Euphytica **120:** 115–125, 2001.
© 2001 *Kluwer Academic Publishers. Printed in the Netherlands.*

Genomic and biological diversity of the African cassava geminiviruses

J.S. Pita[1,2], V.N. Fondong[3], A. Sangaré[2], R.N.N. Kokora[1,2] & C.M. Fauquet[1*]
[1]*International Laboratory for Tropical Agricultural Biotechnology (ILTAB) / Donald Danforth Plant Science Center, UMSL / CME-M308, St Louis MO 63121-4499, U.S.A.;* [2]*Université de Cocody, Laboratoire de Génétique, 22 BP 582 Abidjan 22, Côte d'Ivoire;* [3]*Cornell University, Dept. of Plant Pathology, 3150 Plant Science, Ithaca, NY 14853, U.S.A.; (*author for correspondence, e-mail: iltab@danforthcenter.org)*

Key words: African cassava mosaic disease, geminivirus, synergism, trans-replication

Summary

The virological situation of cassava in Africa is increasing in complexity due to the number and types of viruses isolated from different locations within the continent. Here, we report the complete nucleotide sequences of both A and B components of two geminivirus species infecting cassava in the Ivory Coast and review the current knowledge of the molecular and biological diversity of the African cassava geminiviruses. As a whole, newly obtained sequences are compared with those of the African cassava mosaic geminiviruses identified to date. Results indicate that all isolates of *African cassava mosaic virus* (ACMV), irrespective of their geographical origin are clustered together with little or no variation in their genomic sequence. On the contrary, the genomes of the *East African cassava mosaic virus* (EACMV) are more genetically diverse due to the frequent occurrence of recombinations within their two components. Indeed, the EACMV-like viruses vary so much that their classification is becoming problematic. In addition, there is also a large range of phenotypic symptom variation for each of these virus species, irrespective of the location of isolation. Furthermore, it has been shown that ACMV and EACMV can be synergistic in cassava, resulting in a greater DNA accumulation and consequently inducing severe symptoms. For all these reasons, this paper initiates a discussion concerning the species demarcation for cassava geminivirus.

Introduction

Cassava (*Manihot esculenta* Crantz) is the primary food crop in sub-Saharan Africa. A serious constraint to cassava production is African cassava mosaic disease (ACMD), which is considered as the most important crop disease in the continent (Fargette et al., 1988). Three geminivirus species belonging to the genus *Begomovirus*, family *Geminiviridae*, are responsible for ACMD. These include *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and a newly identified virus named *South African cassava mosaic virus* (SACMV) (Berrie et al., 1997). These geminiviruses possess two DNA molecules called DNA-A and -B. DNA-A encodes all viral proteins necessary for replication and encapsidation of both components (Rogers et al., 1986; Towsend et al., 1986; Sunter et al., 1987), while the DNA-B component encodes for two proteins necessary for efficient systemic spread of the virus throughout the plant (Brough et al., 1988; Etessami et al., 1988; Von Arnim et al., 1993; Ingham et al., 1995). The two DNAs share a 'common region' (CR) which is approximately 200 base pairs (bp) in size and has 90–100% sequence homology between the A and B components.

Until 1994 ACMV and EACMV (Tanzania-like) were the main geminiviruses known to infect cassava in Africa. They were considered to be limited to specific geographical regions, with ACMV and EACMV occurring respectively to the west and east of the African Rift Valley (Swanson & Harrison, 1994). Over the last six years, several emerging strains and/or species of cassava geminiviruses have been identified in different regions of the African continent. Deng et al. (1997), Zhou et al. (1997) and Pita et al. (2000) identified an EACMV strain, named EACMV-UG2 (UgV), which has been confirmed as a recombinant between ACMV and EACMV and which has been associated

with the severe epidemic of cassava mosaic disease in Uganda since 1986. Likewise, Fondong et al. (1998) isolated an EACMV-like virus (EACMV-CM) from infected cassava plants in the Cameroon, while a virus similar to EACMV-CM was described from isolates from the Ivory Coast (Pita et al., 1999). In 1997 Berrie et al. (1997) also identified and confirmed a new geminivirus, know as SACMV infecting cassava in South Africa.

Evidence that EACMV-CM was a double recombinant capable of synergism with ACMV was shown by Fondong et al. (2000). More recently, a new virus EACMV-UG3 was identified in Uganda (Pita et al., 2000). Its DNA-B was reported to be always associated with EACMV-UG2 DNA-A with these two molecules being most prevalent wherever the epidemic occurred in Uganda. Their association with the severe cassava mosaic disease epidemic was obvious although no explanations for the advantage of the heterologous combination EACMV-UG2 DNA-A / EACMV-UG3 DNA-B, over the preexisting homologous association EACMV-UG1 DNA-A / EACMV-UG1 DNA-B, could be provided.

This paper describes the complexity of the current African cassava mosaic geminiviruses as they are known at this time. We present the pattern of their distribution by indexing the variations within their genome structure, describe some biological features of their infection and propose criteria for species demarcation of cassava geminiviruses.

Material and methods

Virus sources

Infected cassava cuttings were collected in the southern region of the Ivory Coast between the towns of Bonoua and Dabou and from the savannah area in the central part of that country around Yamoussoukro, Bouaké and Dabakala in the August 1999. Sampling was carried out as described by Pita et al. (2000). The cuttings were transported to the USA, and planted in a controlled growth room and maintained at 24 °C with a 16-hour daylength at the Danforth Plant Science Center, St. Louis, MO.

