DISTRIBUTION OF BEGOMOVIRUSES INFECTING CASSAVA IN AFRICA

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SUMMARY

Cassava mosaic disease (CMD) caused by cassava begomoviruses is the major constraint to cassava production in Africa. To gain an overview of the identities and distribution of viruses causing the disease, 230 leaf samples and 185 hardwood stem cuttings from major cassava growing countries in sub-Sahara Africa were collected and analyzed. Symptoms of diseases caused by different begomoviruses in cassava were indistinguishable, however, the most distinct and outstanding feature of Uganda variant cassava mosaic virus (UgV) infections was the huge reduction in tuber formation by the infected plants. Symptoms in *Nicotiana benthamiana* plants infected with an ACMV isolate from Kenya, ACMV-KE, could be distinguished from those induced by an ACMV isolate from Nigeria, ACMV-NG, as the former induced bright yellow blotches on the leaves of infected plants. *N. benthamiana* plants doubly infected with ACMV and UgV developed the severest symptoms. Serological and molecular analysis of the samples revealed that all three cassava begomoviruses were found in Western Kenya. All samples from the coastal province were infected with EACMV only and no other begomovirus was found in this area. Analysis of samples from other countries showed that ACMV was the only virus found in West Africa, except for EACMV in a few samples from Nigeria, Cameroon and Guinea. UgV was detected in samples from Uganda and Democratic Republic of the Congo (DRC). The movement of this virus and how it possibly displaces ACMV in cassava is discussed.

Key words: Africa, begomoviruses, cassava, cassava mosaic disease, characterization, distribution.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the major food crop in sub-Saharan Africa. Cassava mosaic disease is the most devastating disease of cassava in Africa, causing an estimated annual yield loss of over US$ 1.5-2.3 billion (Thresh *et al*., 1998). The disease has been associated with at least four whitefly-transmitted geminiviruses (WTGs). Three species, *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and *South African cassava mosaic virus* (SACMV) occur in Africa. SACMV is closely related to EACMV but probably resembles more a virus from tomato since a recombination event likely to have been with a tomato virus was detected in its AC1, Rep-gene (Berrie *et al*., 2001). Improved diagnostic techniques have resulted in the identification of other begomoviruses. In Uganda and the neighbouring countries, a new virus variant has been detected and identified. This virus resembles EACMV because most of its genome is derived from EACMV except for the core region of the coat protein gene, which is identical to that of ACMV. The virus has been referred to either as a distinctive strain of EACMV (EACMV-UG) (Deng *et al*., 1997) or as the Uganda variant cassava mosaic virus (UgV) (Zhou *et al*., 1997).

Until recently (Harrison *et al*., 1991, 1995), it was thought that EACMV and ACMV had distinct but largely non-overlapping geographical distributions with ACMV occurring in West, Central and Central Southern Africa, while EACMV was largely restricted to the East African coast, Madagascar, Malawi, Mozambique and Zimbabwe. Recent studies (Gibson, 1996; Ogbe *et al.,* 1996, 1997; Legg *et al*., 1999; Fondong *et al*., 2000) showed that EACMV occurs over a much wider area including Western Kenya, Western Tanzania, Zambia, Nigeria, Togo, Guinea, Ivory Coast and Cameroon. However, epidemiological data for EACMV occurring in West Africa from cassava-growing regions where the virus was found is lacking. The highly aggressive and rapidly spreading UgV has been detected in samples from Uganda, Tanzania, Rwanda and Southern Sudan (Harrison *et al*., 1997; Legg and Okoa-Okuja, 1999). It is worth noting that until now, a begomovirus causing indistinguishable disease on cassava in India, the *Indian cassava mosaic virus* (ICMV) has not been reported from Africa. We report here serological and molecular characters as well as the distribution of viruses causing CMD in Africa.

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MATERIALS AND METHODS

Sample collection. Virus-infected cassava samples used in this study were provided by collaborators and colleagues working in various cassava growing countries of Africa or collected during an intensive CMD survey conducted in Kenya between May and July 1999 (Table 1).

