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Complete genome sequencing of two causative viruses of cassava mosaic disease in Ghana

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Summary. – Cassava mosaic disease (CMV), caused by one or a combination of cassava mosaic geminiviruses, is ranked among the most important constraints to profitable and efficient production of cassava. Effective control measures require in-depth knowledge of the viral causative agent. Using rolling-circle amplification and unique enzymes, the full genome of two species of cassava mosaic geminivirus isolated from infected cassava plants in Ghana were cloned into pCambia 1300 and pET-28b. The sequences of the genome were determined on an ABI sequencer and a pairwise comparison was performed with other cassava-infecting geminiviruses from different countries. It was revealed that cassava grown in Ghana is attacked by two species of geminivirus in either single or mixed infections. These are the African cassava mosaic virus (ACMV) and the East African cassava mosaic virus (EACMV)-like, with high sequence similarity of 94% and 80%, respectively, between the DNA-A and DNA-B components of each virus, and 66% and 41% similarity of the common region (CR) (for A and B accordingly). The DNA-A of ACMV and EACMV-like contained 2781 and 2800 nucleotides, respectively, while their DNA-B components had 2725 and 2734 nucleotides, respectively. ACMV DNA-A was over 97% similar to those of other ACMVs from the continent. In contrast, EACMV-like DNA-A was over 98% similar to the isolates from Cameroon and other West African countries, and less than 88% similar to other EACMV species. Thus ACMV and EACMV-like were named African cassava mosaic virus-Ghana and East African cassava mosaic Cameroon virus-Ghana. Computer analysis revealed that their genome arrangement follows the typical old world bipartite begomovirus genome. The association of these two species and their interaction might account for the severe symptoms observed on infected plants in the field and in the greenhouse.

Keywords: cassava mosaic disease; begomovirus; rolling-circle amplification

Introduction

Cassava (*Manihot esculenta* Crantz) is a woody perennial shrub, which grows in tropical and subtropical areas of the world (Sayre *et al*., 2011). It is a staple crop grown throughout Africa, serving as the most important source of dietary carbohydrate in sub-Saharan Africa (Alabi *et al*., 2008), and it is the third most important source of calories after rice and maize (Adepoju *et al*., 2010). It is considered the most important food crop in Ghana (Manu-Aduening *et al*., 2007), where it is used for domestic and industrial purposes (Nassar and Ortiz, 2006).

Cassava is vulnerable to several different viral diseases, in part because it is vegetatively propagated (Patil and Fauquet, 2009). CMD, caused by begomoviruses of the family *Geminiviridae,* is considered the most serious constraint to cassava production (Legg and Fauquet, 2004). Due to the apparent propensity of cassava mosaic geminiviruses

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Abbreviations: ACMV = African cassava mosaic virus; CMD = cassava mosaic disease; CMG = cassava mosaic geminivirus; CP = capsid protein; CR = common region; EACMV = East African cassava mosaic virus; EACMCV = East African cassava mosaic Cameroon virus; ICMV = Indian cassava mosaic virus; RCA = rolling-circle amplification; SACMV = South African mosaic virus; SLCMV = Sri Lankan cassava mosaic virus

(CMGs) to exchange genetic material (Legg and Fauquet, 2004), new species have been identified, which differ in their biological characteristics and in the severity of the resultant disease.

Incidence of both ACMV and EACMV, and the importance of CMD in Ghana have been reported (Lamptey *et al.*, 1998, 2000; Offei *et al.*, 1999; Manu-Aduening *et al.*, 2007; Torkpo, 2009). Though those studies report on CMD in Ghana, the molecular features of the virus have not been conclusively determined. This paper reports the full-genome sequences of two species of CMG infecting cassava in Ghana.

Materials and Methods

DNA extraction. Total DNA was extracted from young symptomatic leaves harvested from cuttings established in plastic pots in the greenhouse using the CTAB-based DNA extraction method (Doyle and Doyle 1987). The cuttings were from infected cassava plants collected from fields in Ghana assembled during surveys in 2005 and 2008 (Torkpo, 2009).

Confirmation of CMG in the infected cassava samples. Standard PCR (Sambrook *et al.*, 1989) was performed with the samples using cassava mosaic virus conserved primers (Table 1) (Pita *et al.*, 2001) in a Biometra® T Gradient Thermal Cycler. The general reaction cycles were: 94°C for 2 min followed by 30 cycles of 94°C for 1 min, 52–63°C for 1 min (depending on the primer), 72°C for 1 min and a final extension of 72°C for 10 min. PCR products were separated at 110 V for about 45 min on a 1% agarose gel stained with ethidium bromide (10 mg/ml) alongside a 100 bp DNA ladder (GeneRuler).

