeria:2006] |FJ685621.1|	98%
42	Kara Region	20-215b/2014 (Tomato)	Tomato leaf curl Nigeria virus-[Nigeria:2006] |FJ685621.1|	98%
43	Kara Region	20-216/2014 (*Solanum macrocarpon)*	No found	NS
44	Kara Region	20-248/2014 (Tomato)	Tomato leaf curl Nigeria virus-[Nigeria:2006] |FJ685621.1|	99%
45	Kara Region	20-266a/2014 (Tomato)	Pepper yellow vein Mali virus-Ouaga |FM876851.1|	99%
46	Kara Region	20-266b/2014 (Tomato)	Pepper yellow vein Mali virus-Bazegahot |FM876848.1|	99%
47	Savannah Region	31-112a/2014 (Tomato)	No found	NS
48	Savannah Region	31-112b/2014 (Tomato)	No found	NS
49	Savannah Region	31-153/2014 (Tomato)	Tomato leaf curl Nigeria virus-[Nigeria:2006]|FJ685621.1|	99%

NS = No similarit

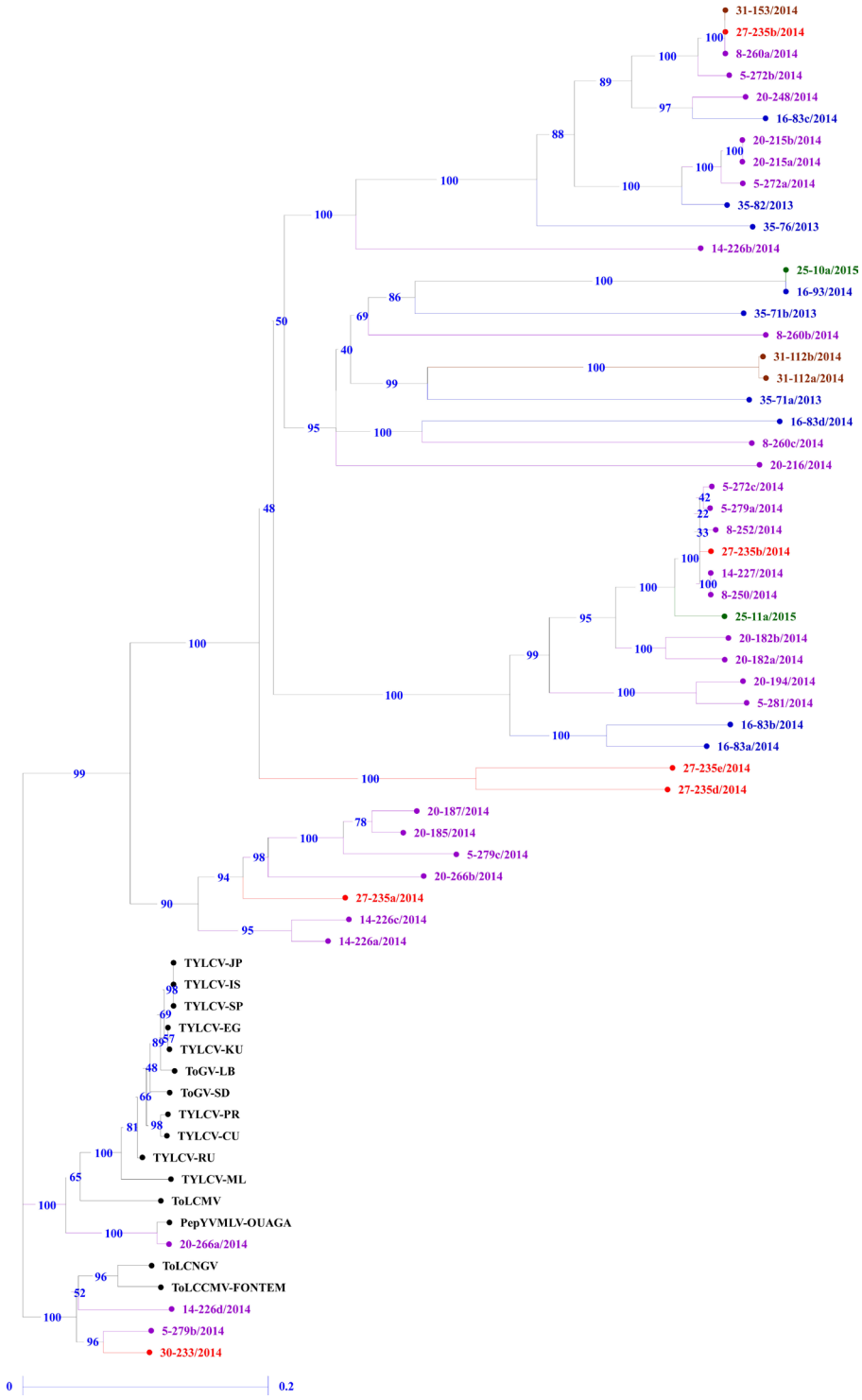

Figure 3. Phylogenetic tree obtained with the DNA sequences of the CP 48 isolates Begomovirus Togo, after Bootstrap analysis with 1000 repetitions performed using the software DARWin5. ToLCMV: *Tomato leaf curl Mayotte virus* (AJ620914), TOGV-LB: *Tomato geminiviruses* Lebanon (AF065823), TOGV-SD: *Tomato geminiviruses* Gezira (AF058030), TYLCV-PR: *Tomato yellow leaf curl virus*—Puerto Rico (AY134494), TYLCV -RU: *Tomato yellow leaf curl virus*-Reunion (AJ842307), TYLCV-EG: *Tomato yellow leaf curl virus*—Egypt (AY594174), TYLCV-IS *Tomato yellow leaf curl virus*—Israel (AB110218), TYLCV-SP *Tomato yellow leaf curl virus-Mild* [Spain7297] (AF071228), TYLCV-JP: *Tomato yellow leaf curl virus—Mild* [Shizuokua] (AB014346), TYLCV-CU *Tomato yellow leaf curl virus*-Cuba (AJ223505), TYLCV-ML: *Tomato yellow leaf curl Mali virus* (AY502934), ToLCCMV: *Tomato leaf curl Cameroon virus*—Fontem (HE659516), PepYVMLV-OUAGA: *Pepper yellow vein Mali virus*-Ouagadougou (FM876851), TYLCV-KU: *Tomato geminiviruses* Kuwait (AF065822), ToLCNGV: *Tomato leaf curl Nigeria virus* (FJ685621).

4. Discussion

In West Africa several viruses infecting tomato crops have been reported, including strains of begomoviruses such as: *Tomato leaf curl Cameroon virus* (ToLCCMV), *Tomato yellow leaf curl Mali virus* (TYLCMLV), *Tomato leaf curl Nigeria virus* (ToLCNGV), *Tomato leaf curl Ghana virus* (ToLCGHV), *Tomato leaf curl Kumasi virus* (ToLCKuV) et *Tomato leaf curl Togo virus* (ToLCTGV) [4] [14] [15] [23] belong the West African tomato infecting begomoviruses (WATIBs) clade. The results in this study revealed that the PCR with virus-specific primers for the Mediterranean TYLCV isolates used by [21], did not succeed in detecting the TYLCD-associated viruses in symptomatic tomato plants and wild plants collected in fields in Togo. However, the degenerate primers of Avcore/ACcore, Deng A/Deng B, Av494/Ac1048, PALIc1960/PARI722 and PAR1c496/ PCRv181 designed respectively by [17] [19] [18] and [20] for geminiviruses succeeded in amplifying the expected band size of geminiviruses of the tested infected samples.