DNA extraction and polymerase chain reaction (PCR)

Young leaves were removed for DNA extraction 30 days after striking the stake cuttings. Total DNA was extracted from the leaf tissue by the method of Dellaporta et al. (1983). Nucleic acids obtained were characterized by PCR using the conditions and primers JSP001, JSP002, and JSP003 as described by Pita et al. (2000). The full-length DNA-A and -B of ACMV-IC was amplified using the primers JSP004, JSP005, JSP008, JSP009 (Pita et al., 2000), while primers, VNF003, VNF004, EB03, EB04 (Fondong et al., 2000) were used to amplify full-length molecules of EACMV-CM/IC DNA-A and -B.

Sequences determination and analysis

PCR products were recovered, cloned and sequenced as described by Pita et al. (2000). Sequences obtained were compared using the cluster option of multiple sequence alignment (MegAlign program) with DNASTAR package (Wisconsin, Madison) for the Apple Macintosh computers and by a cladistic parsimony method using the computer program PAUP 3.1.1 (Swofford, 1993). One hundred bootstrap replications were performed to place confidence estimates on the groups contained in the tree.

The GenBank accession numbers of the published cassava geminivirus DNA-A and -B sequences used in this paper are as follows: ACMV-CM (AF112352, AF112353); ACMV-KE (J02057, J02058); ACMV-NG (X17095, X17096); ACMV-UG/Mld (AF126800, AF126801); ACMV-UG/Svr (AF126802, AF126803); EACMV-CM (AF112354, AF112355); EACMV-KE (AJ006458); EACMV-MW/K (AJ006460); EACMV-MW/MH (AJ006459); EACMV-TZ (Z83256); EACMV-UG2/Mld (AF126804); EACMV-UG2/Svr (AF126806); EACMV-UG1 (AF230375); EACMV-UG3 (AF230374, AF126807); SACMV (AF1555806, AF1555807).

Results

Characterization of the Ivorian cassava infected samples

A total of 62 cassava samples, 29 from the southern Dabou-Bonoua region and 33 from the central part around Yamoussoukro, Bouaké and Dabakala, were analysed for the presence of geminiviruses. Using primers specific to the coat proteins of ACMV and EACMV (Pita et al., 2000), we detected virus in 94% of the cassava samples tested. Of these, 54% were found to be infected with ACMV alone and 40%

Table 1. Comparison of nucleotide sequences of selected African cassava mosaic geminiviruses

	AC-CM	AC-IC	AC-KE	AC-NG	AC-UG	EA-CM	EA-IC	EA-UG1	EA-UG2	EA-TZ	EA-UG3	SACMV	ICMV
AC-CM	–	96 (92)	95 (93)	96 (95)	96 (93)	60 (37)	60 (43)	– (42)	68 (40)	63 (40)	– (42)	68 (40)	61 (41)
AC-IC	91	–	96 (93)	98 (93)	97 (93)	60 (37)	61 (43)	– (41)	68 (42)	64 (41)	– (43)	69 (41)	62 (43)
AC-KE	90	91	–	96 (91)	97 (93)	60 (38)	61 (43)	– (45)	68 (40)	64 (41)	– (43)	68 (40)	62 (40)
AC-NG	92	94	92	–	96 (90)	60 (38)	61 (42)	– (43)	68 (44)	64 (44)	– (46)	69 (43)	62 (43)
AC-UG	91	92	94	93	–	60 (41)	61 (44)	– (45)	68 (42)	64 (41)	– (46)	69 (42)	62 (42)
EA-CM	33	33	32	32	32	–	96 (90)	– (84)	81 (93)	84 (84)	– (56)	66 (66)	57 (40)
EA-IC	33	32	33	32	32	91	–	– (85)	82 (90)	85 (82)	– (47)	66 (60)	58 (46)
EA-UG1	33	32	32	32	33	64	64	–	– (90)	– (90)	– (65)	– (70)	– (45)
EA-UG2	–	–	–	–	–	–	–	–	–	91 (85)	– (60)	75 (68)	59 (41)
EA-TZ	–	–	–	–	–	–	–	–	–	–	– (65)	– (71)	59 (46)
EA-UG3	32	32	32	31	32	63	63	96	–	–	–	76 (80)	– (40)
SACMV	32	32	33	32	32	62	62	83	–	–	90	–	62 (38)
ICMV	27	27	27	27	27	27	28	28	–	–	27	27	–

(Numbers) correspond to percentage of nucleotide sequence homologies between the respective CR. Numbers in the upper and the lower parts of the matrix correspond respectively to the percentage of nucleotide sequence homologies between selected A and B components. AC-CM = ACMV-Cameroon; AC-IC = ACMV-Ivory Coast; AC-KE = ACMV-Kenya; AC-NG = ACMV-Nigeria; AC-UG = ACMV-Uganda; EA-CM = EACMV-Cameroon; EA-IC = EACMV-Ivory Coast; EA-UG1 = EACMV-Uganda 1; EA-UG2 = EACMV.

to have a mixed infection of EACMV and ACMV. EACMV-CM/IC was therefore found to be present only in association with ACMV-IC. This mixed infection was equally distributed throughout the regions visited. Disease symptoms were usually extremely severe on the plants containing the mixed infection (ACMV and EACMV) compared to those singly infected with ACMV in both the greenhouse and field conditions.

Sequence analysis and comparison

Analysis of all the available nucleotide sequences of African cassava geminiviruses is presented in Table 1. Following Padidam et al. (1995), the criteria utilised to distinguish between different viruses were; 90%–100% for isolates, 80%–90% for strains and less than 80% for species demarcation. Due to the number of genetic recombinations now known to occur among cassava geminiviruses, we did not consider individual ORFs sequences as a criteria for the establishment of cassava geminivirus species demarcation (Figure 1), but instead, used sequences of the complete genome A and B. The CR sequences were considered for this analysis due to their importance for geminivirus replication.