Rolling-circle amplification (RCA) of total DNA extracted from infected plants. The circular genomic DNA was amplified by RCA using the TempliPhi Amplification kit (GE Healthcare, Piscataway, NJ, USA) following the manufacturer′s protocol outlined in (Haible *et al.*, 2006; Guenoune-Gelbart *et al.*, 2010) as follows: 1 µl of the plant′s total DNA preparation was added to 5 µl sample buffer (containing random hexamers that prime DNA synthesis non-specifically), heated to 95°C for 3 min to denature the DNA, snap-chilled on ice, and combined with 5 µl of reaction buffer (containing salts and deoxynucleotides with an adjusted pH to support DNA synthesis) plus 0.2 µl of enzyme mix (containing Phi29 DNA polymerase and random hexamers in 50% glycerol). The reaction mixtures were incubated for 18 hrs at 30°C, followed by inactivation of the enzyme at 65°C for 10 min. The quality (and estimated quantity) of the RCA products was analyzed by gel electrophoresis (1% agarose stained with ethidium bromide). The RCA products were used immediately or kept at -20°C.

Cloning of CMGs. Viral DNA was digested with the appropriate restriction enzyme at a unique site in the viral genome following RCA. The enzymes were selected after screening their banding patterns. All enzymes used were Fast Digest® enzymes from Fermentas with the exception of *Xba*I, which was acquired from New England Biolabs (Ipswich, MA, USA). The reaction mixture contained 8 µl (approximately 4 µg) DNA from RCA, 4 µl of 10X Fast Digest® green buffer (Fermentas), 4 µl of the selected enzyme and 24 µl water (molecular biology grade from Bio-Lab), for a total reaction volume of 40 µl. This was incubated at 37°C for 15 to 20 min, after which it was resolved on a 1% agarose gel and the DNA eluted.

Plasmid DNA (pCambia 1300 and pET-28b) was digested with the appropriate enzyme (*Bam*HI, *Hind*III, *Xba*I, and *Nde*I) as described above and dephosphorylated by addition of 3 µl alkaline phosphatase (Fast AP), 4 µl of 10X Fast AP buffer and 3 µl water (molecular biology grade, Bio-Lab). This was followed by additional 15 min incubation at the same temperature. After the incubation period, the DNA was resolved on a 1% agarose gel.

Eluted viral DNA was ligated to eluted dephosphorylated plasmid DNA. Ligation conditions were as follows: 5 µl vector, 10 µl insert (CMG fragment), 2 µl ATP, 2 µl of 10X T4 ligase buffer (Fermentas) and 1 µl enzyme (T4 DNA ligase, 500 U). The reaction was carried out in a 200-µl microtube in a PCR apparatus overnight under the following conditions: 1 hr at 16°C followed by 22°C for 1 hr, 16°C for 1 hr and 10°C for 15 hrs. Control reactions were run containing water instead of DNA.

Competent *Escherichia coli* cells (DH5α) were transformed with recombinant DNA using the heat-shock method (Sambrook *et al.*, 1989). Single colonies were picked to verify the presence of recombinant plasmid with the putative viral sequence. Clones containing positive inserts were identified by PCR screening with respective PCR primers on bacterial colonies as described above. Positive, as well as some negative clones identified through PCR were further screened with miniprep followed by restriction mapping and sequence comparison. Double restriction was performed with the enzymes *Xho*I and *Bam*HI or *Nde*I, *Xba*I or *Hind*III for the respective clones.

Sequencing of CMG fragments. The dideoxynucleotide chain termination method was used on an ABI automatic sequencer platform to determine the complete nucleotide sequence of the virus in both orientations. Sequencing was outsourced to Hay-Labs Company Ltd. (Rehovot, Israel). Forward and reverse primers were synthesized to target the multiple cloning sites of pC-1300. Primer pair pET upstream primer and T7 terminator primer were used to sequence the pET-28b clones. Effectively, a sequence length of less than 700 nucleotides (nt) was obtained in both directions. Single primers (Table 2) were synthesized for genome walking to obtain overlapping sequences for the complete genome of the virus.

Analysis of CMG sequences. The authenticity of the obtained sequences was determined using the basic local alignment search tool (BLAST) from the National Center for Biotechnology Information (NCBI) website. Viral DNA sequences were edited using DNAMAN software (LBDraw Version 4.20, Lynnon Biosoft) and BioEdit Sequence Alignment Editor (Ibis Therapeutics, Carlsbad,

Table 1. Primers used to detect the presence of CMG DNA in cassava samples collected from Ghana

Primer name	Direction	Sequence (5′→3′)	Target
CP-For	Forward	ATGTCGAAGCGACCAGGAGATAT	CMG AV1
CP-Rev	Reverse	CCATATACAGAAGCAAAGCATTCTC	CMG AV1
JSP001	Forward	ATGTCGAAGCGACCAGGAGAT	ACMV AV1
JSP002	Reverse	TGTTTATTAATTGCCAATACT	ACMV AV1
UV-AL1/F1	Forward	TGTCTTCTGGGACTTGTGTG	EACMV AV1 & AC1
ACMV-CP/R3	Reverse	TGTCTCCTGATGATTATATGT	EACMV AV1 & AC1
EAB555F	Forward	TACATCGGCCTTTGAGTCGCATGG	BC1
EAB555R	Reverse	CTTATTAACGCCTATATAAACACC	BC1

