J Gen Plant Pathol (2004) 70:243–248
DOI 10.1007/s10327-003-0119-y

VIRAL AND VIROID DISEASES

Short communication

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Variations and taxonomic status of begomoviruses causing severe epidemics of cassava mosaic disease in Kenya, Uganda, and Democratic Republic of the Congo

Received: May 9, 2003 / Accepted: December 17, 2003

Abstract The molecular variability in the DNA-A of cassava-begomoviruses, *East African cassava mosaic virus* (EACMV), East African cassava mosaic virus – Uganda variant (EACMV-UG), *African cassava mosaic virus* (ACMV), and *East African cassava mosaic Zanzibar virus* (EACMZV) in Kenya, Uganda, and Democratic Republic of the Congo (DRC) was investigated. Most samples from western Kenya, Uganda, and eastern DRC contained EACMV-UG. Fewer than half of the samples had a mixed infection of ACMV and EACMV-UG, and a small percentage had only ACMV infections. EACMV and EACMZV were the only begomoviruses detected in samples from the Kenyan coast. The coat protein gene nucleotide (nt) and the deduced amino acid sequence analyses revealed a high degree of sequence identity within each virus type and that EACMV-UG was most related to ACMV. However, analysis of the retrieved complete DNA-A (2781–2801 nt) sequences of selected virus types revealed that EACMV-UG DNA-A share more than 90% identity with EACMV and less than 80% with ACMV, confirming that the virus is a strain of EACMV.

Key words Begomovirus · Cassava · Distribution · EACMV-UG · Taxonomic status

Cassava mosaic disease (CMD) is the most important constraint to cassava (*Manihot esculenta* Crantz) production in Africa, causing an estimated loss of more than US $2.4 billion per annum (Legg et al. 1999). The disease is associated with several whitefly-transmitted geminiviruses belonging to the genus *Begomovirus*. Most begomoviruses have a bipartite genome with A and B components that share only a "common region" (CR) of approximately 200 bp of 90%–100% sequence identity.

The disease has been reported in all cassava-growing countries in Africa and the Indian subcontinent. Six viral species – *African cassava mosaic virus* (ACMV) (Bock and Woods 1983), *East African cassava mosaic virus* (EACMV) (Hong et al. 1993; Swanson and Harrison 1993), *East African cassava mosaic Cameroon virus* (EACMCV) (Fondong et al. 2000), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Zanzibar virus* (EACMZV) (Maruthi et al. 2002), *South African cassava mosaic virus* (SACMV) (Berrie et al. 1997, 2001; Rey and Thompson, 1998) – occur in Africa. In addition, a new virus variant referred to as either Uganda variant (UgV) (Zhou et al. 1997) or a distinctive strain of EACMV (EACMV-UG) (Deng et al. 1997) was identified in Uganda. This virus is highly aggressive and was reported to be moving at a rate of 20 km/year (Gibson 1996; Otim-Nape et al. 1997), devastating cassava fields. The virus is said to have arisen by way of interspecific recombination between ACMV and EACMV in the coat protein (CP) gene (Zhou et al. 1997). The nucleotide sequence of its DNA-A is essentially identical to that of EACMV except that the central 60% of the CP is virtually the same as that of ACMV and only 75% identical to the equivalent EACMV sequence (Zhou et al. 1997). Serologically, EACMV-UG is indistinguishable from ACMV but quite different from EACMV (Were 2001).

Based on observations of infected cassava plants in the field and greenhouse and based on the polymerase chain reaction (PCR) results obtained with cassava begomovirus-specific primers, we report here the distribution and molecular variability in the DNA-A among field isolates of begomoviruses infecting cassava in Kenya, Uganda, and the DRC. Additionally, the taxonomic status of EACMV-UG is elucidated.

Between 1998 and 2001, a total of 161 leaf samples and 98 hardwood stem cuttings (52 from Kenya, 17 from the

