American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic
Journals Inc.

www.academicjournals.com

American Journal of Biochemistry and Molecular Biology 3 (4): 341-355, 2013
ISSN 2150-4210 / DOI: 10.3923/ajbmb.2013.341.355

Unexpected Molecular Variability of Begomovirus Infecting Cassava (*Manihot esculenta* Crantz) in Togo

[1,2]Adjata Kossikouma Djodji, [2]Emmanulle Muller, [2]Michel Peterschmitt and [1]Yawovi Mawuena Dieudonné Gumedzoe

[1]Laboratory of Plant Virology and Biotechnology, High School of Agronomy, University of Lomé, P.O. Box 1515 Lomé, Togo

[2]Biology and Genetics of Plant-Pathogen Interactions for Integrated Protection, (CIRAD-UMR BGPI), TA 41/K, International Campus of Baillarguet, 34398 Montpellier Cedex 5, France

Corresponding Author: Adjata Kossikouma Djodji, Laboratory of Plant Virology and Biotechnology, High School of Agronomy, University of Lomé, P.O. Box 1515 Lomé, Togo

ABSTRACT

The major constraint for cassava production in Africa in general and particularly in Togo, is Cassava Mosaic Disease (CMD), caused essentially by different species of Begomoviruses. Thus, to undertake any strategy of effective control of this disease, it is very important to know the different components of the species of Begomoviruses behind the disease. For this purpose, a molecular characterization of Begomovirus responsible viruses of CMD in Togo was carried out by using specific primers targeting the Coat Protein (CP). A total of 114 of various isolates of these Begomoviruses were sequenced. Phylogenetic sequence analysis and comparison of the sequences obtained of the various isolates of these Begomoviruses with those coming from the GenBank database was performed. The results of these analyses revealed a molecular variability among the different groups of Begomoviruses infecting cassava in Togo. Although the results obtained in this study are not exhaustive, given the emergence of new viral diseases due to Begomovirses, it is important to take them into account in the search for resistant/tolerant clones to cassava virus diseases.

Key words: Begomovirus, cassava, characterization, mosaic, variability

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the first African staple food crop and the third greater source of carbohydrates for human consumption in the world (Pita *et al*., 2001). In Togo, the yields of this crop are generally weak as in the majority of African countries, and several constraints among which viral diseases are not the least, could explain this weak performance (Otim-Nape *et al*., 1998). Several Begomoviruses causing Cassava Mosaic Disease (CMD) were reported in Africa (Fauquet and Fargette, 2005; Atiri *et al*., 2004; Pita *et al*., 2001; Polston and Anderson, 1997; Moriones and Navas-Castillo, 2000). Surveys were undertaken in all the producing zones of this tuber plant during the period of July-August 2004 and 2005 and the major Begomovirus (ACMV, EACMV and ICMV) were characterized with specific primers. The main outcome was that the different Begomovirus were in mixed infection in Togo (Adjata *et al*., 2009) as the case reported in Tanzania (Harrison and Robinson, 1999), Cameroon (Fondong *et al*., 2000) and Côte d'Ivoire (Pita *et al*., 2001), with most plants exhibiting severe disease symptoms. Moreover, Begomovirus are able to implement recombination and harbour frequent footprints of

recombination events within their genomes (Padidam *et al*., 1999; Jeske *et al*., 2001). The aim of this present work is to highlight the evidence of molecular variability among Begomovirus infecting cassava in cassava production zones of Togo.

MATERIELS AND METHODS

Sources of virus sequences and analysis: Sources of virus sequences: Coat Protein (CP) gene sequences were considered in this study due to their importance for Begomoviruses replication gene expression and the sequences used in our present study were partial sequences obtained from CP gene (~770 bp) of a previous work done by Adjata *et al*. (2009, 2008). These various sequences (Table 1 and 2) were obtained by the method of direct sequencing carried out by the company "Genome express, Cogenics", in Grenoble. The accession numbers of CP sequences of cassava Begomovirus we used were from the GenBank.

Table 1: Length of sequences of ACMV isolates identified in Togo

Sequences of ACMV isolates		
Reference of the laboratory	Source/prefecture	Fragment length (bp)
0704C (22-171)	Sotouboua	724
2804C (22-637)	Sotouboua	725
3204C (25-133)	Tchaoudjo	715
5204C (24-77)	Tchamba	720
6604K (05-99)	Bassar	721
6704K (05-104)	Bassar	738
7404K (05-115)	Bassar	719
8604C (07-612)	Blitta	725
9604M (27-53)	Vo	738
9704M (27-23)	Vo	725
9804M (27-08)	Vo	754
10904M (04-505)	Avé	714
12004M (30-271)	Zio	721
12104M (30-587)	Zio	742
13404M (18-64)	Lacs	719
16104M (04-512)	Avé	734
18304M (27-29)	Vo	727
18404M (27-22)	Vo	719
0405C (25-126)	Tchaoudjo	749
0505C (25-140)	Tchaoudjo	725
2505C (22-602)	Sotouboua	717
3805K (16-73)	Kozah	721
4205S (21-77)	Oti	725
4505C (03-60)	Assoli	725
5005S (21-75)	Oti	725
5305M (12-121)	Golfe	727
13305P (15-461(glaz))	Kloto	723
13605P (15-460(glaz))	Kloto	727
14005P (15-462(glaz))	Kloto	721
14205P (15-463(glaz))	Kloto	723
14905P (15-464(glaz))	Kloto	716

Table 1: Continue

Sequences of ACMV isolates		
Reference of the laboratory	Source/prefecture	Fragment length (bp)
15005P (15-466(glaz))	Kloto	723
15205P (01-282)	Agou	719
15605P (11-585)	Est-mono	722
16105P (13-199)	Haho	721
18305P (28-300)	Wawa	724
19205P (02-390)	Amou	721
22905P (28-331)	Wawa	725
28005M (29-09)	Yoto	727
28905M (29-11)	Yoto	716
29205M (30-02)	Zio	719
29305P (15-489)	Kloto	739
30505P (02-416)	Amou	726
30605P (02-399)	Amou	715
33005M (30-114)	Zio	717
33105P (20-218)	Ogou	724
33205P (20-675)	Ogou	721
36505P (09-541)	Est-mono	736
39805P (13-186)	Haho	691
45805C (07-592)	Blitta	724
48605M (12-161)	Golfe	726
49005M (12-147)	Golfe	727