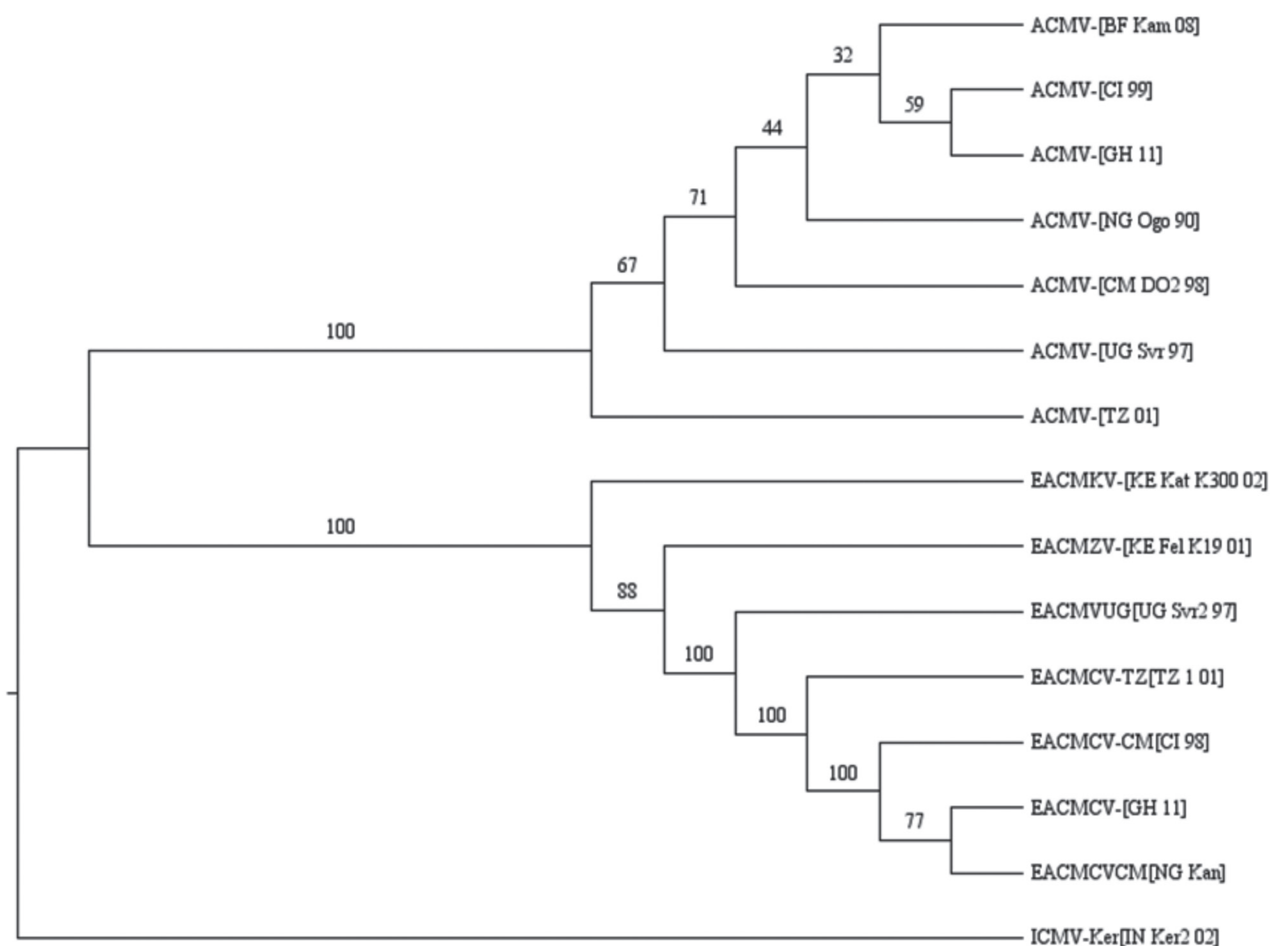

Fig. 1

Phylogenetic tree showing DNA-A complete nucleotide sequence relationships between the Ghanaian cassava mosaic geminivirus isolates and other cassava mosaic geminiviruses

ICMV was used as an outgroup. Tree was constructed for the full-length viral sequence from the multiple alignments by maximum likelihood framework using the phyml software with 100 bootstrap replicates.

California, USA) to obtain a consensus sequence. Full-length DNA-A and DNA-B geminivirus sequences were extracted from available sequences in GenBank (Acc. Nos. are provided in the appendix) and these were used as reference sequences. The CR and capsid protein gene (AV1) sequences of these geminiviruses were also extracted.

Multiple sequence alignment of the full-length CR DNA sequence and AV1 amino acid sequences was performed using MUSCLE program (Edgar, 2004). Phylogenetic trees were constructed for the full-length viral sequence and AV1 from the multiple alignments by maximum likelihood framework using the phyml software with 100 bootstrap replicates (Guindon and Gascuel, 2003). The CR was compared with selected geminiviruses, and virus-specific iterons were identified and compared. The predicted open reading frames (ORFs) were determined. Percentage similarity between individual ORFs and those of selected geminiviruses was also determined.

Results and Discussion

We determined the full-length nucleotide sequences of both DNA-A and DNA-B of CMGs infecting cassava in Ghana and compared them to other known cassava mosaic viruses.

Nucleotide sequence characteristics of CMG from Ghana

ACMV alone was detected in 36 (21%) out of 171 symptomatic cassava samples, using ACMV-specific primer pair (Table 1). Mixed infections by ACMV and EACMV were detected in 79% of symptomatic cassava leaves using EACMV primer pairs (Table 1).