The identification of *Tomato leaf curl Cameroon virus*-Fontem [24] first in Cameroon and now in Togo suggests that this virus is widely distributed in Central and West Africa. Furthermore, since cultivated tomato is not indigenous to Africa and begomoviruses are not seed transmissible [25], it is more likely that WATIBs originated from an endemic alternate host. The identification of *Ageratum leaf curl Cameroon virus* first in *Ageratum conyzoides* and now on tomato in Togo suggests that this begomovirus can infect more than one host. The identification of ALCCMA first in *Ageratum conyzoides* and now in tomato suggests that ALCCMA may infect more than one host and may be trans-replicated by other begomoviruses. The identification of *Tomato leaf curl Nigeria virus* [26] on the wild *Euphorbia heterophylla* in our study suggests that it is a reservoir plant for this virus.

Analysis of the genetic diversity of begomoviruses infecting tomato was carried out by direct sequencing of the PCR products. This strategy, after the PCR parameters were optimized, was extremely efficient for analyzing a large number of sequences in a short period of time. The sequenced region comprises the 5 end of the CP gene. This is the most variable region of the CP gene and according to [10], is representative of the variability of the nucleotide sequence of the viral genome. Therefore, phylogenetic analysis based on the region is usually sufficient to establish the taxonomic position of a given isolate of begomoviruses.

From the results obtained from the phylogenetic analysis, sequences from the begomoviruses CP gene that infect tomato in Togo form seven large groups of begomoviruses. Thus, in groups 6 and 7 there are isolates from Togo mixed with GenBank isolates. But it should also be noted that the elements of the Maritime Region, Central Region, Savannah Region and those of the Kara region can be found together in the same group (G1) and so on. It should be noted that, according to our study, the isolates of begomovirus infecting tomato in Togo are specific to Togo (as shown in the tree of **Figure 3** except for two cases where Togo

isolates are mixed with isolates from GenBank). The proximity of *Pepper yellow vein Mali virus*-Ouagadougou [27] with a high degree of similarity of 99% with one of our isolates (20-266a/2014) from the Kara region suggests that this begomovirus has been introduced into this area through the vector insect or trade.

Although, new distinct species of begomovirus were identified in the early 2000s and this, due to increased interest in begomovirus research that has been strengthened, identification and the determination of begomoviruses as emerging viruses and the discovery of new species by viral genome sequencing is the major concern nowadays. [28] have suggested that the South-East of continental Asia could be an important center of diversity for begomoviruses based on the great diversity of strains and species of local monopartite Begomoviruses and associated betasatellite molecules identified in these regions.

5. Conclusion

It appears from this study that geographically separated viruses meet and are in mixed infection in Togo. So for the future it would be important to sequence more begomovirus isolates from tomato and wild plants to monitor viral genotypes and to be able to track possible changes in the population structure of these virus. This should be considered when screening programs for virus resistance are established because recombination is permanent with begomoviruses and it is known that when there is recombination, the new recombinants are usually more infectious than their parents.

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