Table 1. Countries, locations and number of CMD-infected cassava samples/cuttings collected from each location.

Country	Location	No. of samples
Democratic Republic of the Congo	Bas Msokomgulu / Mboka	5
	Kinshasa	5
	Kisangani	17
Ghana	Kumasi	4
Guinea	Conakry	8
India	Trivananthuparan	6
Kenya	Bungoma	2
	Busia	6
	Kakamega	4
	Kilifi	10
	Kwale	12
	Luanda	3
	Siaya	6
	Suba	10
	Teso	6
	Vihiga	5
Madagascar	Antananarivo	6
Nigeria	Ibadan	3
	Isumi K.	5
Togo	Boke	11
	Foulaya	7
	Linsan	4
	Sinta	5
Uganda	Busiu	7
	Kamuli	2
	Ngiya	11
	Nyenga	4
Zambia	Chanda	3
	Kalipopo	5
	Ndasemana	3

Glasshouse conditions. Cuttings collected from the field were planted in a glasshouse at the Biologische Bundesanstalt, Braunschweig, Germany. For symptom reproduction under controlled environment, the cuttings were planted in pots containing sterilised Floradur®:sand mixture (3:1 v/v) and kept in an insect-free glasshouse at 25-27°C with a 16 h light period. Plants were regularly observed for the presence of insects particularly mites and were sprayed at weekly intervals with pirimicarb (Pirimor, ICI) or Pyrethrin Spruzit, Neudorff. All viruses were maintained in the original cassava varieties in which they were collected.

Inoculation to *Nicotiana benthamiana*. To determine the severity of symptoms induced by different viruses and/or strains in *N. benthamiana*, plants were inoculated at the 3-5 leaf stage either with sap diluted in 0.1 M phosphate buffer, pH 7.5 and rubbed onto the surface of the first 3 expanding carborundum-dusted leaves or by biolistic bombardment of total DNA extracted from young symptomatic cassava leaves.

ELISA. TAS-ELISA was conducted essentially as described by Thomas *et al.* (1986). For serological differentiation of cassava begomoviruses by TAS-ELISA, a panel of differentially reacting monoclonal antibodies (MAbs) produced previously (Were *et al.,* 2000) was used. Polyclonal antiserum against ACMV was used for coating and the MAbs that reacted specifically with respective viruses (Table 2), for detection.

PCR. Since it was not possible to differentiate cassava begomoviruses, ACMV, UgV, and EACMV in mixed virus infections, a polymerase chain reaction (PCR) approach was followed. Published sequences available at GenBank and the European Molecular Biology Laboratory (EMBL) were compared for DNA-A genomic sequences of ACMV Nigeria (Gen1G), ACMV Western Kenya (Ge1G), UgV (CVUV39) and EACMV Tanzania (EACMVT). We designed some primers and used them together with those designed by Zhou *et al.* (1997) (Table 3) to detect viral DNA-A fragments in infected cassava. Two primers, Begomo 146 and 672, from sequences that are common to all cassava (and most) begomoviruses were included for virus detection purposes. Depending on the virus to be detected, primer combinations shown in Table 4 were used. Total DNA was extracted from young expanding symptomatic cassava leaves essentially as described by Dellaporta *et al.* (1983).

DNA-A fragments of the respective viruses and/or strains were amplified using primers indicated in Tables 3 and 4. One µl of total DNA was subjected to PCR in a reaction volume of 50 µl consisting of 2.5 µl $MgCl_2$, 1 µl (100 pmol.) of each primer, 5 µl of 10x *Taq* polymerase buffer, 1 µl of 25 mM dNTPs, and 0.5 µl (2.5 units) of *Taq* DNA polymerase (Boehringer Mannheim, Germany). The cycling profile was 3 min of denaturation at

Table 2. Differential reactions of four monoclonal antibodies in TAS-ELISA for definition of an African cassava mosaic begomovirus type in an infected plant.