Full-length nucleotide sequences in both the forward and reverse direction obtained from the Ghanaian isolates were aligned with other geminivirus sequences. Sequences obtained from recombinant plasmid ligated to the *Bam*HI site aligned with ACMV DNA-A, whereas those ligated to the *Nde*I site aligned with ACMV DNA-B. Similarly, recombinant plasmids created at the *Xba*I and *Hind*III sites aligned to EACMV-like

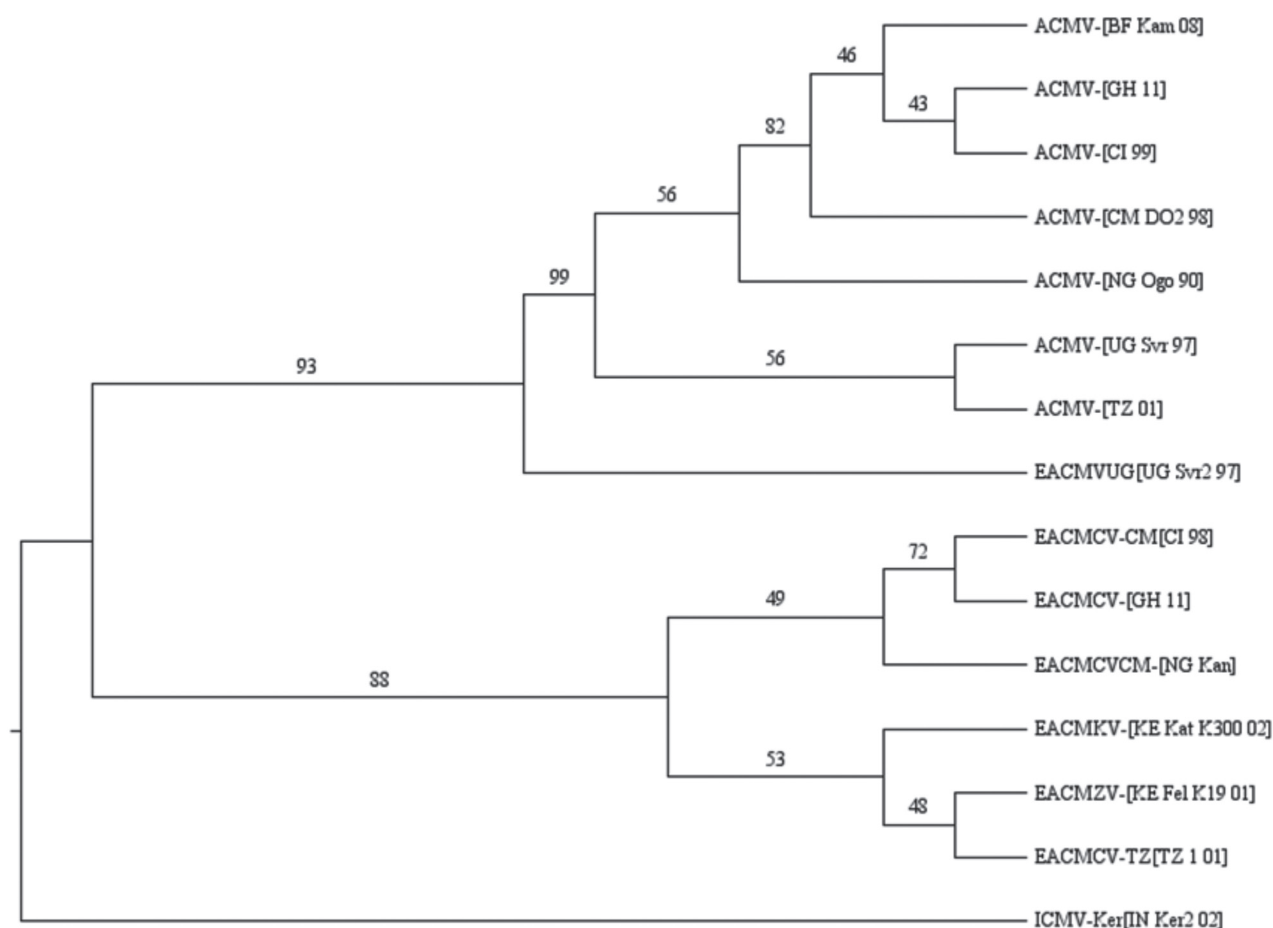

Fig. 2

Phylogenetic tree showing the capsid protein gene (AV1) amino acid sequence relationship between the Ghanaian cassava mosaic geminivirus isolates and other cassava mosaic geminiviruses

ICMV was used as an outgroup. Tree was constructed for AV1 from the multiple alignments by maximum likelihood framework using the phyml software with 100 bootstrap replicates.

DNA-A and B, respectively. All of the clones were obtained from a mixed-infected cassava plant collected from Djanpokrom in the Western Region of Ghana. All sequences were submitted to the NCBI database with GenBank Acc. Nos. JN165088, JN165086, JN165089, and JN165087, respectively.