Table 2: Length of sequences of EACMV isolates identified in Togo

Sequences of EACMV isolates		
Reference of the laboratory	Source/prefecture	Fragment length (bp)
04-16C (22-175)	Sotouboua	720
p04-28C (22-637)	Sotouboua	709
0p4-43C (24-649)	Tchamba	619
04pp-65K (05-119)	Bassar	701
04-68K (05-107)	Bassar	699
04-81K (05-98)	Bassar	719
04-84C (07-606)	Blitta	698
04-90C (07-589)	Blitta	765
04-94M (27-27)	Vo	713
04-98M (27-08)	Vo	703
04-118M (30-13)	Zio	743
04-126M (30-01)	Zio	710
04-139M (04-513)	Avé	756
04-155M (04-661)	Avé	701
04-156M (04-529)	Avé	643
04-157M (04-506)	Avé	711
04-166M (30-266)	Zio	709
04-175M (27-43)	Vo	688
04-181M (27-18)	Vo	700
05-06C (25-149)	Tchaoudjo	760

Table 2: Continue

Sequences of EACMV isolates		
Reference of the laboratory	Source/prefecture	Fragment length (bp)
05-35K (05-105)	Bassar	829
05-38K (16-73)	Kozah	752
05-67K (06-94)	Doufelgou	712
05-89C (03-68)	Assoli	710
05-111K (06-92)	Doufelgou	714
05-123S (21-82)	Oti	767
05-132P (15-440)	Kloto	708
05-152P (01-282)	Agou	674
05-161P (13-199)	Haho	711
05-192P (02-390)	Amou	714
05-197P (02-391)	Amou	709
05-289M (29-11)	Yoto	711
05-305P (02-416)	Amou	673
05-306P (02-399)	Amou	711
05-330M (30-114)	Zio	701
05-331P (20-218)	Ogou	698
05-332P (20-675)	Ogou	710
05-333P (20-220)	Ogou	702
05-338P (20-222)	Ogou	701
05-455C (07-598)	Blitta	702
05-456C (07-590)	Blitta	749
05-485M (12-167)	Golfe	712
05-486P (12-161)	Golfe	744
05-491K (05-122)	Bassar	702
05-506K (05-123)	Bassar	691
04-168P (13-260)	Haho	617
04-173P (15-501)	Kloto	638
04-183P (28-300)	Wawa	654
04-213P (15-430)	Kloto	670
04-229P (28-331)	Wawa	681
04-250P (15-482)	Kloto	613
05-271M (12-19)	Golfe	677
05-274M (12-17)	Golfe	663
05-283M (29-13)	Yoto	650
04-311P (02-404)	Amou	673
04-325P (02-389)	Amou	653
05-353P (20-566)	Ogou	630
05-405P (13-671)	Haho	665
05-464C (07-595)	Blitta	616
05-468P (13-679)	Haho	648
04-77K (05-100)	Bassar	646
04-07C (25-145)	Tchaoudjo	650

Sequence analysis: Phylogenetic analyses were conducted on matrices of aligned sequences by using the neighbour-joining and bootstrap options of DARWin5 (Perrier *et al.*, 2003). All sequences of the virus isolates selected were aligned using the ClustalV method of aligning multiple sequences

of BioEdit7 (Hall, 1999). The phylogenetic tree was estimated using the neighbor-joining method with the unweighted pair-group method with arithmetic average (UPGMA) distance matrix (MegAlign program) by using DARWin5. One-thousand bootstrap replications were performed to place confidence estimates on major groups resolved in the tree. The aligned sequences were subjected to the same multiple alignments approaches to detect intermolecular recombination events. Dendrograms were viewed, manipulated and printed by using DARWin5 (Perrier *et al*., 2003).

Pairwise identity analysis between isolates: The calculation of the percentage of identity between sequences was carried out by using the Align program of Vector NTI Advance 10 of Invitrogen after alignment in BioEdit7 (Hall, 1999). The criteria used for the analysis of identity between the various viruses are those of Padidam *et al*. (1996), i.e., the identity between the various viruses is: 90-100% for isolates; 80-90% for strains and less than 80% for the delimitation of species.

RESULTS

Sequences from Togo: Table 1 and 2 present CP gene (~770 bp) sequences obtained from cassava isolates fom Togo. These sequences were compared to sequences chosen in the GenBank by using the unweighted pair-group method with arithmetic average (UPGMA) as described in the methods.

GenBank sequences: The GenBank accession numbers used in this study were in particular: for ACMV (AY211462, AY211462, AY211464, AY211465, AY211466, AF423177 and AY562421); ACMVUG/Mld (AF126800); ACMV-UG/Svr (AF126802, AY 562429). And in the case of EACMV, there were: EACMV of Cameroun and Ivory Coast (AF259896, AY211467); from Cameroun alone (AY211460, AY211463, AY211468, AY211887); from Kenya (AJ717531, AJ717532, AJ17553); from Malawi (AJ006461); from Uganda (AF230374, AF423178, AY562425, Z83257; EACMVUG /Mld (AF126804, AY562428); EACMVUG/Svr (AF126806, AY562422, AY562423, AY562424, AY562427, NC 004674); from Tanzania (AY795985, AY795987, AY828226, Z83256).

Phylogenetic analyses of ACMV and EACMV taken separately: The alignment of the sequences of ACMV on one hand and of EACMV on the other hand followed by an analysis of 1000 repetitions of Bootstrap by using DARWin5 (Perrier *et al*., 2003) allowed to obtain the phylogenetic trees presented in Fig. 1-3. The ACMV isolates identified in Togo are divided into three groups among which, one contains almost exclusively the sequences of the GenBank. The results obtained from the matrix of pairwise identity percentages of some of these Begomovirus are ranging between 80 and 99% as shown in Table 3. In the case of EACMV isolates, the situation is completely different; the pairwise identity percentages between the CP gene sequences of the various isolates (Table 3) revealed a strong heterogeneity. Indeed, these percentages vary from 77 to 97%. Referring to the phylogenetic dendrogram, the only distant isolate of the major group EACMV of Togo, belongs to the same isolates group as the Uganda variant of EACMV (EACMV-UG) which is in fact a recombinant issued from ACMV and EACMV; it seems closer to UgV, with a pairwise identity percentage of 96% as it can be seen on the dendrogram.