The DNA-A molecules

Analysis of the complete DNA-A molecule sequences indicated that EACMV-CM/IC is nearly identical to EACMV-CM with 96% of sequence homology. The lowest percentage homology between EACMV-CM/IC and EACMV-CM with respect to the different ORFs was 96%. The AC2 and AC3 gene showed 98% and 97% of sequence homology respectively, confirming that EACMV-CM/IC and EACMV-CM posses the same recombination in the AC2-AC3 region (Fondong et al., 2000; Pita et al., 1999). As expected, ACMV-IC DNA-A was similar in nucleotide sequence to all ACMVs known to date. Taking into account all the sequences selected, apart from the *Indian cassava mosaic virus* (ICMV) which was included as an out-group, we can distinguish the three known ACMD causing species: ACMV, EACMV and SACMV. However, the percent identity between some EACMVs is very low, with that between EACMV-CM/IC and EACMV-TZ or between EACMC-IC/CM and EACMV-UG at 85% and 82% respectively. Despite the degree of similarity, EACMV-CM (including EACMV-CM/IC), EACMV-TZ, EACMV-KE, EACMV-UG1, EACMV-UG2, EACMV-MW (including K and MH isolates) can be differentiated as strains of the EACMV species (Figure 1).

Apart from the AC2 and AC3 gene sequences, for which SACMV presents a high homology (93%–95%) with the EACMVs, the rest of its DNA-A molecule is very different in nucleotide sequence to that of the other cassava geminiviruses. With only 60% and 75% of sequence identity with ACMV and EACMV

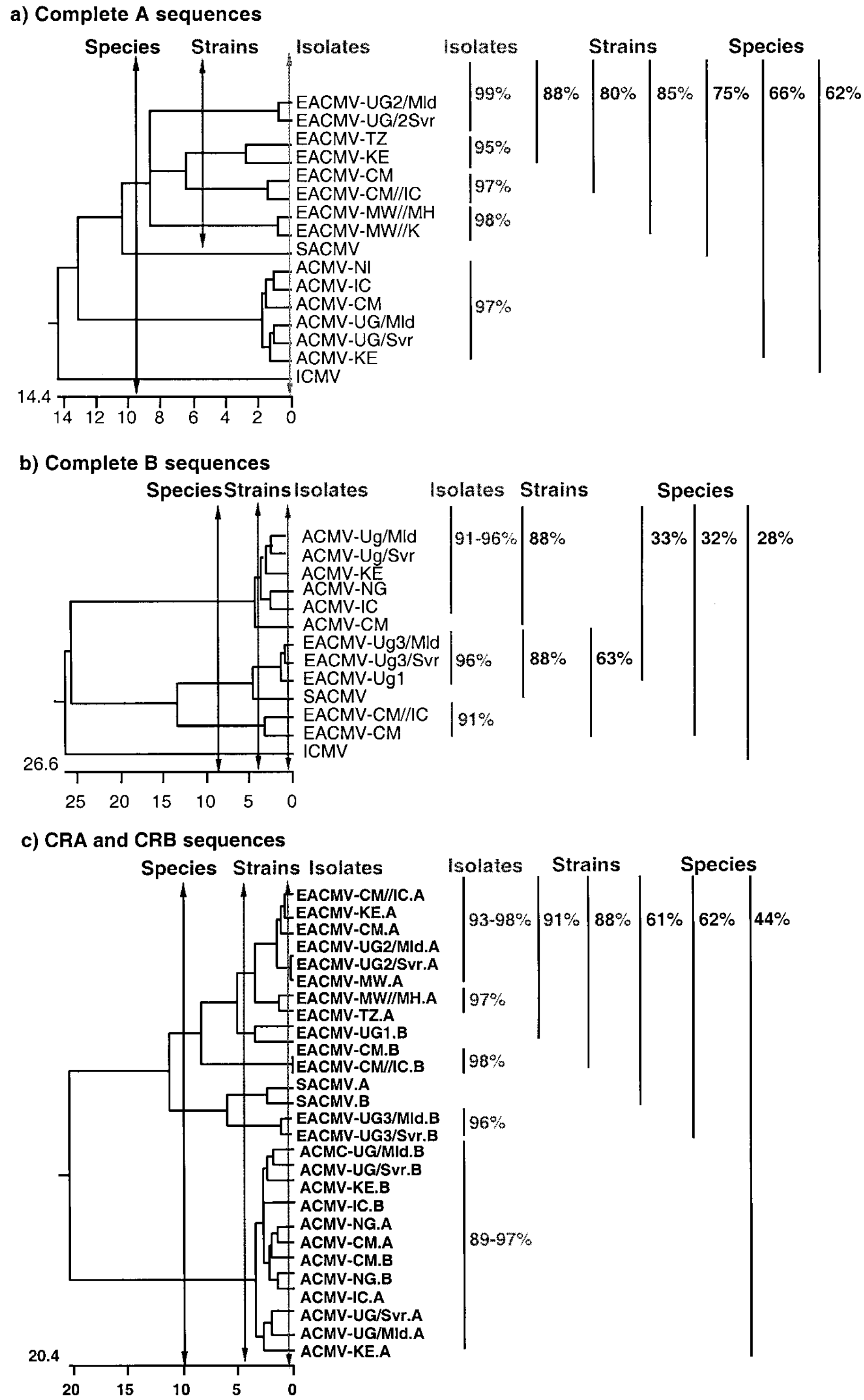

Figure 1. Determination of species amongst geminiviruses infecting cassava. SACMV and either EACMV-CM or EACMV-CM/IC clustered into different groups when either DNA or DNA B sequences were used for sequence comparison.

respectively, it can be concluded that SACMV is definitively a separate species (Berrie et al. 1997).