MAb 1C1	MAb 6E9	MAb 4F10	MAb 1H2	MAb 4G7	Virus defined
+	+	+	+	-	ACMV-NG
+	+	+	-	-	ACMV-KE
+	-	+	+	-	UgV
+	-	-	+	-	EACMV
-	-	-	-	+	ICMV

Table 3. Primers used to detect viral DNA-A fragments in cassava samples from Africa.

Primer	Sequence (5′→ 3′)	Strand	Target
ACMV AL1/F [a]	gcggaatccctaacattatc	Sense	AL1
ACMV ARO/R [a]	gctcgtatgtatcctctaaggcctg	Complementary	AR2
EACMVT588U20	cactggtatggtccgatgtg	Sense	CP
UV ECO/CP	gaattcttaatttgtcactgcat	Complementary	CP
UV CP/R [a]	gttacggagcaacatgcaat	Complementary	CP
UV AL1 /F1 [a]	tgtcttctgggacttgtgtg	Sense	AL1
UgV1129L20	cactacaagttacggagcaa	Complementary	CP
Begomo 146	taatattacckgwkgvccsc	Sense	AR2
Begomo 672	tggacyttrcawggbccttcaca	Complementary	CP

[a] Primers designed by Zhou *et al.* (1997).

Table 4. Primer combinations and annealing temperatures used to detect different begomoviruses in cassava samples.

Virion sense primer	Complementary primer	Annealing temperature (°C)	Virus detected
1. ACMV AL1/F	ACMV ARO/R	52	ACMV
2. EACMV T588U20	UV ECO/CP	55	EACMV
3. UV AL1/F1	UgV 1129	52	UgV
4. BEGOMO 146	BEGOMO 672	52	begomoviruses

95°C followed by 35 cycles of: 1 min at 95°C denaturation, 1.5 min at 52°C primer annealing, and 1 min at 72°C strand extension.

RESULTS

Sample collection. In total, 230 leaf samples from the Kenyan survey and 185 hardwood stem cuttings from farmers' fields in countries shown in Table 1 were collected and analyzed. In Western Kenya, Uganda and the DRC, a disease incidence of up to 100% was evident in most farms and there was hardly a farm with a CMD incidence below 50%.

Symptomatology. In cassava, symptoms ranged from barely perceptible mosaic to bright mosaic with mild to severe leaf distortions, extreme reduction in the leaf blades and complete stunting of the plant. Observations made in the field and in the glasshouse indicate that all the three viruses induce similar symptom patterns on the foliage of infected cassava. Symptom severity of CMD in cassava plants that were maintained in the glasshouse over a 2-year period and which were singly or doubly infected with ACMV, EACMV and UgV showed the following: 1) plants infected with ACMV developed mild to severe symptoms and often recovered from the disease, 2) plants infected with either EACMV or UgV exhibited moderate to severe symptoms on a

Table 5. Percent country composition of Begomoviruses infeeting cassava in Africa as per sample analysis.

Country	ACMV	EACMV	UgV	ACMV+UgV	ACMV+EACMV
DR Congo	29.6	0	22.3	48.1	0
Ghana	85.7	0	0	0	14.3
Guinea	75	0	0	0	25
Kenya	15	31.7	38.3	13.3	1.7
Madagascar	75	25	0	0	0
Nigeria	83.3	16.7	0	0	0
Togo	100	0	0	0	0
Uganda	0	0	87.5	12.5	0
Zambia	100	0	0	0	0

continuous basis and rarely recovered from the disease and 3) plants doubly infected with both ACMV and UgV developed symptoms similar to those in plants infected with UgV alone.

Two cassava plants obtained from Kisangani in the DRC in 1998 doubly infected by ACMV and UgV and whose virus status was checked at 6-month intervals, 'lost' ACMV in or around June 2000. Subsequent checks have since failed to trace ACMV in the plants and CMD symptoms can hardly be seen. Some varieties obtained from the field when infected with ACMV and showing CMD symptoms recovered from the disease completely after having been planted in the glasshouse. It is worth noting that some mild isolates of ACMV and EACMV turn severe and vice versa depending on environmental and nutritional conditions of the plant. However, this phenomenon rarely occurred in samples infected with UgV isolates. In addition, most field samples from the DRC, Kenya and Uganda with a double infection of either ACMV and UgV or ACMV and EACMV did not develop severe symptoms. Based on these observations it can be concluded that cassava begomoviruses induce similar, non-distinctive symptoms in host cassava plants and are therefore symptomatically indistinguishable.