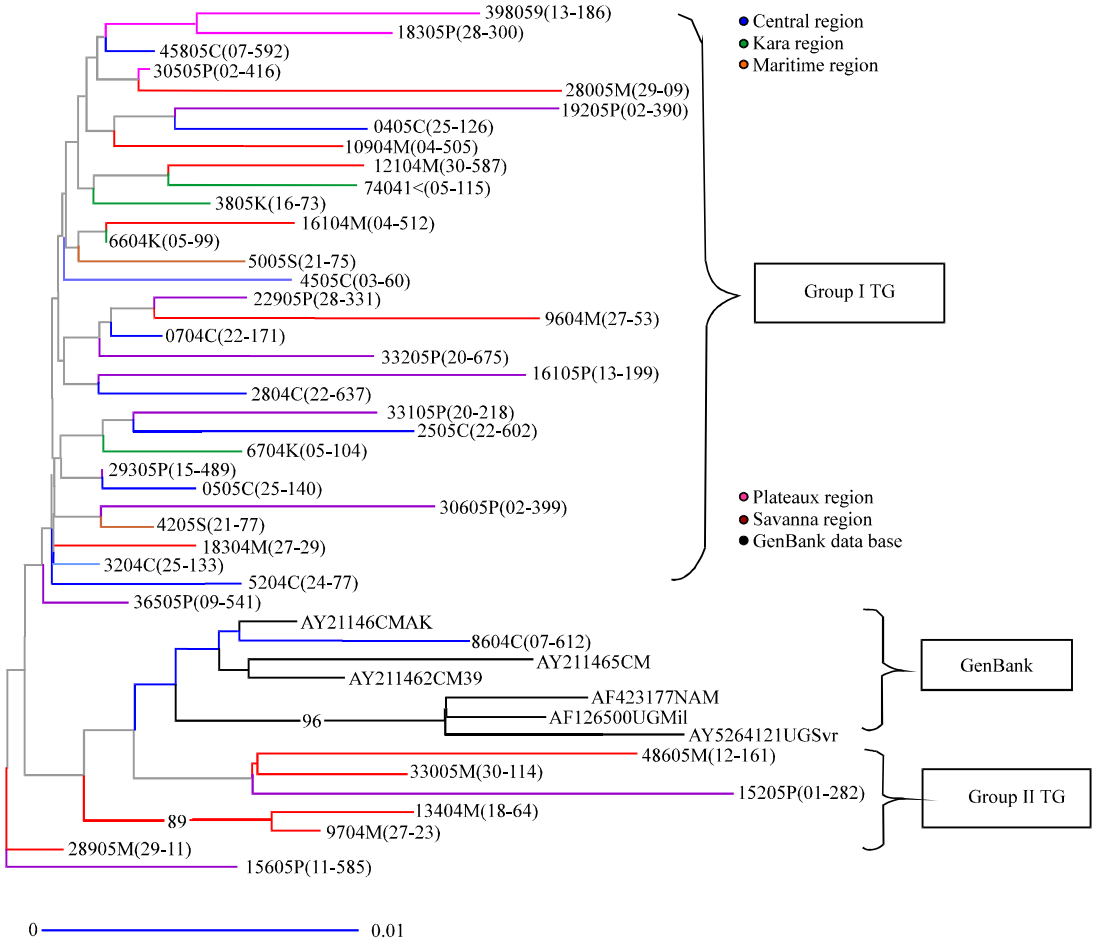

Fig. 1: Consensus phylogenetic tree (1000 bootstrap replications) obtained from comparison of the CP sequences of selected ACMV identified in Togo and the GenBank accession number of the published CP Begomovirus sequences from various part of Africa (black bullet)

Global phylogenetic analysis of all the Begomovirus isolates identified in Togo: Sequences obtained from ACMV and EACMV isolates were compared to each other and with ACMV and EACMV sequences available in GenBank. The aim in view was to demonstrate that ACMV and EACMV isolates identified in Togo are identical to these two species. The dendrogram obtained is presented in Fig. 3. It can be observed clearly on the figure that all EACMV isolates of Togo except one are joined together in one group. The isolate 05-484 M (12-167) identified in Togo and not belonging to the major group is closer to the Uganda variant of EACMV (EACMVUG/Svr) with a pairwise identity percentage of 96%. The same isolate has a pairwise identity percentage of 80-83% with sequences from the isolates of EACMV major group and 92-94% with ACMV isolates of Togo (Table 1 and 2).

DISCUSSION

Phylogenetic analysis of begomovirus infecting cassava in Togo: The phylogenetic analysis of the CP gene sequences of Begomovirus isolates identified in Togo revealed that there are two great groups of Begomovirus infecting cassava in this country: The group of ACMV isolates