The relationship of the Ghanaian isolates to other cassava-infecting geminiviruses was determined by comparing full-length sequences with other geminivirus sequences in the database. The results show that the ACMV grouped with the other ACMV sequences, while the EACMV-like grouped with the East African cassava mosaic Cameroon virus (EACMCV) species (Fig. 1).

General features of ACMV isolated from infected cassava plants in Ghana

ACMV isolated from Ghana possessed both the DNA-A (2781 nt) and B (2725 nt) components. The DNA-A aligned closest to ACMV-Cote d'Ivoire (AF259894) with 98% nucleotide identity. This was followed by ACMV-Burkina Faso (FM877473), ACMV-Nigeria (AJ427910) and ACMV-Uganda Mild (AF126800) with sequence identities of 97%. The virus showed high overall sequence identity (>90%) with all other published sequences of ACMV isolates (Table 3). There is no relationship between the origins of the ACMV isolates, and this makes it difficult to determine, which of them are more closely related (Ndunguru *et al.*, 2005). Because this is the first time that a viral isolate from an infected cassava plant in Ghana has been fully sequenced, it was named African cassava mosaic virus-Ghana (ACMV-[GH]) as suggested by the ICTV (Fauquet *et al.*, 2008).

The gene arrangement of ACMV isolated from Ghana followed a typical old world bipartite begomovirus gene arrangement (Patil and Fauquet, 2009). The sequence predicted the typical six ORFs with four in the complementary sense and two in the viral sense on DNA-A. DNA-B had two ORFs: one in the viral sense and the other in the complementary sense. There was a CR in both DNA-A and DNA-B with sequence similarity of 94%.

General features of EACMCV isolated from Ghana

The EACMCV isolated from Ghana had both DNA-A (2800 nt) and B (2734 nt) components. It aligned closest to EACMCV-Cote d′Ivoire (AF259896) with a sequence identity of 97%. It also showed high overall sequence identity (96%) with another EACMCV isolate from Nigeria (AJ259896, Table 4).

Phylogenetic analysis of the full-length nucleotide sequence of EACMCV from Ghana and other geminivirus sequences in the Genbank showed closest alignment with EACMCV relatives isolated from West Africa (Fig. 1). This might be due to the geographical relationship between them,

Table 2. Primers used for genome walking of the geminivirus inserted into different sites of the cloning vector

Cloning at *Bam*HI site of pC-1300

Primer name	Direction	Primer sequence (5′→3′)
pC2300 8300 Fw	Forward	GTGTGGAATTGTGAGCGG
pC2300 844 Rev	Reverse	CCAGTCACGACGTTGTAA
9BamHICF 833	Forward	GGTTTCAGGTGTTGAGGAA
9BamHICR 2446	Reverse	CTGCATCAGAATGGGGAACC TCACT

Cloning at *Hind*III site of pC-1300

Primer name	Direction	Sequence (5′→3′)
pC2300 8300 Fw	Forward	GTGTGGAATTGTGAGCGG
pC2300 844 Rev	Reverse	CCAGTCACGACGTTGTAA
EACMV9II CF 1895	Forward	TGCGTCTTCAAGGCTGACGC

Cloning at *Xba*I site of pC-1300

Primer name	Direction	Primer sequence (5′→3′)
pC2300 8300 Fw	Forward	GTGTGGAATTGTGAGCGG
pC2300 844 Rev	Reverse	CCAGTCACGACGTTGTAA
9XbaICF 1000	Forward	AGAATCATACCGAGAATGCG
9XbaICR 1294	Reverse	ACTTCCGGATCTGGACTCGT
9XbaICF 2710	Forward	GGCATTATATAGGATGTCCC

Cloning at *Nde*I site of pET-28b

Primer name	Direction	Primer sequence (5′→3′)
pET upstream	Forward	ATGCGTCCGGCGTAGA
T7 terminator	Reverse	GCTAGTTATTGCTCAGCG G
9NdeICF 1200	Forward	CTATGTTGGATGATTTATTA
9NdeICF 1840	Forward	TGATCCTGTTGCTCATCATC
9NdeICF 2416	Forward	ATAGACATATATTTCTTATC

although the EACMV-Uganda was isolated and sequenced in Burkina Faso (Tiendrébéogo *et al.*, 2009).

Since this isolate was closest in the overall nucleotide identity to EACMCV species (>98%) (Table 4) and it is the first EACMCV to be sequenced in Ghana, it was named East African cassava mosaic Cameroon virus-Ghana. (EACMCV-[GH]).

The gene arrangement followed the typical begomovirus arrangement as described in section 3.2. The CR between the DNA-A and DNA-B shared a nucleotide similarity of 80%.