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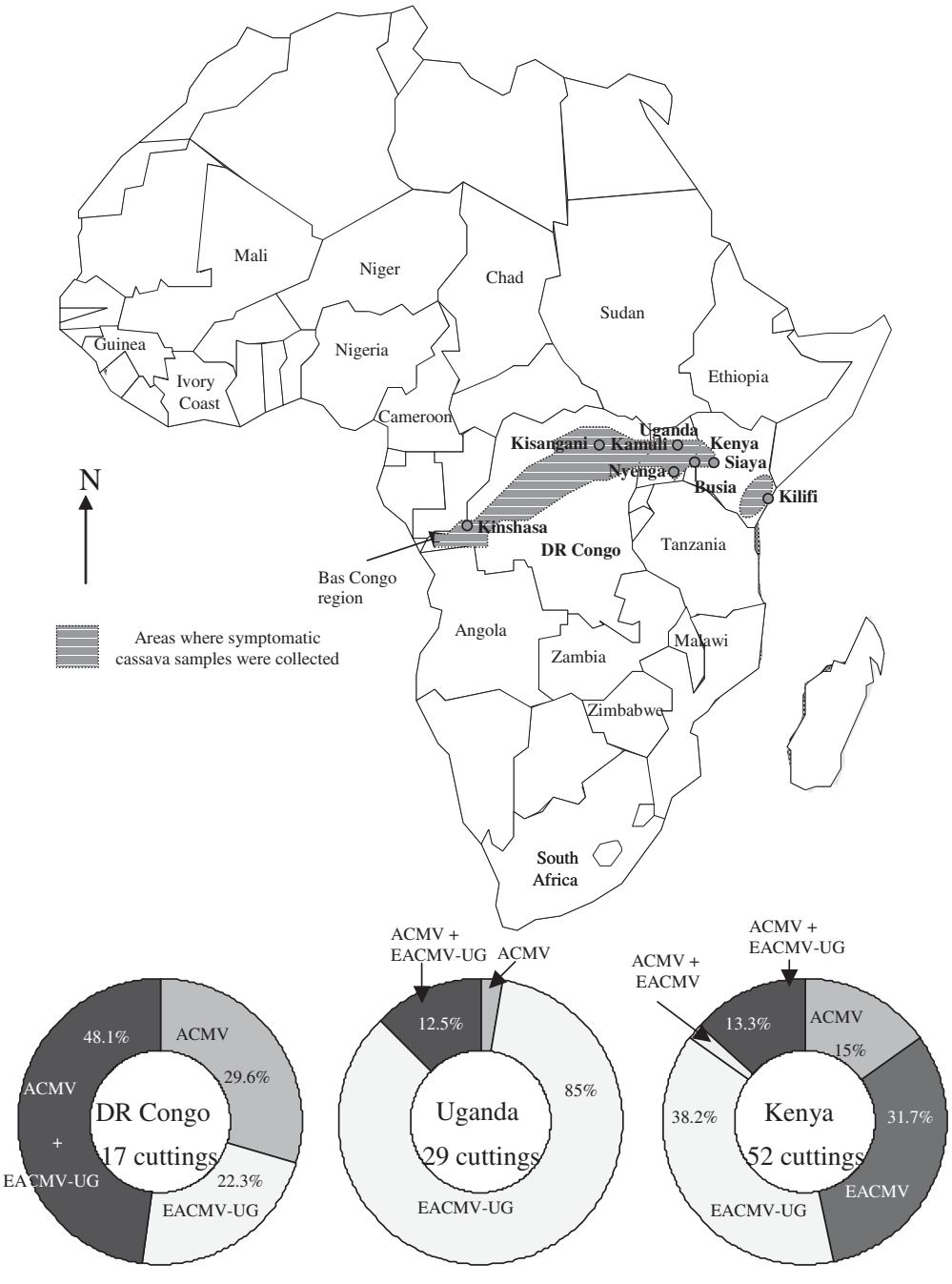

Fig. 1. Map of Africa showing areas where symptomatic cassava samples were collected and pie charts showing country composition of cassava begomoviruses. *ACMV*, *African cassava mosaic virus*; *EACMV*, *East African cassava mosaic virus*; EACMV-*UG*, Uganda variant

DRC, 29 from Uganda) were collected from symptomatic CMD-infected cassava plants growing in Bas Msokongulu/Mboka, Kinshasa, and Kisangani of the DRC; Bungoma, Busia, Kakamega, Luanda, Siaya, Suba, Teso, and Vihiga of western Kenya; Kwale and Kilifi from coastal Kenya and Busiu, Kamuli, Ngiya and Nyenga of eastern and central Uganda (Fig. 1). The cuttings were potted and maintained in an insect-free greenhouse at 25°–27°C with a 16h light period.