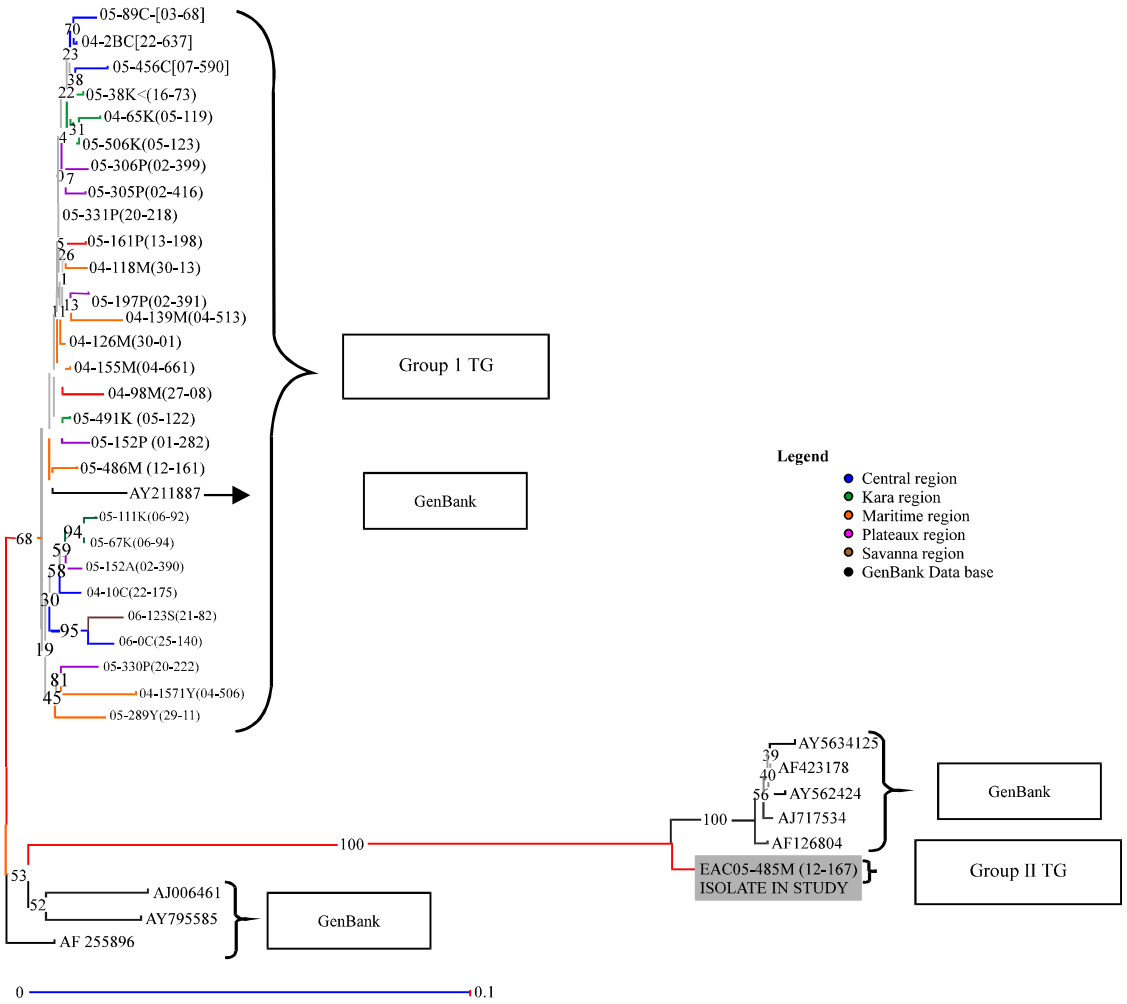

Fig. 2: Consensus phylogenetic tree (1000 bootstrap replications) obtained from comparison of the CP sequences of selected EACMV identified in Togo and the GenBank accession number of the published CP Begomovirus sequences from various part of Africa (black bullet)

and that of EACMV. Considering each isolate group, there are subgroups; but the study of the phylogenetic relationship between the different isolates always showed that all these isolates belong to the group of African cassava bipartite Begomovirus. The pairwise identity between these different isolates was 97 to 99%. Hence, within the group of ACMV isolates, there are three subgroups including one limited to ACMV isolates of Togo among the GenBank isolates. But it should be noted that the elements of these three subgroups are not specific to the regions though it is not unusual to see or to note a certain gathering according to the proximity of the regions. Thus, it is easy to find some isolates of the Maritime region and those of the of the Plateaux region together in the same subgroup. It is important to notice that according to our study, the ACMV isolates infecting cassava in Togo, seemed be specific to Togo as the dendrogram shows it in Fig. 1; except one case where an isolate of the Central region (8604C(07-612)) is found with a sequence of the GenBank database (Fig. 1). This study therefore makes it possible to bring to light new groups of ACMV isolates not yet emphasized. These results confirm that ACMV isolates detected

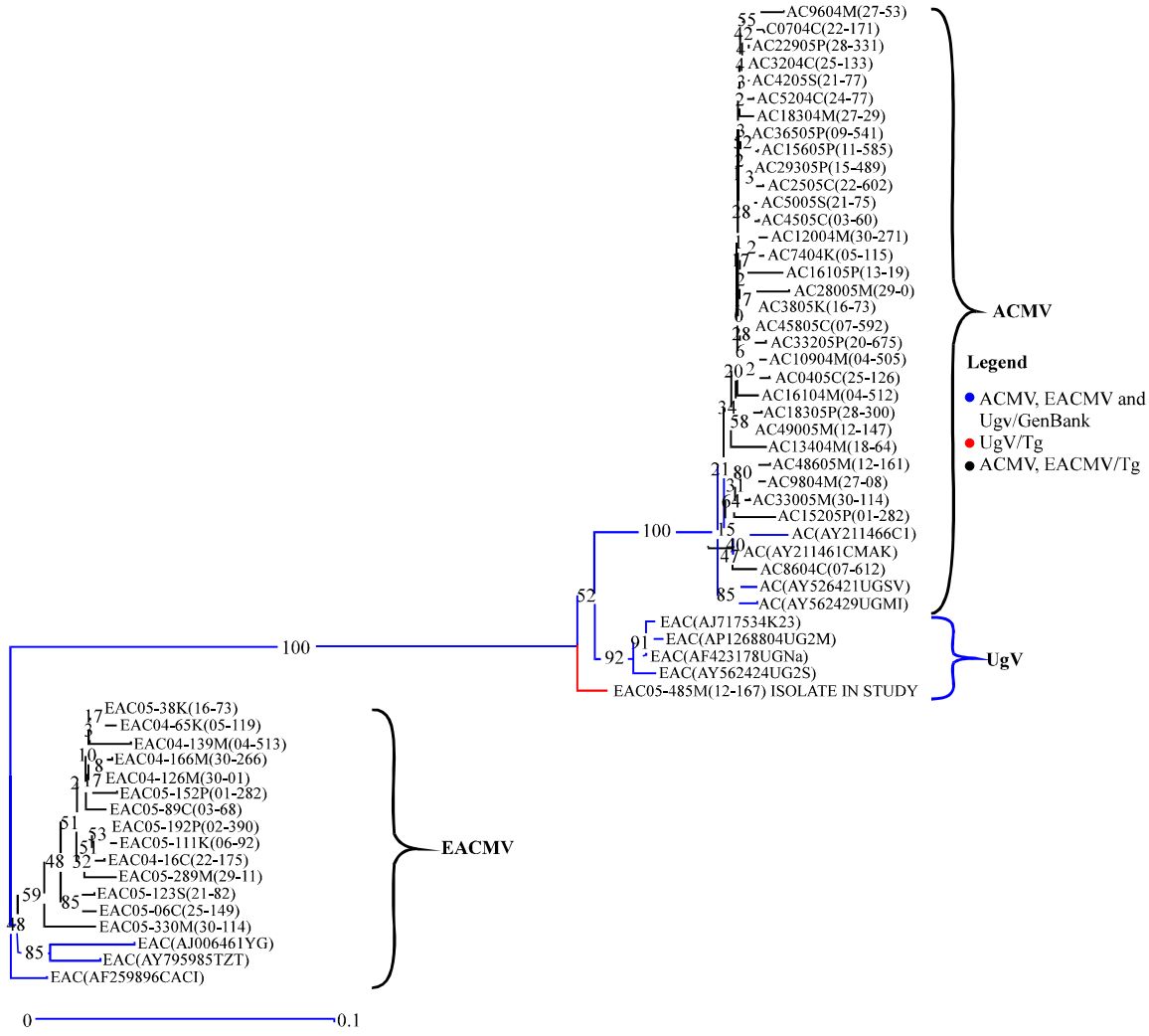

Fig. 3: Consensus phylogenetic tree (1000 bootstrap replications) obtained from comparison of the CP sequences of selected cassava Begomovirus occurring in Togo (black and red bullet) and the GenBank accession number of the published CP Begomovirus sequences from various part of Africa