The DNA-B molecules

Analysis of B component sequences allowed the same conclusions as those described above to be made concerning the viruses isolated in the Ivory Coast. EACMV-CM/IC and EACMV-CM were found to be nearly identical and ACMV-IC, as expected, is shown to be similar to all other ACMVs known to date. However, SACMV which was considered as a different species according to the A component analysis clustered here with the EACMVs. Moreover, SACMV is 83% to 90% identical to EACMV-UG1 or EACMV-UG3. Its BC1 and BV1 gene are 93% to 95% identical to that of EACMV-UG1 and EACMV-UG3. In contrast, EACMV-CM (including EACMV-CM/IC) which was classified as strain of EACMV according to the A component analysis, appeared here as a new species different from ACMV and EACMV due to very low percentage of identity with ACMV and the EACMVs ($\leq$ 64%).

The common region

The CR contains various features characteristic of begomoviruses and presents a high sequence homology ($\geq$ 90%) between the A and B components. When the CRs of the known cassava geminiviruses are analyzed, we can distinguish the three distinct species, ACMV, EACMV and SACMV with EACMV-UG3 DNA-B being shown as a strain of SACMV (Figure 1) relative to the CR. Three types of iteron sequences were found. The type EACMV: GGTGG-AAT-GGGGG, the type SACMV and EACMV-UG3: GGGGG-AAW-GGGGG and the type ACMV with only one iteron unit, GGAGA.

Recombination among cassava geminiviruses

Recombination is considered to be one of the driving forces for virus evolution (Padidam et al., 1999). Cassava geminiviruses provide prime examples of this phenomena with recombination occurring in both DNA-A and -B molecules. Figure 2 presents maps of known recombinations and the different types of variation within cassava geminivirus genomes in Africa. Recombination events are important because they create significant variations in the cassava geminiviruses genomes. That complicate the classification and nomenclature of the cassava geminiviruses. The first known inter-species recombination between two geminiviruses was described in 1997 in Uganda between ACMV and EACMV (Deng et al., 1997; Zhou et al., 1997). Since that time, several other examples have been identified in the region AC2-AC3 of EACMV-CM (and EACMV-CM/IC), acquired from an as yet undetermined virus (Fondong et al., 2000). Evidence of recombination in the BC1 gene was described between EACMV-CM and EACMV-UG3 (Fondong et al., 2000) while a similar event as also suggested to have occurred in the CR of EACMV-UG3 between EACMV and an unknown virus (Pita et al., 2000). Finally, EACMV-MW presents a double recombinant site in the AV1 and AC1 regions. Today, only a partial sequence of EACMV-UG3 DNA-A has been determined. We have not found a B component for EACMV-UG2 since it seems that the A component of this virus is always present and therefore acting in trans-replication with the B component of EACMV-UG3 (Pita et al., 2000).

Trans-replication among cassava geminiviruses

Cassava geminiviruses are known to undergo recombination, a phenomenon facilitated by common occurrence of mixed infections in the field (Pita et al., 2000). Survival of the recombined molecules depends on several factors among which is their ability to replicate. Trans-replication, whereby the A component of a given geminivirus is capable of replicating a B component of a heterologous virus, offers an additional possibility of survival for new molecules and thereby provides another source of viral biodiversity. Natural trans-replications between EACMV-UG2 DNA-A and EACMV-UG3 DNA-B or between ACMV-UG DNA-A and EACMV-UG3 DNA-B were reported in Uganda with epidemiological implications (Pita et al., 2000). For as yet unknown reasons, the association of EACMV-UG2 DNA-A with EACMV-UG3 DNA-B was favored over the homologous combination of EACMV-UG1 DNA-A and -B. This has resulted in the disappearance of EACMV-UG1 from Uganda and an associated increase in the severity of cassava mosaic disease symptoms in that country (Pita et al., 2000). Evidence for trans-replication between EACMV-CM DNA-A and ACMV-CM DNA-B and between EACMV-CM DNA-A and EACMV-UG3 DNA-B has also obtained in tobacco protoplasts in our laboratory (data not shown).

Synergistic interaction between ACMV and EACMV

Synergism between ACMV and EACMV has been demonstrated between EACMV-CM and ACMV-CM