Total DNA was extracted from young symptomatic leaves essentially as described by Dellaporta et al. (1983). For identification, five primer pairs [ACMV AL1/F// ACMV ARO/R (Zhou et al. 1997) specific for ACMV; EACMVT588//UV Eco cp specific for EACMV; Ca122fl A Bams//Ca122fl A Bamc specific for EACMZV; UV AL1/F1//UgV 1129 specific for EACMV-UG; and BEGOMO 146 and BEGOMO 672 general for all cassava and most begomoviruses] (Were 2001) were used. The four cassava begomoviruses (ACMV, EACMV, EACMZV, EACMV-UG) were detected in samples from Kenya, where 38.2% of the samples tested positive for EACMV-UG, 15.0% for ACMV, 31.7% for EACMV, 13.3% for mixed infections of EACMV-UG and ACMV, and 1.7% for mixed infection of ACMV and EACMV. EACMV and EACMZV were the only begomoviruses detected on the coast of the country, but a few samples from western Kenya also had EACMV.

Although most samples tested positive for EACMV-UG, ACMV was previously known to be the predominant virus in western Kenya. No EACMV was detected in samples collected from Uganda, where 85.0% of the samples were infected with EACMV-UG and only 12.5% had a double infection with ACMV and EACMV-UG. The situation in the DRC was different: 29.6% of the samples had ACMV, 22.3% had EACMV-UG, and 48.1% had a mixed infection of ACMV and EACMV-UG; no sample from the DRC tested positive for EACMV (Fig. 1). The presence of a large number of samples with mixed infection (ACMV + EACMV-UG) in the DRC shows that the epicenter of EACMV-UG has rapidly shifted from Uganda to the DRC. The rapid virus movement in the DRC could be attributed to the transport of infected cassava cuttings by people displaced by war. At the virus front, now located in western DRC, a large number of tested samples had a mixed infection of ACMV and EACMV-UG, indicating a recent invasion by EACMV-UG of an area originally colonized by ACMV (Were 2001).

Coat protein genes of the respective viruses or strains were amplified essentially as described by Zhou et al. (1997) using two primer pairs (UV Bam CP//ACM Xba for ACMV and UV Bam CP//UV Eco cp for both EACMV and EACMV-UG); full-length DNA-A components were amplified using two overlapping abutting primer pairs (ACMV/F A//ACMV/R A for ACMV, Ca122fl A Bams// Ca122fl A Bamc for EACMZV, and UV/EA/F A//UV/EA/R A for EACMV and EACMV-UG) in a touchdown PCR utilizing a mixture of proof-reading polymerase (TaqPlus Precision; Stratagene, La Jolla, CA, USA) and *Taq* polymerase (1:15) as described by Were (2001). PCR products of CPs and of full-length DNA-A components were purified and cloned into pBluescript II SK-vector (Stratagene) at compatible ends and at the unique *Bam*HI site, respectively. The generated CP clones as well as those of full-length (Ca002, Ca011, Ca024, Ca068, Ca070, Ca103, Ca122, Ca127, Ca185) (Table 1) were sequenced by a commercial company (Sequiserve, Vaterstetten, Germany).

Cassava begomovirus CP and DNA-A sequences were phylogenetically compared to DNA-A of other cassava begomoviruses (GenBank and EMBL) using the multiple sequence alignment application of DNAMAN version 4.0 (Lynnon Biosoft, Quebec, Canada) and the full optimal sequence alignments and neighbor-joining method options of Saitou and Nei (1987) with 1,000 bootstrap (Felsenstein 1985) replications. Percentage DNA-A nucleotide (nt) and open reading frame (ORF) amino acid sequence identities between virus isolates were calculated using the distance between all pairs of sequences in the multiple alignments. Cassava begomovirus DNA-A sequences used for comparison as well as their database accession numbers are as follows: ACMV – [Cameroon] (ACMV-CM; AF112352); ACMV – [Kenya] (ACMV-KE; J02057, GEIG); ACMV – Kenyan isolate (ACMV-KE2; AF001467); ACMV – [Nigeria] (ACMV-NG; X17095, GENIG); ACMV – [Uganda Mild] (ACMV-UGMld; AF126800); ACMV – [Uganda Severe] (ACMV-UGSvr; AF126802); *East African cassava mosaic Cameroon virus* (EACMCV; AF112354); EACMV – [Kenya–K2B] (EACMV-KE-K2B; AJ006458); EACMV – [Malawi] (EACMV-MK; AJ006460); EACMV – [Tanzania] (EACMV-TZ; Z83256); EACMV – [Uganda] (EACMV-UG1; Z83257, CVUV39, EACMV-UG1a; Z83254, CVC70); EACMV – [Uganda2Severe] (EACMV–UG2Svr; AF126806); EACMV – [Uganda2Mild] (EACMV–UG2Mld; AF126804); and SACMV – [South Africa] (SACMV-ZA; AF155806). The sequence of *Cowpea golden mosaic virus* – [Nigeria] (CPGMV-NG; AF029217) was used as an outgroup.