Capsid protein (CP) gene sequence analysis and comparison with selected viruses

The amino acid sequence predicted by the AV1 gene for both ACMV-Ghana and EACMCV-Ghana was determined using the DNAMAN protein translation feature. The

Table 3. Comparison of nucleotide and deduced amino acid sequences of ACMV-[GH] ORFs with those of other geminiviruses (values of 89% identity and above are in bold)

Viral isolate	Nucleotide sequence				Amino acid sequence							
	A	B	CRA	CRB	AV1	AV2	AC1	AC2	AC3	AC4	BV1	BC1
ACMV-[CM:98]	**96**	**93**	**96**	**93**	**98**	**94**	**96**	**94**	83	**90**	**94**	**97**
ACMV-[CI:99]	**98**	**93**	**96**	**95**	**99**	**96**	**98**	**97**	**93**	-	**92**	**98**
ACMV-[NG:Ogo:90]	**97**	**94**	**98**	**92**	**97**	**95**	**96**	**98**	**93**	**94**	**92**	**97**
ACMV-[UG:Svr:97]	**97**	**94**	**97**	**90**	**97**	**94**	**97**	**97**	**92**	-	**96**	**97**
ACMV-[BF:Kam:08]	**97**	-	**97**	-	**98**	**94**	**97**	**97**	**92**	**95**	-	-
ACMV-[TZ:01]	**95**	-	**96**	-	**97**	**95**	**95**	**94**	**89**	**92**	-	-
ICMV-Ker[IN:Ker2:02]	71	41	45	37	75	73	77	-	63	48	28	42
SACMV-[ZA]	75	49	43	43	79	76	82	67	70	56	38	56
SLCMV-IN[IN:Adi]	74	42	70	69	75	73	83	62	63	53	24	42
EACMCV-CM[CM:98]	68	47	44	61	82	53	71	57	59	36	38	59
EACMCV-CM[CI:98]	68	46	39	57	82	-	71	-	-	-	38	59
EACMVKE[KE:Boa:K48:01]	70	48	35	57	82	54	72	69	69	34	37	58

Table 4. Comparison of nucleotide and deduced amino acid sequences of EACMCV-[GH] ORFs with those of other geminiviruses (values of 89% identity and above are in bold)

Viral isolate	Nucleotide sequence				Amino acid sequence							
	A	B	CRA	CRB	AV1	AV2	AC1	AC2	AC3	AC4	BV1	BV2
EACMCV-CM[CM:98]	**97**	**94**	**97**	**95**	**94**	**99**	**97**	**96**	**94**	**95**	**93**	**97**
EACMCV-CM[CI:98]	**97**	**96**	**98**	**98**	**96**	-	**98**	**96**	-	-	**99**	**96**
EACMCVCM[NG:Kan]	**97**	-	**97**	-	**93**	**98**	**97**	-	**93**	-	-	-
EACMKV[KE:Kat:K300:02]	78	71	56	55	**94**	**93**	70	**97**	62	69	63	85
EACMKV[KE:Mit:K298:02]	79	70	63	56	**94**	**92**	70	59	63	36	65	88
EACMMV-[MW:K:96]	79	-	**93**	-	84	56	**94**	62	-	-	-	-
EACMVKE[KE:Boa:K48:01]	87	71	**96**	61	**94**	**93**	**94**	61	62	10	64	87
EACMV-[BF:Kou:08]	83	-	**99**	-	85	**92**	**95**	61	63	**91**	-	-
EACMVUG[UG:Svr2:97]	83	-	**97**	-	85	**92**	-	-	-	-	-	-
EACMZV-[KE:Fel:K19:01]	78	70	58	37	**94**	**91**	73	61	62	12	64	87
ICMV-Ker[IN:Ker2:02]	68	42	72	36	76	62	69	-	59	-	24	46
SACMV-[ZA]	72	68	70	61	84	56	71	60	64	36	65	85
SLCMV-IN[IN:Adi]	67	41	36	56	76	60	65	60	60	10	24	46
ACMV-[CI:99]	69	47	38	57	81	57	65	59	59	-	37	59

predicted polypeptide formed the virus CP. The CP of the Ghanaian isolates was analyzed by comparison with the CPs of other geminiviruses (Fig. 2).

The amino acid sequence of ACMV-Ghana CP was almost identical (98%) to that of ACMV-Cote d΄Ivoire (Table 3). It showed overall high nucleotide similarity (>96%) with other ACMV isolates. On the other hand, the CP showed low amino acid sequence identity (~80%) with EACMV and EACMCV species. The same held true for the CPs of South African cassava mosaic virus (SACMV), Sri Lankan cassava mosaic virus (SLCMV) and Indian cassava mosaic virus (ICMV), with sequence similarities between 71 and 75%.

The CP of EACMCV-Ghana showed the highest amino acid sequence identity (96%) with the CP of EACMCV-Cote d΄Ivoire (Table 4). Other species of EACMCV also shared high CP sequence identity (93–95%). Interestingly, the CP of East African cassava mosaic Kenya virus (EACMKV) and East African cassava mosaic Zanzibar virus (EACMZV) also showed high sequence similarity (94%) to the Ghanaian isolate. This can also be attributed to the recombination events that characterize viruses in this species, as has been seen in isolates from Cameroon, Cote d΄Ivoire, Tanzania and Togo (Fondong *et al.*, 2000; Pita *et al.*, 2001; Ndunguru *et al.*, 2005; Adjata *et al.*, 2009) among others, although this particular sequence was not analyzed for recombination.