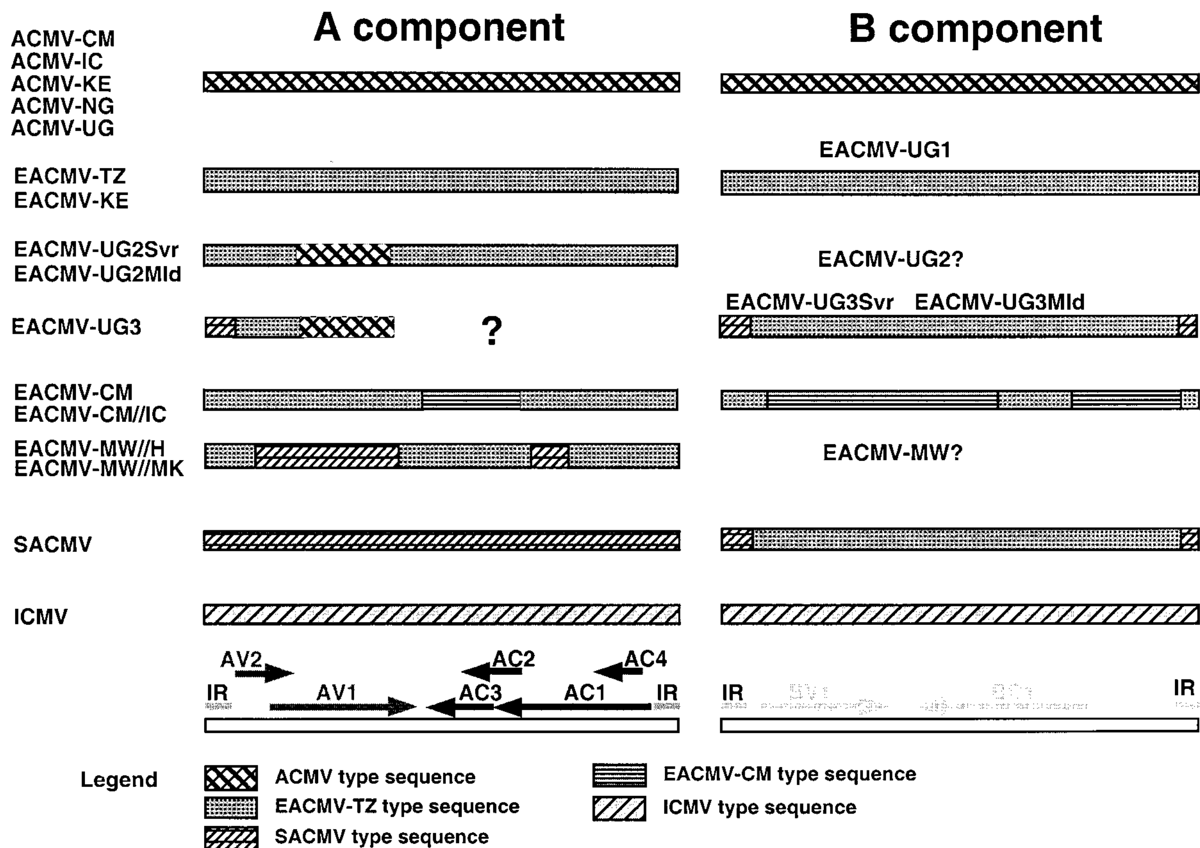

Figure 2. Schematic representation of variation amongst genomes of geminiviruses infecting cassava. Blocks with similar shading represent regions of high homology.

(Fondong et al., 2000) and between EACMV-UG2 and ACMV-UG (Pita et al., 2000). In both cases, double infections resulted in very severe symptoms. Likewise, symptoms were usually extremely severe on the plants detected with a mixed infection of ACMV-IC and EACMV-CM/IC compared to those singly infected by ACMV-IC (Figure 3). Ten *N. benthamiana* plants were mechanically inoculated using the sap extracted from leaves of cassava plants with such mixed infections. Results showed that irrespective of the order of inoculation, the inoculated plants displayed different levels of symptom severity. PCR results from these tissues indicated that plants presenting very severe symptoms were infected with both ACMV-IC and EACMV-CM/IC, while plants with mild symptoms were singly infected by ACMV. None of the *N. benthamiana* plants were found to be infected by EACMV-CM/IC alone. This synergistic interaction between EACMV-CM/IC and ACMV-IC was foreseeable because of their nucleotide sequence homology with ACMV-CM and EACMV-CM respectively.

Host range

Host range is an important criterion that can be used to differentiate geminiviruses species (Van Regenmortel et al., 1997). While ACMV-KE, ACMV-UG, ACMV-IC, EACMV-CM and EACMV-CM/IC can be transmitted easily and repeatedly to *Nicotiana benthamiana* plants, difficulty in mechanical transmission and multiplication of EACMV-UG (Pita unpublished results) and SACMV (Berrie et al., 1997) in *N. benthamiana* appeared to place them biologically in a different group as identified by Harrison et al. (1986).