In *N. benthamiana*, symptoms of diffuse chlorotic lesions followed by systemic curling, leaf deformation and yellow blotching were observed. Plants infected with EACMV and UgV often developed similar and mild symptoms beginning with systemic curling and crinkling of leaves followed by stunting but failed to develop chlorotic lesions and/or yellow blotching. In contrast, ACMV and ICMV always induced severe symptoms in *N. benthamiana* plants and the symptoms of ACMV-KE and ACMV-NG, though similar in severity, were different in appearance in that ACMV-KE induced early yellow blotching symptoms whereas ACMV-NG induced late and milder yellow blotches in the plants.

ELISA and PCR. All samples received from or collected in several cassava-growing countries of Africa (Table 1) were tested by TAS-ELISA as well as by PCR. For detection and discrimination among cassava begomoviruses, a panel of MAbs, 4F10, 1C1 and 4G7 (Table 2) and the differential primer pair combinations described in (Tables 3 and 4) were used. Results of this study are presented in Table 4 and Fig. 1.

Distribution of ACMV, EACMV and UgV in Africa. Based on ELISA- and PCR- results of samples collected between 1998 and 2001 from Africa, a distribution map of cassava begomoviruses was drawn (Fig. 1). Cassava-

Fig. 1. Map of Africa showing the distribution of cassava begomoviruses in cassava growing countries.

growing regions in Africa range from the savannah zones of the Sahel to the northern regions of South Africa. ACMV was detected in at least one sample from all cassava-growing countries in the continent. EACMV was the only cassava begomovirus detected in samples collected along the coast of Kenya and Madagascar, although there were a few isolated cases of the virus in Western areas of Kenya around Lake Victoria. However, no EACMV was detected in samples obtained from Uganda. EACMV was also detected in samples from Guinea and Nigeria. The recombinant virus UgV was found in samples from Western Kenya, Uganda and the Democratic Republic of the Congo. The virus was most prevalent in the Oriental region around Kisangani, and the Bas Congo area extending from Kinshasa southwestwards towards Republic of the Congo and Angola. This was the Western-most region of Africa where UgV was found in the year 2000.

South African cassava mosaic virus (SACMV) has been reported in South Africa and Swaziland (Berry and Rey, 2001) but with a hitherto undefined epidemiological significance. The authors also analysed samples from Angola, Botswana and Zimbabwe, the results of which are included in Fig. 1.

The distinct species ICMV was not detected in any of the cassava samples from Africa but only in those from India.

DISCUSSION

This is the first report on the presence of UgV in Western Kenya and it confirms reports of other workers (Otim-Nape *et al*., 1997; Gibson, 1996; Legg and Okoa-Okuja, 1999), who based their observations on increased incidence and severity of CMD in the region. From the results, it is clear that UgV has displaced ACMV from Western Kenya and Uganda, and it is threatening to do the same in Eastern DRC. The questions that arise from the disease analyses are: how did UgV displace ACMV and why did it (UgV) spread or why is it spreading so quickly?