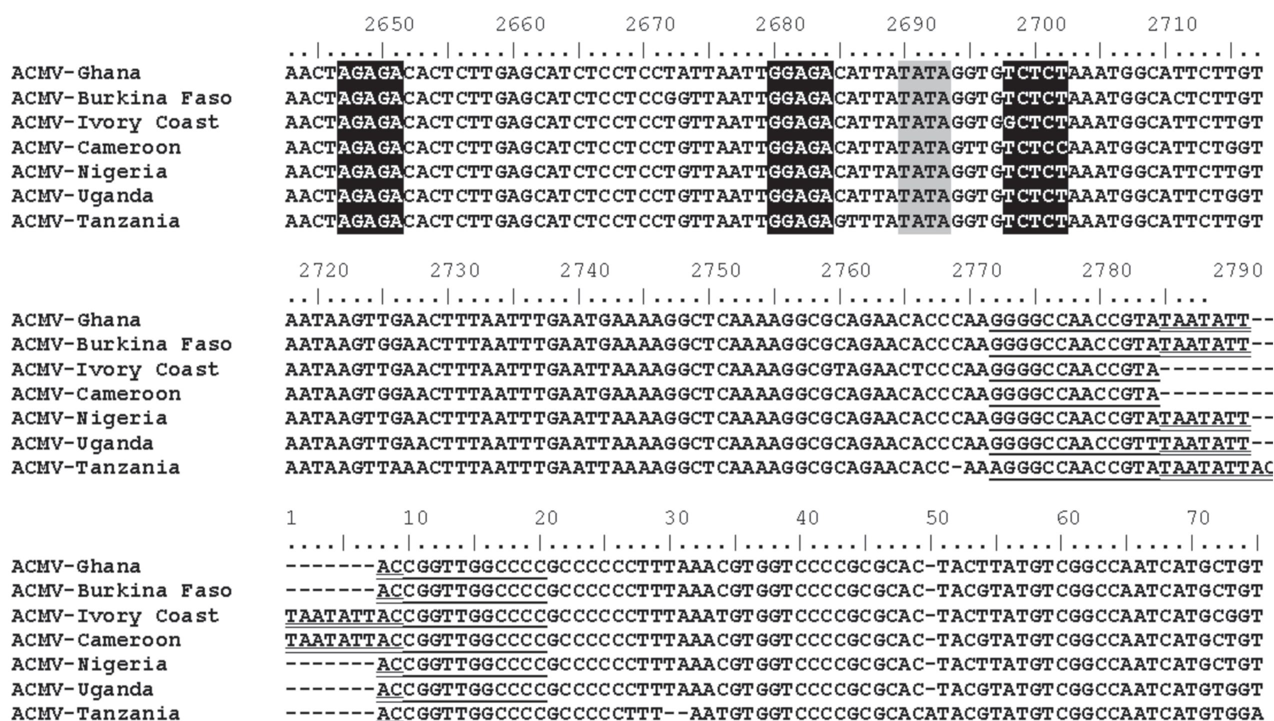

Fig. 3

Alignment of the CR nucleotide sequence of DNA-A (CRA) of ACMV-[GH] with other isolates of ACMV from the database sequences

The TATA box for AC1 is highlighted in grey. The putative Rep-binding iterative sequences (iterons) are highlighted in black. The conserved nonanucleotide sequence TAATATTAC, together with its stem loop, are underlined once and twice, respectively.

The EACMV species were less similar (<85%) to the Ghanaian isolate, as were East African cassava mosaic Malawi virus (EACMMV) and SACMV (84% similar). As expected, the similarity with species of ACMV, ICMV and SLCMV was much lower (<80%).

The CRs of the Ghanaian CMGs

The CR of ACMV-Ghana was 180 nt long, while that of EACMCV-Ghana was 174 nt in length. The conserved nonanucleotide in the hairpin-loop, TAATATTAC, which is characteristic for the members of the family *Geminiviridae*, and the AC1 TATA box, were identified in the CR sequences of both Ghanaian CMGs. When the CR sequence of ACMV-Ghana was compared and aligned to the published CR sequences of other cassava-infecting ACMV isolates from Africa, it became apparent that ACMV-Ghana is very similar to all ACMV isolates, with nucleotide similarities over 93% (Table 3).

The iteron sequence GGAGA, as well as TCTCT, were also present, as in all published ACMV sequences, representing the putative Rep-binding iteron (Fig. 3). There was also an inverted-repeat sequence (GGGGCCAACCGTA**TAATA TTAC**CGGTTGGCCCC) that predicts a hairpin-loop structure within the CR.

EACMCV-Ghana also had characteristics similar to its counterpart ACMV in the CR. The difference arose in the iteron sequences, which were GGGGG and CCCCC (Fig. 4). It also had the conserved nonanucleotide (TAATA TTAC) within the hairpin-loop-predicting sequence (GC GGCCATCCGAT**TAATATTAC**CGGATGGCCGC). This difference could be attributed to the loss or insertion of nucleotides at the 5′ end of the conserved hairpin-loop motif (Fondong *et al.*, 2000). The CRs also had the Rep-binding iteron sequences (Eagle *et al.*, 1994; Fontes *et al.*, 1994; Roberts and Stanley, 1994; Chatterji *et al.*, 1999) upstream of the promoter region (TATA box) of AC1.