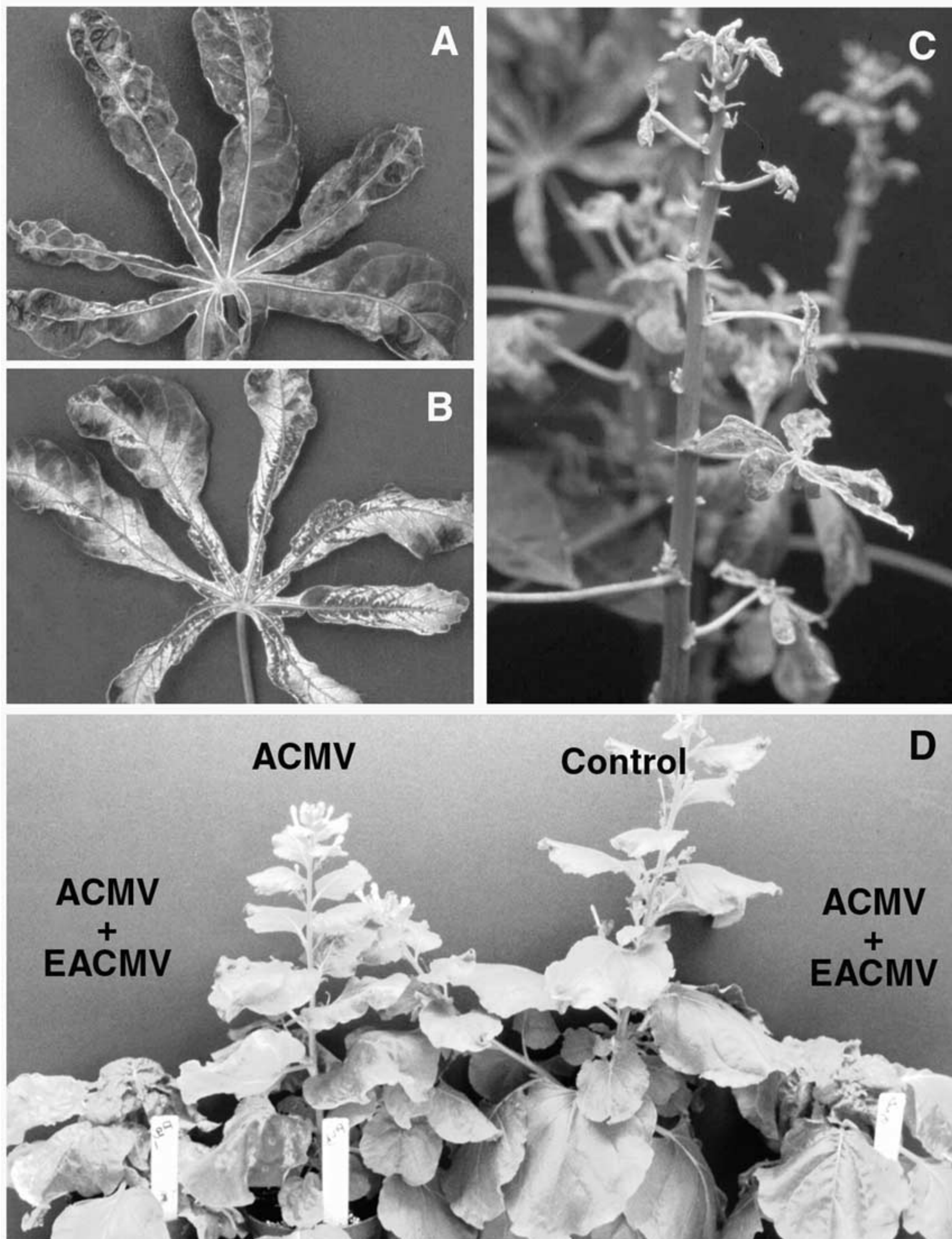

Figure 3. Viral symptoms on cassava and *N. benthamiana* plants. (a) CMD symptoms on a leaf from a plant infected by ACMV. (b) CMD symptoms on a leaf from a plant infected by EACMV. Chlorosis on leaves from plants infected by EACMV is of a brighter yellow color compared to that of leaves infected with ACMV. (c) Defoliation of a shoot from a cassava plant infected by both EACMV-IC and ACMV IC. (d) CMD symptoms on *N. benthamiana* plants mechanically inoculated with sap from a plant infected by both ACMV-IC and EACMV-IC. Plants displaying very severe symptoms (severe stunting, chlorosis, leaf epinasty) were infected by both ACMV-IC and EACMV-IC. Plant infected by ACMV alone displayed very mild symptoms (slight reduction in height when compared to mock inoculated plants).

Symptoms expression on cassava and N. benthamiana *plants*

A biological consequence of the molecular diversity of the cassava geminiviruses could be the variability of the cassava mosaic disease (CMD) symptoms on infected plants (Figure 3). These symptoms can vary between different regions of the same country or depending on the virus isolate, strain and species. In Uganda, different strains of ACMV and EACMV have been identified in different ecological zones (Pita et al., 2000). ACMV-UG/Mld was found along the Lake Victoria in the southern part of the country while ACMV-UG/Svr was located in the highlands of the Northwestern region. Mild and severe CMD symptoms were also observed on cassava plants of the same cultivar infected by different strains of EACMV-UG2 DNA-A and EACMV-UG3 DNA-B. In the Ivory Coast and Cameroon as well as in Uganda, plants containing mixed infections of ACMV and EACMV were characterized by extremely severe and completely systemic symptoms that occasionally resulted in defoliation. Irrespective of the strains, symptoms produced by ACMV and EACMV (-CM, -CM/IC, and -UG) were distinguishable by the color and degree of chlorosis observed on infected leaves. Cassava plants infected with EACMV displayed a brighter yellow colored mosaic patterns on the leaves.

Leaves of ACMV infected *N. benthamiana* plants usually remain green during the time of the infection (except for plants infected by ACMV-KE) and plants start to recover at approximately 20 to 25 days after inoculation. EACMV infected plants never recovered. In the case of EACMV-CM/IC the bright yellow chlorosis is restricted to the veins.

Discussion

In an earlier publication, Pita et al. (1999) reported that PCR characterization using CP-specific primers confirmed the presence of ACMV and EACMV in cassava plants grown in the Ivory Coast. Here we provide the complete nucleotide sequence components A and B of ACMV-IC and EACMV-CM/IC and compare them to other African cassava mosaic geminivirus sequences known at this time.

ACMV-IC was found to be similar (>90%) to all other ACMV isolates which are very homogeneous in their genome sequence. However, as for other EACMV geminivirus EACMV-IC (EACMV-CM/IC) was found to contain recombination events. EACMV-CM/IC was nearly identical to the double recombinant isolated in Cameroon (EACMV-CM) (Fondong et al., 2000) with 96% and 91% of homology respectively between their DNA-A and -B molecules. In addition, EACMV-CM/IC and EACMV-CM posses the same recombined fragment in the AC2-AC3 region and in the BC1 gene. EACMV-CM/IC was detected with the same frequency in the south and the central part of the Ivory Coast and was always present as a mixed infection with ACMV-IC. Although EACMV-IC has yet to be identified in another West African countries, Cameroon and the Ivory Coast are separated by four different countries (Nigeria, Benin, Togo and Ghana), where we would predict EACMV-CM/IC to be present and therefore to be widespread in West Africa as a whole. Although a geographical distribution of geminiviruses had been established by Harrison et al., in 1986, the actual distribution of the African cassava viruses is no longer geographically distinct. For example ACMV is found in all parts of the continent and EACMV is now found in West Africa in addition to East Africa.