Table 1. Begomovirus isolates selected for genetic analysis, their geographical origin, and virus species/variant affiliation as determined from the respective DNA-A genomic component

Virus isolate DNA-A	Geographical origin	Virus affiliation
Ca002	Siaya, Kenya	EACMV-UG[KE]
Ca068	Kisangani, DRC	EACMV-UG[CDR]
Ca070	Kisangani, DRC	EACMV-UG[CDR]
Ca185	Kisangani, DRC	EACMV-UG[CDR]
Ca127	Nyenga, Uganda	EACMV-UG
Ca024	Busia, Kenya	ACMV
Ca011	Kinshasa, DRC	ACMV[CDR]
Ca103	Kilifi, Kenya	EACMZV-[KEKil]
Ca122	Kilifi, Kenya	EACMV

Detailed analysis of the aligned CP nucleotide (nt) sequences revealed a varying degree of sequence identity grouping at the 5′-terminal, at the core, and at the 3′-terminal regions. Altogether, 222 nt at the 5′-terminal and 88 nt at the 3′-terminal sequences of EACMV-UG and EACMV were highly identical, with an invariable sequence element present in both sequences. In contrast, the core region (approximately 470 nt) of EACMV-UG CP was identical to that of ACMV. Translation of the CP nt sequence of various viruses into putative proteins revealed a high similarity in the 257 deduced amino acids in ACMV, EACMV-UG, and EACMV sequences. Key amino acid sequence motifs (blocks) provided evidence of recombination in the coat protein gene. In the first block, ACMV sequences were well separated from those of EACMV and EACMV-UG. The middle block comprised 17 invariable amino acids shared between ACMV and EACMV-UG, which indicated the putative recombination site. There was a unique amino acid motif for EACMCV whose affiliation was not known (data not shown). Phylogenetic analysis of the CPs split the isolates into three types. Isolates Ca185, Ca002, Ca068, Ca070, and Ca127 were clustered with the EACMV-UG type; Ca011 and Ca024 were clustered with the ACMV type; and Ca103 and Ca122 were with the EACMV type (Fig. 2).

Clustal W alignments revealed a high degree of nucleotide sequence identity: more than 97% within the ACMV type, about 96% within the EACMV type, and 99% within the EACMV-UG type. The clusters formed defined groups, with the ACMV and EACMV-UG types, respectively, falling into one group (with 89% bootstrap support) that was well separated from that of EACMV (with 75%–77% bootstrap support) and SACMV (with 75% bootstrap support). Interestingly, EACMV-MK fell into the SACMV type

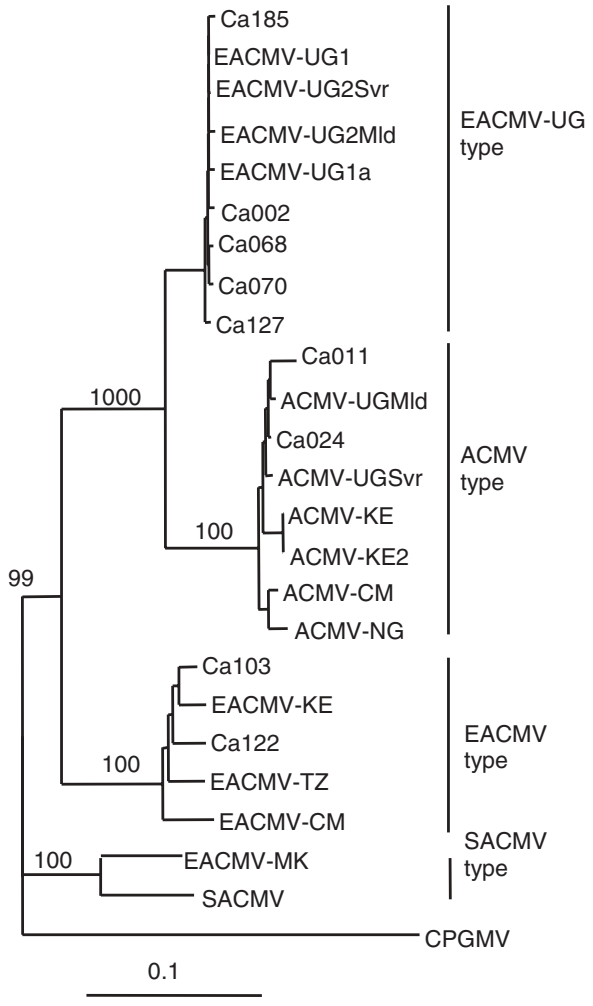

Fig. 2. Phylogenetic tree constructed from nucleotide sequence alignments of coat protein genes of cassava begomoviruses. The coat protein gene of *Cowpea golden mosaic virus* (*CPGMV*) was used as an outgroup. *Mld*, mild; *TZ*, Tanzania; *CM*, Camaroon; *KE*, Kenya; *Svr*, severe; *NG*, Nigeria; *Mk*, Malawi; *SACMV*, *South African cassava mosaic virus*