Several arguments have been put forward to answer the above questions but experimental evidence is lacking. First, it has been speculated that there is a preferential transmission of UgV by whiteflies. Work done by Briddon *et al*. (1990), Höfer *et al*. (1997) and Noris *et al*. (1998) showed that the geminivirus coat protein is responsible for vector transmission because exchanging amino acids in key positions of the coat protein completely abolished or significantly reduced the efficiency of whitefly transmission. The recombination events in the UgV coat protein gene do not pinpoint the amino acid positions specified for TYLCV transmission (Noris *et al*., 1998). However, since the interaction of the whitefly receptors with the virus capsid appears not to be determined by a motif in the protein (like the DAG involved in aphid transmission of potyviruses) but rather by the composition of a domain, an altered capsid protein structure (through recombination) might result in preferred virus uptake by, or translocation in, the whitefly vector. However, the obvious problems of biotype incompatibility and the feeding preferences of *B. tabaci* render vector/virus transmission studies in cassava almost impossible (Abdullahi, 2001). Another possibility for a selective transmission of UgV from mixed virus infected plants can be highly attributed to the recovery and reversion phenomenon (Pacumbala, 1985; Fargette *et al*., 1988; Rossel *et al*., 1988) observed in cassava plants infected with ACMV. These workers discovered that some cassava varieties have the ability to localise the distribution of cassava begomoviruses so that whole branches or individual shoots may develop free of any detectable virus. This phenomenon was rarely observed in UgV-infected plants, which remained symptomatic almost throughout the 3-year experimental period. The example of two plants obtained from the Democratic Republic of the Congo having a mixed virus infection of UgV and ACMV but that lost ACMV after 18 months, can help explain how UgV displaces ACMV in the field. In a field situation with mixed ACMV/UgV infections, a down-regulation or, uneven distribution of ACMV through recovery and reversion, would leave only UgV to be spread by the whiteflies. Consequently this virus then becomes the predominant virus in the area.

Studies on the distribution of cassava begomoviruses in Africa showed that ACMV occurred everywhere except on the Kenyan coast while UgV was detected in samples from an area stretching from western Kenya and Uganda to the Bas-Congo region of the DRC. DRC is currently regarded as the epicenter of the epidemic with frontier areas. At the virus fronts a large number of samples were infected with both ACMV and UgV and a few plants were infected with ACMV alone but rarely with only UgV, which is an indication of a recent invasion of the area by the UgV. This mixed virus situation seems transient and due to the recovery phenomenon explained above, UgV may remain the single dominant virus in infected cassava after the previously doubly infected plants recover from ACMV. This was already evident in Uganda and the Eastern provinces of the DRC where UgV invasions took place much earlier and the virus has now displaced ACMV in cassava.

It is most likely that the virus has already spread to neighboring countries such as Angola and Congo Brazaville, from where no cassava samples were collected. Since the first association of UgV as the causal agent of the severe form of CMD in Uganda (Zhou *et al*., 1997), the disease has been reported to be spreading at a rate of 20-30 km/year in all directions from the place where it was first diagnosed (Otim-Nape *et al*., 1996). The rapid virus movement is fuelled by human activities such as transport of infected cassava cuttings over long distances like that witnessed in the DRC due to rapid displacement of people by war. If the *status quo* is maintained, UgV is most likely to displace ACMV as the pre-

dominant virus in Africa in a couple of years. In summary, UgV spread in the field is characterized by the invasion by the recombinant virus of areas where ACMV already exists, then mixed virus infections of ACMV and UgV are transiently formed, which finally transforms into UgV-only infections. In this study begomoviruses ACMV, EACMV and UgV were only rarely found outside their traditional areas of distribution. EACMV was distributed along the coast of the continent including Guinea in West Africa, where it was found in only one sample. However, contrary to earlier reports (Legg *et al.,* 1999), EACMV was not detected in samples from Togo. The absence of EACMV in these samples could have been due to the fact that samples were not representative of all cassava growing areas of these countries. A thorough diagnostic survey such as that done in Kenya is recommended in order to assess the presence of this virus in Togo and other countries. Although SACMV has been reported occurring in South Africa and Swaziland (Berry and Rey, 2001), it is highly probable that the virus exists in neighboring countries as well. As with EACMV, a thorough survey using improved diagnostic tools is needed to determine the distribution of this virus in Africa.

The fact that ICMV was detected only in samples from India and not in those from Africa is not surprising given the huge distance between the two continents. Additionally it is very unlikely that, farmers from both continents can share planting material or that viruliferous whiteflies will move from India to Africa and vice versa.

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