In order to classify geminiviruses, several types of criteria should be taken in account (Van Regenmortel, 1998) including the host range, type of transmission vector and genomic sequences. As far as we know, all cassava geminiviruses are transmitted through the same type of vector, the Aleyrodidea *Bemisia tabaci*, although precise data concerning the mode of transmission and the nature of the natural whitefly populations is not available. There is also little information for hosts other than cassava and *N. benthamiana*. Plants infected with ACMV recover much better from infection than those infected with EACMV, although this has yet to be fully documented. We could categorize EACMV-CM and EACMV-CM/IC as members of separate EACMV strains because of their aptitude to be easily and repeatedly mechanically transmitted to *N. benthamiana.* This is opposite to the case for other EACMV isolates and SACMV for which it is difficult to achieve infection of *N. benthamiana*. Despite our findings that EACMV and ACMV can produce differing mosaic symptoms in the greenhouse we consider that symptoms apparition on cassava leaves induced by African cassava geminiviruses is not a reliable classification criteria. Indeed, we have identified strains of ACMV and EACMV (Pita et al., 2000) that can induce a range of symptoms varying from very mild to extremely severe cassava plants in the field and in the greenhouse. To compare viruses causing symptoms

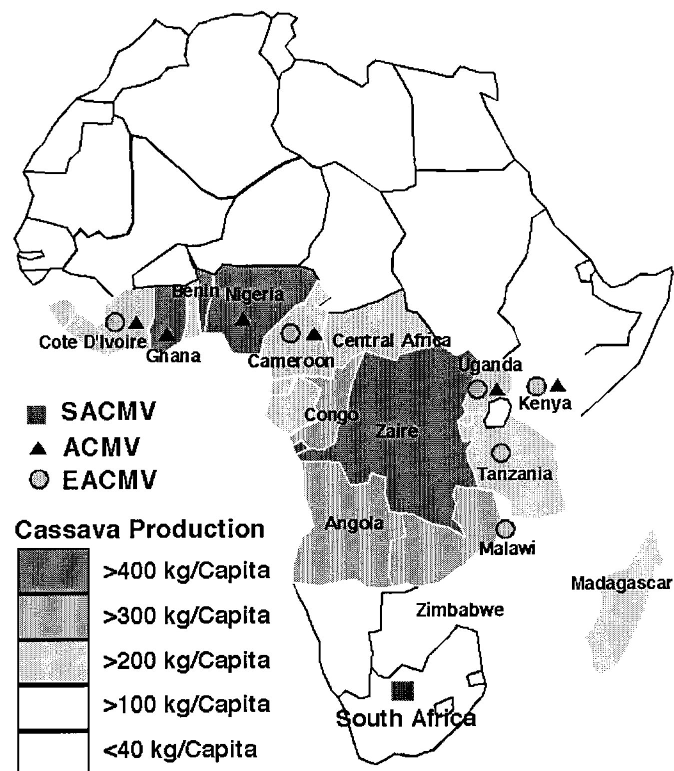

Figure 4. Distribution of the cassava production in Africa and geographical repartitioning of the African cassava geminiviruses known to date.

in different countries, under different climatic conditions and infecting a range of cassava genotypes with different degrees of virus susceptibility is therefore inadvisable. The situation is further complicated by the occurrence of mixed infection and synergistic interactions. For example, it is known that synergism occurs between ACMV and EACMV at least into three different countries in Africa, Uganda, Cameroon and the Ivory Coast, leading to a very severe symptomatology (Fondong et al., 2000; Pita et al., 2000).

Consequently, molecular information constitutes the easiest and most reliable source of information for diagnosis differentiation and classification of African cassava geminiviruses. Low percentage nucleotide homology (±60% and ±33% respectively for the A and B components) provides clear-cut evidence for separation of ACMV from the two other cassava-infecting geminiviruses, EACMV and SACMV at the species level. Nevertheless, it still remains difficult to make an appropriate demarcation within different EACMV isolates and also between the EACMVs and SACMV using this method. Sequence analysis of the CR classifies EACMV-CM and EACMV-CM/IC as part of the species EACMV which also includes EACMV-UG1, -UG2 -TZ, -KE, -MW but places SACMV as another species together with EACMV-UG3 (Figure 1c). Similarly, analysis of the complete DNA-A nucleotide sequences of all the cassava geminiviruses gives the same classification, with EACMV-CM and EACMV-CM/IC clustering again with the EACMV species and including EACMV-UG, -TZ, -KE, -MW but this time with SACMV standing alone as a separate species (Figure 1a). When considering the complete DNA-B nucleotide sequences, we obtained a different grouping with the species EACMV now clustering to include SACMV but with EACMV-CM and EACMV-CM/IC becoming members of species distinct from ACMV and EACMV (Figure 1b).

In summary, due to the number of recombination events which have taken place between different species of geminiviruses infecting cassava in Africa, and because of the possibility of trans-replication between components A and B belonging to different species or strains, it becomes impossible to have a consistent classification of these viruses. Clearly a ranking of the molecular criteria should be established to generate a

unique classification. The criterion of trans-replication between A and B molecules does not sound logical as it is more and more obvious that it can be achieved by the recognition of the iterons by the N-terminus domain of the replicase associated protein (Rep) (Chatterji et al., 1999; 2000). A single recombination of 300 nucleotides would thus suffice to facilitate trans-replication. The nature of the B component is also confusing as it appears that an A molecule can recruit a B molecule provided there is compatibility between their Rep and their iterons, as it is the case between ACMV and EACMVs and between EACMV-UG2 DNA-A and EACMV-UG3 DNA-B.

Consequently, we propose that the complete DNA-A sequences be used as sole criteria to classify geminiviruses. In the case of cassava geminiviruses there are presently four known species, the *Indian cassava mosaic virus* (ICMV present in India, the *African cassava mosaic virus* (ACMV) present in West and East Africa, the *East African cassava mosaic virus* (EACMV) present in both West and East Africa and finally the *South African cassava mosaic virus* (SACMV) solely present in South Africa at this time (Figure 4).