in Togo and the ACMV isolates published by GenBank, belong well to the same isolates group ACMV because the pairwise identity percentages with ACMV sequences of GenBank are always higher than 90 (Adjata *et al*., 2012). Indeed, the criteria utilised for the analysis of the identity between the various viruses are those of Padidam *et al*. (1996), i.e., the identity between the various viruses is of 90-100% for isolates, 80-90% for strain and less than 80% for the delimitation of species. Relating to EACMV isolates, the phylogenetic analysis of the CP gene sequences of Begomovirus isolates in comparison with that of other EACMV isolates of GenBank, also revealed, the existence of groups and subgroups within these isolates which are not specifically associated with the regions similar to what was noticed in the case of ACMV isolates. It has been observed that the majority of the EACMV isolates published in GenBank are not found in EACMV ordinary group detected in Togo; except a case where a GenBank sequence was found in a cluster of group I of

Table 8: CP sequences identity (%) of selected cassava Begomovirus

	AC (AY211 461CMAK)	AC (AY2114 66CMDO8)	AC (AY562 421 UGSvr)	AC (AY562 429 UGM11)	AC0405C (25-126)	AC0704C (22-171)	AC18404 M (18-64)	AC15605 P (11-585)	AC16104 M(04-512)	AC16105 P (13-199)	AC18304 M (27-29)	AC18305 P (28-300)	AC2505C (22-602)	AC28005 M (29-09)	AC29305 P (15-489)	AC32404 (25 -133	AC33205 P (20-675)
AC (AY211461CMAK)		98	98	98	98	99	98	98	98	98	98	98	98	97	99	99	98
AC (AY211466CMDO8)			97	97	96	97	96	97	97	96	97	96	97	96	97	97	97
AC (AY562421UGSvr)				98	97	98	97	97	98	97	98	97	97	97	98	98	97
AC (AY562429UGM11)					97	98	97	97	97	97	98	97	97	96	98	98	97
AC0405C (25-126)						98	98	98	98	98	98	98	98	97	99	99	98
AC0704C (22-171)							98	99	99	98	99	99	99	98	100	100	99
AC18404M (18-64)								98	98	97	98	98	98	97	98	98	98
AC15605P (11-585)									98	98	99	98	99	98	99	99	98
AC16104M (04-512)										98	99	98	98	98	99	99	98
AC16105P (13-199)											98	98	98	97	98	98	98
AC18304M (27-29)												98	99	98	99	99	98
AC18305P (28-300)													98	98	99	99	98
AC2505C (22-602)														97	99	99	98
AC28005M (29-09)															98	98	98
AC29305P (15-489)																100	99
AC32404 (25-133)																	99
AC33205P (20-675)																	
AC3605P (09-541)																	
AC3805K (16-73)																	
AC4505C (03-60)																	
AC45805C (07-592)																	
AC48605M (12-161)																	
AC5005S (21-75)																	
AC5204C (24-77)																	
AC7404K (05-116)																	
AC8604C (07-612)																	
EAC (AF126804UG2M)																	
EAC (AF259896CAC1)																	
EAC (AF423178UGNa)																	
EAC (AJ006461YG)																	
EAC (AJ717584K28)																	
EAC (AY562424UG2S)																	
EAC (AY795985T2T)																	
EAC04-139M (04-513)																	
EAC04-166M (30-266)																	
EAC04-16C (22-176)																	
EAC04-66K (05-119)																	
EAC05-06C (25-149)																	
EAC05-111K (06-92)																	
EAC05-128S (21-82)																	
EAC05-152P (01-282)																	
EAC05-192P (02-390)																	
EAC05-38K (16-73)																	
EAC05-485M (12-167)																	
EAC05-89C (03-68)																	
ICMV-AY312989																	
SACMV-AJ575560																	