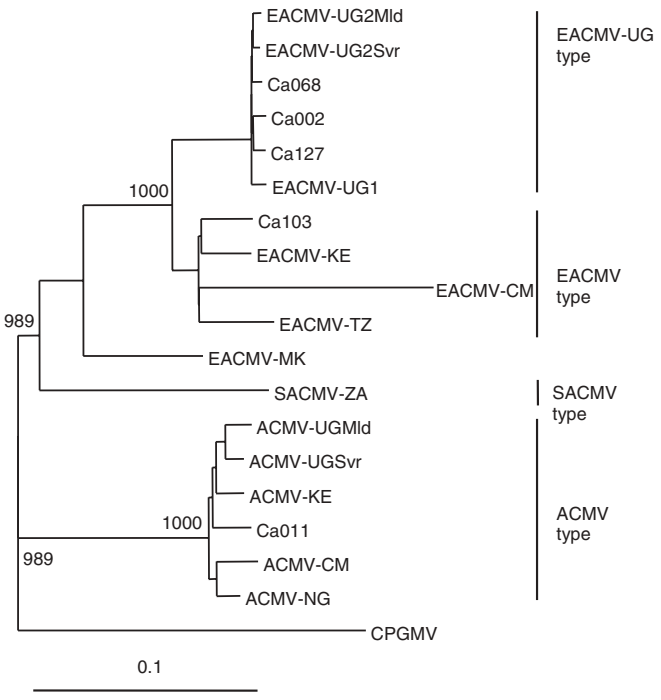

Fig. 3. Phylogenetic tree constructed from nucleotide sequence alignments of DNA-A genomic components of cassava begomoviruses. DNA-A of *Cowpea golden mosaic virus* (*CPGMV*) was used as an outgroup

cluster but not into that of the EACMV type. Overall, the EACMV type was more diverse than either the ACMV type or the EACMV-UG type. Phylogenetic analysis of the deduced 257 amino acids comprising the CP revealed an identical tree structure (data not shown), with EACMV and SACMV amino acid sequences forming a cluster well separated from that of ACMV and EACMV-UG. This provided further evidence that the EACMV-UG CP is more closely related to ACMV than to EACMV or SACMV.

Analysis of the complete DNA-A (2781–2801 nt) sequences showed that EACMV isolates, including SACMV and EACMCV, were clustered with those of EACMV-UG (Fig. 3). However, there was a higher percentage sequence identity (approximately 99%) within EACMV-UG isolates than between EACMV-UG and EACMV (up to 92%). Moreover, these begomoviruses shared less than 80% identical nucleotide sequences with ACMV isolates. Interestingly, ACMV isolates from the same geographical location had higher percentage nucleotide sequence identity than those from different geographical locations. For instance, ACMV isolates from East Africa (ACMV-KE) were clearly separated from those of West Africa (ACMV-NG).

A detailed comparison of a EACMV-UG clone Ca002 DNA-A ORFs with those of other cassava begomoviruses is shown in Table 2. The virus, EACMV-UG1 is the reference virus isolate first described as the Uganda variant virus (Deng et al. 1997). The isolate together with Ca068 and Ca127 had high percentage nucleotide and amino acid sequence identities (>98%) in all the five ORFs compared. However, when the virus was compared with Ca011, ACMV-UG-Svr, or other ACMV isolates, there was a strikingly high nucleotide and amino acid sequence similarity among the CP genes only. A comparison of Ca002 with EACMV isolates (e.g., Ca103, EACMV-KE) revealed a reverse situation. There was higher sequence similarity in all ORFs across DNA-A than among the CP genes. Because the sixth ORF [AC4, which lies within but is not part of AC1 (Rep)] was not present in EACMV or EACMV-UG, it was excluded from the comparative analysis. Generally, sequence analysis revealed that three virus species and one strain (ACMV-KE, EACMV, EACMZV, EACMV-UG) were predominantly present in cassava in East and Central Africa.