Acknowledgements

This work was funded by the Rockefeller Foundation and the Institute for Research and Development (IRD), a French public research institute. The authors would like to thank Dr Nigel Taylor for critical reading and correcting the manuscript. This is manuscript DDPSC 00–13. The GenBank accession numbers of the sequences reported in this paper are AF259894, AF259895, AF259896, and AF259897.

References

Berrie, L.C., K. Palmer, E.P. Rybicki & M.E.C. Rey, 1997. A New Isolate of African Cassava Mosaic Virus in South Africa. Proc 3rd Intl Sci Meeting, Cassava Biotechnology Network (CBN III) 26–31 August 1996. Kampala, Uganda.

Brough, C.L., R.J. Hayes, A.J. Morgan, R.H.A. Coutts & K.W. Buck, 1988. Effects of mutagenesis *in vitro* on ability of cloned tomato golden mosaic virus DNA to infect *Nicotiana benthamiana* plants. J Gen Virol 69: 503–514.

Chatterji, A., M. Padidam, R.N. Beachy & C.M. Fauquet, 1999. Identification of replication specificity determinants in two strains of tomato leaf curl virus from New Delhi. J Virol 73: 5481–5489.

Chatterji, A., U. Chatterji, R.N. Beachy & C.M. Fauquet, 2000. Defining sequence parameters that determine specificity of binding of the replication associated protein to its cognate sites in two strains of tomato leaf curl virus. Virology (In press).

Dellaporta, S.L., J. Wood & J.B. Hicks, 1983. A plant DNA mini preparation: Version II. Plant Mol Biol Rep 1: 19–21.

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

Etessami, P., R. Callis, S. Ellwood & J. Stanley, 1988. Delimitation of essential genes of cassava latent virus DNA 2. Nuc Acids Res 14: 1253–1265.

Fargette, D., C.M. Fauquet & J.C. Thouvenel, 1988. Yield losses induced by African cassava mosaic virus in relation to the mode and date of infection. Trop pest Manag 34: 123–133.

Fondong, V.N., J.S. Pita, C. Rey, R.N. Beachy & C.M. Fauquet, 1998. First report of the presence of East African cassava mosaic virus in Cameroon. Plant Dis 82: 1172.

Fondong, V.N., J.S. Pita, M.E.C. Rey, A. DeKochko, R.N. Beachy & C.M. Fauquet, 2000. Evidence of Synergism Between African Cassava Mosaic Virus and a New Double Recombinant Geminivirus Infecting Cassava in Cameroon. J Gen Virol 81: 287–297.

Harrison, B.D., A.M. Lennon, P.R. Massalski, D.J. Robinson & J.E. Thomas, 1986. SCRI (Scottish Crop Research Institute. 1986. Annual Report 1986. Dundee, Scotland. pp. 179–180.

Ingham, D.J., E. Pascal & S.G. Lazarowitz, 1995. Both bipartite geminivirus movement proteins define viral host range, but only BL1 determines viral pathogenicity. Virology 207: 191–204.

Padidam, M., R.N. Beachy & C.M. Fauquet, 1995. Classification and identification of geminiviruses using sequence comparisons. J Gen Virol 76: 249–263.

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

Pita, J.S., V.N. Fondong, A. Sangaré, G.W. Otim-Nape, S. Ogwal & C.M. Fauquet, 1999. Biodiversity of Cassava Mosaic Disease in East and West Africa: Special Cases in Uganda and Ivory Coast. In: Proc 4th Intl Sci Meeting of the Cassava Biotechnology Network, Salvador, Bahia, Brazil. November 3–7, 1999.

Pita, J.S., V.N. Fondong, A. Sangaré, G.W. Otim-Nape, S. Ogwal & C.M. Fauquet, 2000. Recombination, pseudo-recombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. Submitted.

Rogers, S.G., D.M. Bisaro, R.T. Fraley, N.L. Hoffmann, L. Brand, J.S. Elmer & A.M. Lioyd, 1986. Tomato golden mosaic virus A component DNA replicates autonomously in transgenic plants. Cell 45: 593–600.

Sunter, G., W.E. Gardinier, A.E. Rushing, S.G. Rogers & D.M. Bisaro, 1987. Independent encapsidation of tomato golden mosaic virus A component DNA in transgenic plants. Plant Mol Biol 8: 477–486.

Swanson, M.M. & B.D. Harrison, 1994. Properties, relationships and distribution of cassava mosaic geminiviruses. Trop Sci 34: 15–25.

Swofford, D.L., 1993. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1.1. Champaign, IL: Illinois Natural History Survey.

Townsend, R., J. Watts & J. Stanley, 1986. Synthesis of viral DNA forms in *Nicotiana plumbaginifolia* protopasts inoculated with cassava latent virus (CLV): Evidence for the independent replication of one component of CLV genome. Nuc Acids Res 14: 1253–1265.

Von Arnim, A., T. Frischmuth & J. Stanley, 1993. Detection and possible functions of African cassava mosaic virus DNA B gene products. Virology 192: 264–272.

Van Regenmortel, M.H.V., D.H.L. Bishop, C.M. Fauquet, M.A. Mayo, J. Maniloff & C.H. Calisher, 1997. Guidelines to the demarcation of virus species. Arch Virol 142: 1505–1518.

Van Regenmortel, M.H.V., 1998. From absolute to exquisite specificity. Reflections on the fuzzy nature of species, specificity and antigenetic sites. J Immunol Meth 216: 37–48.

Zhou, X., Y. Lui, L. Calvert, C. Munoz, G.W. Otim-Nape, D.J. Robinson & B.D. Harrison, 1997. Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by inter specific recombination. J Gen Virol 78: 2101–211.