Table 3: Continue

	AC36505P (09-541)	AC3805K (16-73)	AC4505C (03-60)	AC45805C (07-592)	AC48605M (12-161)	AC5005S (21-75)	AC5204C (24-77)	AC7404K (05-115)	AC8604C (07-612)	EAC (AF12 68 04UG2M	EAC (AF2 59 896CACI	EAC (AF42 3 17 8UGNa	EAC (AJ0 06 461YG)	EAC (AJ7 17534K23)	EAC(AY56 2424UG2S
AC (AY211461CMAK)	99	98	98	99	98	98	98	98	99	94	79	94	77	94	94
AC (AY211466CMDO3)	97	97	97	97	96	97	97	96	97	92	78	93	76	92	92
AC (AY562421UGSvr)	98	98	97	98	97	97	98	97	98	94	79	94	76	94	94
AC (AY562429UGMil)	98	98	97	98	97	97	98	98	98	94	78	94	76	94	94
AC0405C (25-126)	98	98	98	99	97	98	98	98	97	93	78	93	76	93	93
AC0704C (22-171)	99	99	99	99	98	99	99	99	98	93	79	94	77	93	93
AC13404M (18-64)	98	98	98	98	97	98	98	98	97	93	78	94	76	93	93
AC15605P (11-585)	99	99	99	99	98	99	99	99	98	93	78	93	76	93	93
AC16104M (04-512)	99	99	98	99	97	99	99	98	98	93	78	94	76	93	93
AC16105P (13-199)	98	98	98	98	97	98	98	98	97	92	78	93	76	92	92
AC18304M (27-29)	99	99	99	99	98	99	99	98	98	93	79	93	76	93	93
AC18305P (28-300)	99	98	98	99	97	98	98	98	97	93	78	93	77	93	93
AC2505C (22-602)	99	99	98	99	97	98	99	98	98	93	79	94	77	93	93
AC28005M (29-09)	98	98	98	98	97	98	98	98	97	92	78	93	76	92	92
AC29305P (15-489)	100	99	99	100	98	99	99	99	98	93	79	94	77	94	93
AC3204C (25-133)	100	99	99	100	98	99	99	99	98	93	79	94	76	93	93
AC33205P (20-675)	99	98	98	99	97	98	98	98	97	93	79	93	76	93	93
AC36505P (09-541)		99	99	99	98	99	99	99	98	93	79	94	76	93	93
AC3805K (16-73)			99	99	97	99	99	99	98	93	79	94	77	93	93
AC4505C (03-60)				99	97	99	99	98	98	93	79	93	76	93	93
AC45805C (07-592)					98	99	99	99	98	93	78	94	76	93	93
AC48605M (12-161)						97	98	97	98	93	77	94	75	93	93
AC5005S (21-75)							99	98	98	93	78	93	76	93	93
AC5204C (24-77)								98	98	93	79	93	76	93	93
AC7404K (05-115)									98	93	78	93	76	93	93
AC8604C (07-612)										93	79	94	77	94	94
EAC (AF126804UG2M)											82	100	80	99	99
EAC (AF259896CACI)												82	95	82	81
EAC (AF423178UGNa)													80	100	100
EAC (AJ006461YG)														80	80
EAC (AJ717534K23)															99
EAC (AY562424UG2S)															
EAC (AY795985TZT)															
EAC04-139M (04-513)															
EAC04-166M (30-266)															
EAC04-16C (22-175)															
EAC04-65K (05-119)															
EAC05-06C (25-149)															
EAC05-111K (06-92)															
EAC05-123S (21-82)															
EAC05-152P (01-282)															
EAC05-192P (02-390)															
EAC05-38K (16-73)															
EAC05-485M (12-167)															
EAC05-89C (03-68)															
ICMV-AY312989															
SACMV-AJ575560															

Table 3: Continue

	EAC (AY795985TZT)	EAC04-139M (04-513)	EAC04-166M (30-266)	EAC04-16C (22-175)	EAC04-65K (05-119)	EAC05-06C (25-149)	EAC05-111K (06-92)	EAC05-123S (21-82)	EAC05-152P (01-282)	EAC05-192P (02-390)	EAC05-38K (16-73)	EAC05-485M (12-167)	EAC05-89C (03-68)	ICMV-AY312989	SACMV-AJ575560
AC (AY211461CMAK)	77	77	77	78	77	78	77	78	77	77	78	94	78	74	78
AC (AY211466CMDO3)	77	77	77	78	77	78	77	78	77	77	78	94	78	74	78
AC (AY562421UGSvr)	76	76	76	77	76	77	76	77	76	77	77	92	77	74	77
AC (AY562429UGMil)	77	76	77	77	77	77	77	77	77	77	77	93	77	73	78
AC0405C (25-126)	77	76	76	77	77	77	76	77	76	77	77	93	77	77	78
AC0704C (22-171)	77	76	77	77	77	78	77	78	77	77	77	94	78	74	78
AC13404M (18-64)	77	77	77	78	77	78	77	78	77	77	78	94	78	74	77
AC15605P (11-585)	77	76	77	77	77	78	77	78	77	77	77	94	77	74	78
AC16104M (04-512)	77	76	77	77	77	77	77	78	77	77	77	94	77	74	77
AC16105P (13-199)	77	76	77	77	77	78	76	77	76	77	77	94	77	73	77
AC18304M (27-29)	77	76	76	77	76	77	76	77	76	77	77	93	77	73	77
AC18305P (28-300)	77	76	77	77	77	78	77	78	77	77	77	94	78	74	77
AC2505C (22-602)	78	76	77	77	77	78	77	78	77	77	77	94	77	74	78
AC28005M (29-09)	77	77	77	78	77	78	77	78	77	77	78	93	78	74	77
AC29305P (15-489)	77	77	77	77	77	77	77	78	77	77	77	94	77	74	78
AC3204C (25-133)	77	77	77	78	77	78	77	78	77	77	78	94	78	74	77
AC33205P (20-675)	77	76	77	77	77	78	77	78	77	77	77	94	77	74	78
AC36505P (09-541)	77	77	77	78	77	78	77	78	77	77	78	94	78	74	78
AC3805K (16-73)	77	76	77	77	77	78	77	78	77	77	77	94	77	74	77
AC4505C (03-60)	78	77	77	77	77	77	77	78	77	77	77	94	77	74	78
AC45805C (07-592)	77	76	77	78	77	78	77	78	77	77	77	94	77	74	77
AC48605M (12-161)	77	76	77	77	77	77	77	78	77	77	77	94	77	74	78
AC5005S (21-75)	76	75	76	76	76	77	76	76	76	76	76	93	76	73	77
AC5204C (24-77)	77	76	77	77	77	77	76	77	77	77	77	94	77	74	77
AC7404K (05-115)	77	76	77	77	77	78	77	78	77	77	77	94	77	74	77
AC8604C (07-612)	77	76	77	77	77	77	76	77	76	77	77	94	77	74	77
EAC (AF126804UG2M)	78	77	77	78	77	78	77	78	77	78	78	93	78	74	77
EAC (AF259896CACI)	81	80	80	80	80	81	80	81	80	81	81	96	80	74	78
EAC (AF423178UGNa)	95	97	97	96	97	96	96	96	97	97	97	83	97	74	78
EAC (AJ006461YG)	81	80	80	80	80	81	80	80	80	80	80	96	80	73	78
EAC (AJ717534K23)	96	94	95	95	95	94	95	95	95	95	95	80	95	73	78
EAC (AY562424UG2S	81	80	80	80	80	81	80	80	80	80	80	96	80	74	78
EAC (AY795985TZT)	81	79	80	80	80	81	79	80	80	80	80	96	80	73	78
EAC04-139M (04-513)		95	94	95	94	95	95	95	95	95	95	81	94	73	78
EAC04-166M (30-266)			98	97	98	96	97	96	98	97	98	81	98	72	78
EAC04-16C (22-175)				98	98	98	98	97	98	98	98	81	98	73	77
EAC04-65K (05-119)					98	98	99	98	98	99	98	81	98	73	78
EAC05-06C (25-149)						97	98	97	98	98	99	81	98	72	77
EAC05-111K (06-92)							98	98	97	98	98	82	97	72	78
EAC05-123S (21-82)								97	98	99	98	81	98	72	78
EAC05-152P (01-282)									97	97	97	82	97	72	78
EAC05-192P (02-390)										98	98	81	98	72	77
EAC05-38K (16-73)											98	81	98	72	78
EAC05-485M (12-167)												81	99	72	78
EAC05-89C (03-68)													81	74	76
ICMV-AY312989														72	78
SACMV-AJ575560															72

EACMV detected in Togo (Fig. 2). These results thus highlight new EACMV isolates mainly distinct from those already published in GenBank. The phylogenetic analysis revealed for the first time, the existence of an isolate closer to the Uganda variant of EACMV (UgV) in Togo elucidating thus, the recent development of more virulent form the CMD in Togo (Adjata *et al*., 2009); this can be explained by the potential existence among the detected isolates of recombination events between ACMV and EACMV.