The finding that the CPs of EACMV-UG isolates from different regions have a high degree of nucleotide and amino acid sequence identity (99% and 98%, respectively) is an indication that the CPs of these isolates are highly conserved compared to those of other cassava begomoviruses. This substantiates the findings of Pita et al.

Table 2. Comparison of nucleotide and deduced amino acid sequences of Ca002 DNA-A and its respective ORFs with those of other begomoviruses from cassava

Virus isolate	Nucleotide sequences (%) identity		Amino acid sequences (%) similarity				
	DNA-A	IR-A	Coat protein	Rep AC1	TrAP AC2	REn AC3	Pre-Coat AV2
Ca068[a]	**98.9**	**98.8**	**99.6**	**98.6**	**97.8**	**99.3**	**99.0**
Ca127[a]	**98.9**	**98.8**	**99.6**	**99.2**	**97.8**	**97.0**	**98.0**
Ca103[a]	92.4	93.9	89.5	**96.7**	94.1	94.0	**97.0**
Ca011[a]	73.3	64.4	91.4	69.5	65.7	71.6	56.4
EACMV-UG1	**98.5**	**95.1**	**100**	**98.6**	**98.5**	**97.8**	**100**
EACMV-UG2Svr	**99.0**	**98.5**	**100**	**98.3**	**98.5**	**99.3**	**100**
EACMV-TZ	91.2	91.1	89.1	**96.3**	91.9	91.0	**95.0**
EACMV-MK	86.6	88.1	82.9	94.2	91.9	86.6	58.8
EACMV-UG2Mld	**98.9**	**98.8**	**100**	**98.9**	**98.5**	**99.3**	**100**
EACMV-KE	92.4	94.5	89.5	**96.4**	93.3	92.5	**98.0**
EACMCV	84.0	88.6	88.7	91.8	57.8	63.2	93.8
ACMV-UGSvr	73.4	64.2	93.4	69.7	66.4	72.4	57.4
ACMV-NG	74.5	63.7	92.6	69.5	67.9	70.9	57.4
ACMV-KE	74.6	63.8	92.2	69.5	64.9	70.9	59.6
ACMV-CM	73.0	64.6	93.0	68.9	64.2	65.7	58.5
SACMV-ZA	79.1	83.6	81.7	80.4	91.9	91.0	58.8
ACMV-UGMld	73.8	64.0	93.4	69.2	65.7	70.9	56.4
ACMV-KE2	73.4	63.9	92.2	69.5	64.9	70.9	59.6

ORFs, open reading frames
[a] Refer to Table 1

(2001), who analyzed a limited number of virus isolates from one region (Uganda). Furthermore, the EACMV-UG CP analysis results showed that the isolates are more related to ACMV (89%) than to EACMV (77%). However, on analysis of the full-length DNA-A component, the reverse situation was revealed: EACMV-UG shared 92% nucleotide sequence identity with EACMV compared to less than 80% with ACMV. If the list of criteria for demarcating geminivirus species in the Seventh Report of the International Committee on Taxonomy of Viruses (ICTV), which requires that viruses with more than 90% CP sequence identity be considered the same species, is followed EACMV-UG would be a strain of ACMV. Furthermore, because it is becoming increasingly clear that interspecies recombination plays a major role in geminivirus diversity (Galvao et al. 2003; Padidam et al. 1999) the ICTV criterion is misleading and inaccurate as far as naming this virus strain is concerned. Therefore, based on the analysis of the complete DNA-A genomic component of EACMV-UG, which revealed a high percentage (>90%) sequence identity with those of EACMV, an unequivocal argument for the taxonomic position of this virus as a strain of EACMV and not that of ACMV has been provided.

In conclusion, as observed with differential PCR tests and the identified sequences of viral genomes or portions thereof, the Uganda variant virus might be a recombinant virus with a predominant EACMV DNA-A genomic component that has a CP chimera formed by insertion of an ACMV segment in the core region. Following the convention for naming geminiviruses (Fauquet et al. 2000, 2003), the species name, EACMV, must precede the country of origin, hence EACMV-UG.

Acknowledgments We sincerely thank Prof. Dr. Y. Takanami and Dr. Lina Katul for their useful discussions and advice. We also thank Prof. Dr. P.O. Ayiecho for his guidance during sample collection in Kenya and Mr. Pierre E. Bolonge for providing infected cassava samples from the DRC. This work was funded in part by the German Academic Exchange Service (DAAD).

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