All in all, the results obtained indicate that at present there are two species of CMG attacking cassava growing in Ghana: ACMV and EACMCV. The genome characteristics of these viruses were those of the typical old world bipartite begomovirus (Stanley and Gay, 1983). Among other threats to the cassava growing we can now specify one, which stems from our findings, that there are mixed infections

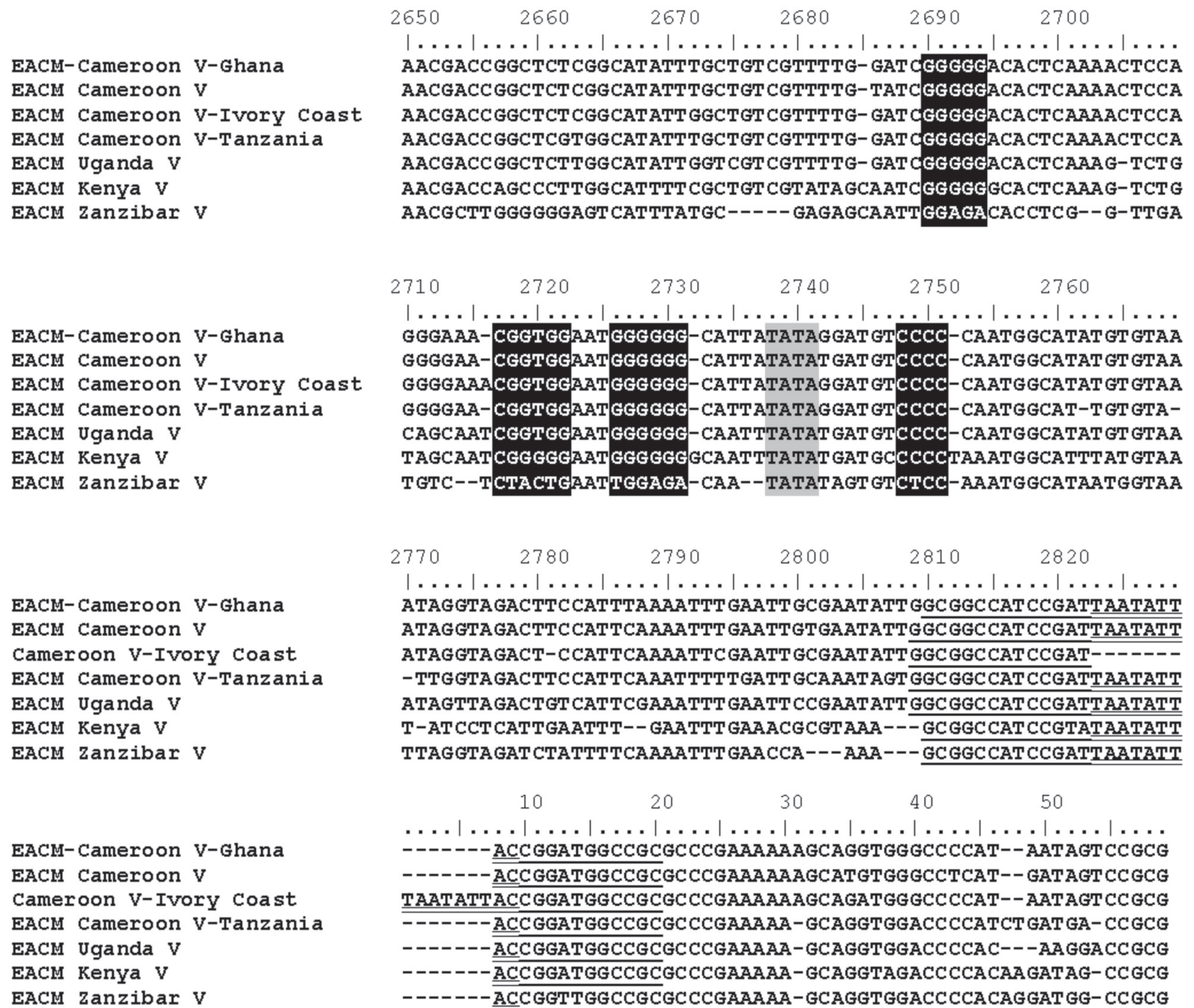

Fig. 4

Alignment of the CR nucleotide sequence of DNA-A (CRA) of EACMCV-[GH] isolate from Ghana with other isolates of EACMV from the database sequences

The TATA box for AC1 is highlighted in grey. The putative Rep-binding iterative sequences (iterons) are highlighted in black. The conserved nonanucleotide sequence TAATATTAC, together with its stem loop, are underlined and double underlined, respectively.

of the two species of CMG in Ghana. Mixed infections in the same host have a tendency to create conditions for new geminiviruses to develop through recombination (Fondong *et al.*, 2000), and these new viruses may be highly virulent. This may cause a synergistic interaction between the viruses resulting in very severe and pronounced symptoms in the host plant. Synergistic interactions have been observed in mixed geminivirus infections (Vanitharani *et al.*, 2004) and have been a major contributor to the epidemic in Uganda; the effect has also been demonstrated in test plants in the greenhouse (Pita *et al.*, 2001). Our results and further studies of CMG will help develop new approaches for cassava protection against this serious problem.

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