Pairwise identity analysis: It arises from the results of the pairwise identity percentages, on the level of ACMV isolates identified in Togo that, though there is a gathering in clusters, the study of the phylogenetic relationship shows that all these isolates belong to the group of African Cassava Begomovirus (ACMV), the pairwise identity was 97 to 99%, that pairwise identity percentage was the same for isolates identified in Central Africa, in East Africa and for that identified in the other part of West Africa. With regard to EACMV isolates, there is heterogeneity on the level of the pairwise identity percentages. The results obtained from the calculation of pairwise identity percentages between EACMV isolates identified in Togo vary between 77 to 97%. If one refers to the phylogenetic tree obtained for this purpose, the distant isolate of the major EACMV group of Togo, belongs to the same group of isolates as the Uganda variant of EACMV (EACMV-UG/Svr) which is in fact a recombinant between ACMV and EACMV. The results also reveal that the EACMV isolate identified in Togo which belongs to the group of the isolates of Uganda variant has a pairwise identity percentage of 77 and 79% with the major EACMV group as it was shown with other EACMV-Ug/Svr isolates by Pita *et al*. (2001). These results brought some answers to the new development of the disease in the various cassava production zones in Togo hitherto and could explain the sensitivity of cultivars like Gbazékouté, Bazoka, Moya known to be resistant/tolerant cultivars to the disease.

UgV is a distinct strain of EACMV: A phylogenetic analysis permits to say that the EACMV isolate that was identified in Togo and that was closer to the Uganda Variant of EACMV isolates, is a strain distinct from the classic EACMV isolates. Indeed, the result obtained from the calculation of the pairwise sequence identity percentages of the CP gene between the UgV isolates from Togo and that of other EACMV isolates of Africa showed in fact that the UgV isolates are strains distinct of the classic EACMV isolates from other countries. Thus, the comparison between the pairwise sequence of UgV isolate of Togo and those of Uganda to the classic EACMV isolates of Tanzania confirms that the UgV isolate in itself is a strain distinct from classic EACMV isolates. The results obtained in this work, join those obtained by Pita *et al*. (2001), which were based on the analysis of a limited number of isolates of Begomovirus coming from Uganda. The phylogenetic analysis, through the calculation of the pairwise sequence identity highlights the characteristic of the sequence obtained from the isolate (05-485 M (12-167) which has molecular characteristics of the Ugandan severe variant of EACMV known as EACMVUG2/Svr or UgV. This sequence has already received from GenBank, the number EU155148. The criteria utilized to distinguish between the various viruses are those of Padidam *et al*. (1996). The results obtained in this study confirm the results of the molecular studies which highlighted that UgV was responsible of the increased severity of the symptoms noted on the infected cassava plants (Zhou *et al*., 1997; Deng *et al*., 1997). Though observations made on a number of cultivars, show that the severity of the symptoms on cassava plants depends initially of the sensibility of the cultivar and then of the virus involved; it is important to point out that recombinants also pose enormous problems in symptom study, hosts

range, especially when they are to show the effect of synergism on certain test plants infected by two species of different viruses of course of the same Genus as in the case of ACMV and EACMV as it was demonstrated by Legg (1999). In similar cases, the question could be: Is the severity of the symptoms on the infected cassava plants in the production zones due to the sensitivity of the cultivars, to the virulence of the virus strain involved, to the synergism of two viruses or the effect of recombinants? Taking into account the explosion of recombinants within the Genus Begomovirus, the question of symptomatology should be reconsidered initially in any study of Begomovirus. For example, in this study, it was difficult to say with precision which virus produces which symptom. This quite simply because according to Padidam *et al.* (1996), there is not really at the taxonomic level, a clear obstruct between the species and strains by using genomic sequences like criterion; studies showed as well that UgV would be a strain of EACMV, but serologically indistinguishable of ACMV (Harrison and Robinson, 1999); if such is the case, how to solve with precision the question of becoming of UgV in term of evolution if it has the possibility to recombine with one of the parents?

Molecular evidence of the existence of Uganda variant of EACMV (UgV) in Togo: On the basis of CP gene sequences analysis of one hundred fourteen (114) Begomovirus sequenced isolates, the results from phylogenetic analysis (Fig. 3) showed that one of the EACMV isolates identified in Togo and not belonging to the major group, is closer to the variant of Uganda strain of EACMV (known as severe EACMV strain of Uganda or UgV or EACMVUG/Svr) which is in fact a recombinant issued from ACMV and EACMV. A pairwise identity percentages of the CP gene sequences between this Togolese EACMV isolate 05-485 M (12-167) and that of the severe Uganda variant of EACMV isolate known as UgV, highlights, the existence of an isolate closer to the severe Uganda variant of EACMV known as UgV/TG in Togo. It is important to note that the pairwise identity percentage of the isolate identified in Togo is in fact, an isolate distinct from those already identified elsewhere. Indeed, this Togolese isolate gives a pairwise identity percentage of 96% with the Uganda variant of EACMV (UgV) isolate. A comparison of this isolate, UG/TG with the major group of EACMV, gives the following pairwise identity percentage varying between 80 and 83%. Compared with ACMV isolate, these pairwise identity percentages vary between 92 and 94%. This highlights the fact that this isolate is at the same time ACMV and EACMV. What confirms that this isolate is a recombinant having a hybrid CP between ACMV and EACMV (Zhou *et al.*, 1997; Brown *et al.*, 2001) and illustrates especially the fact that the CP sequence of 700 bp analyzed in this study is closer to ACMV than of EACMV. The results of the comparison between the EACMVUG2/Svr sequences, the UgV/TG and the other groups of EACMV classic isolates of GenBank and coming from other countries of Africa in particular isolate AY211887 of Cameroun and isolates AY795985, AY795986 and AY795987 of Tanzania, reveal that the UgV isolate of Togo, like all ACMV isolates and other EACMV isolates of GenBank, are very different from the latter (Table 3). The phylogenetic analysis and the pairwise identity percentages highlight the characteristic of the Togolese isolate 05-485 M (12-167) which has molecular and genetic characteristics of the Uganda variant of EACMV (UgV) isolate. The distinction between this sequence and the other EACMV sequences of the GenBank database was confirmed by a pairwise identity percentage of 96% of this particular isolate 05-485 M (12-167) with Uganda variant of EACMV (UgV) isolate. The results obtained in the follow-up of the epidemiologic analysis confirm the results of the molecular studies which highlighted UgV as the potentially responsible of the increased severity of the symptoms noted on the infected cassava plant in the production zones in Togo.

CONCLUSION

The results of our work make it possible to assert that there would exist at least three Begomoviruses which infect cassava in Togo. The studies which were undertaken allowed sequencing the CP gene of these Begomoviruses identified in Togo. Phylogenetic analysis allowed highlighting a great variability between these Begomovirus infecting cassava in Togo; this variability is not only interspecific but also intraspecific. Although the results obtained in this study are not exhaustive, given the emergence of new viral diseases due to Begomovirses, it is important to take them into account in the search for resistant/tolerant clones to cassava virus diseases. So, a more thorough study will allow studying the epidemic of new emerging Begomoviruses in Togo.

ACKNOWLEDGMENT

We are grateful to the 'Agence Universitaire de la Francophonie (AUF)' and the 'Service de Coopération et d'Action Culturelle SCAC' for sponsoring this project.

REFERENCES

Adjata, K.D., E. Muller, M. Aziadekey, Y.M.D. Gumedzoe and M. Peterschmitt, 2008. Incidence of cassava viral diseases and first identification of East African cassava mosaic virus and Indian cassava mosaic virus by PCR in Cassava (*Manihot esculenta* Crantz) fields in Togo. Am. J. Plant Physiol., 3: 73-80.

Adjata, K.D., E. Muller, M. Peterschmitt, O. Traore and Y.M.D. Gumedzoe, 2009. Molecular evidence for the association of a strain of Uganda variant of east African cassava mosaic virus to symptom severity in cassava (*Manihot esculenta* Crantz) fields in togo. Am. J. Biochem. Biotechnol., 5: 196-201.

Adjata, K.D., E. Muller, M. Peterschmitt, M. Aziadekey and Y.M.D. Gumedzoe, 2012. Study of molecular variability of a Begomovirus of cassava (*Manihot esculenta* Crantz) (ACMV) in Togo. J. Rech. Sci. Univ. Lome (Togo), Serie A, 14: 35-43.

Atiri, G.I., F.O. Ogbe, A.G.O. Dixon, S. Winter and O. Ariyo, 2004. Status of cassava mosaic virus diseases and cassava Begomoviruses in the sub-Saharian Africa. Sustainable Agric., 24: 5-35.

Brown, J.K., A.M. Idris, I. Torres-Jerez, G.K. Banks and S.D. Wyatt, 2001. The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. Arch. Virol., 146: 1581-1598.

Deng, D., G.W. Otim Nape, A. Sangare, S. Ogwal, R.N. Beachy and C.M. Fauquet, 1997. Presence of a new virus closely associated with cassava mosaic outbreak in Uganda. Afr. J. Root Tuber Crops, 2: 23-28.

Fauquet, C.M. and D. Fargette, 2005. International committee on taxonomy of viruses and the 3,142 unassigned species. Virol. J., Vol. 2 10.1186/1743-422X-2-64

Fondong, V.N., J.S. Pita, M.E.C. Rey, A. de Kochko, R.N. Beachy and C.M. Fauquet, 2000. Evidence of synergism between African cassava mosaic virus and the new double recombinant Geminivirus infecting cassava in Cameroon. J. Gen. Virol., 81: 287-297.

Hall, T.A., 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acid Symp. Ser., 41: 95-98.

Harrison, B.D. and D.J. Robinson, 1999. Natural genomic and antigenic variation in whitefly-transmitted Geminiviruses (Begomoviruses). Annu. Rev. Phytopathol., 37: 369-398.

Jeske, H., M. Lutgemeier and W. Preiss, 2001. Distinct DNA forms indicate rolling circle and recombination-dependent replication of *Abutilon mosaic* geminivirus. EMBO J., 20: 6158-6167.

Legg, J.P., 1999. Emergence, spread and strategies for controlling the pandemic of cassava mosaic virus disease in East and Central Africa. Crop Prot., 18: 627-637.

Moriones, E. and J. Navas-Castillo, 2000. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. Virus Res., 71: 123-134.

Otim-Nape, G.W., J.M. Thresh and M.W. Shaw, 1998. The incidence and severity of cassava mosaic virus disease in Uganda: 1920-1992. Trop. Sci., 38: 25-37.

Padidam M., R.N. Beachy and C.M. Fauquet, 1996. The role of AV2 (precoat) and coat protein in viral replication and movement in tomato leaf curl geminivirus. Virology, 224: 390-404.

Padidam, M., S. Sawyer and C.M. Fauquet, 1999. Possible emergence of new geminiviruses by frequent recombination. Virology, 265: 218-225.

Perrier, X., A. Flori and F. Bonnot, 2003. Data Analysis Methods. In: Genetic Diversity of Cultivated Tropical Plants. Seguin, X. Perrier and J.C. Glaszmann (Eds.). Enfield Science Publishers, Montpellier, France, pp: 43-76.

Pita, J.S., V.N. Fondong, A. Sangare, G.W. Otim-Nape, S. Ogwal and C.M. Fauquet, 2001. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. J. Gen. Virol., 82: 655-665.

Polston, J.E. and P.K. Anderson, 1997. The emergence of whitefly-transmitted geminiviruses in tomato in the Western hemisphere. Plant Dis., 81: 1358-1369.

Zhou, X., Y. Liu, L. Calvert, C. Munoz, G.W. Otim-Nape, D.J. Robinson and B.D. Harrison, 1997. Evidence that DNA-A of a Geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. J. Gen. Virol., 78: 2101-2111.