Cassava Mosaic Viruses (*Geminiviridae*)☆

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Nomenclature

CMG Cassava mosaic geminivirus
CP Coat protein or capsid protein
CR Common region
dsDNA Double-stranded deoxyribonucleic acid
ELISA Enzyme-linked immuno-sorbent assay
IR Intergenic region
kb Kilobase
kbp Kilobase pair
kDa Kilo Dalton
MP Movement protein
NSP Nuclear shuttle protein
PCR Polymerase chain reaction
PTGS Post-transcriptional gene silencing
RCA Rolling-circle amplification
Ren Replication enhancer protein
siRNA Small interfering RNA
ssDNA Single-stranded deoxyribonucleic acid
TrAP Transcriptional activator protein

Glossary

Nonanucleotide A codon containing nine nucleotides.
Pandemic An outbreak of a disease over several countries or a large part of the world.
Pseudo-recombinant A viral infection involving complementary genome components from different virus species.

Taxonomy

Cassava mosaic viruses belong to the genus *Begomovirus* in the family *Geminiviridae* which comprises viruses with single-stranded circular DNA genomes that are transmitted by *Bemisia tabaci* (Gennadius) whiteflies. The cassava-infecting begomoviruses have two DNA components (DNA-A and DNA-B) of which the DNA-A genome sequences are used for taxonomic assignment of virus species. The species demarcation threshold is at 91% sequence identity, thus virus genomes having less than 91% of nucleotides on DNA-A that are identical to those of their closest neighbor are assigned to a unique species. Eleven cassava mosaic geminiviruses (CMGs) are currently recognized as distinct virus species by the ICTV (See Relevant Websites Section). These are: *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *South African cassava mosaic virus* (SACMV), *Indian cassava mosaic virus* (ICMV), *Sri Lankan cassava mosaic virus* (SLCMV), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Cameroon virus* (EACMCV), *East African cassava mosaic Zanzibar virus* (EACMZV), *East African cassava mosaic Kenya virus* (EACMKV), *Cassava mosaic Madagascar virus* (CMMGV), and *African cassava mosaic Burkina Faso virus* (ACMBFV) (**Table 1**; **Fig. 1**). Several of these originated from recombination events that generated genomic diversities beyond species boundaries. The names given reflect the relatedness to their putative parental virus.

Intraspecific Diversity

New, recombinant cassava viruses described provide evidence for on-going diversification of CMG genomes. Interspecific recombination of DNA-A and DNA-B genome components of cassava viruses also include sequences from other begomoviruses. For example, genome portions of the monopartite Tomato leaf curl virus are present in ACMBFV. Similarly, the genome components of CMMGV include, besides elements from other CMGs, sequences from non-related monopartite begomoviruses infecting tomato in the Indian Ocean islands. Additionally, there is ample evidence for molecular diversity of CMG genomes from complete DNA-A and DNA-B genome sequences of virus isolates available in GenBank. Nevertheless, in spite of contrasting molecular characteristics, there is little evidence for unique biological features of each of the virus species in terms of their symptom phenotypes, natural host range, transmission parameters or the association of particular virus isolates with *B. tabaci* species that would provide additional information for the discrimination of virus strains or the identification of a particular virus species. The 11 acknowledged CMG species and the numerous isolates that are present in Africa and Asia are merely characterized by distinctive sequences of their DNA-A genome components.

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Table 1 Cassava mosaic geminivirus species

Virus	*Reference*	*Genome*	*Occurrence*
African cassava mosaic virus	Bock and Woods (1983)	NC_001467 NC_001468	Africa
East African cassava mosaic virus	Swanson and Harrison (1994)	NC – 004674 NC – 004676	East Africa
Indian cassava mosaic virus	Hong *et al.* (1993)	NC_001932 NC_001933	India
Sri Lankan cassava mosaic virus	Saunders *et al.* (2002)	NC_003861 NC_003862	India, Sri Lanka, Vietnam, Cambodia, China
South African cassava mosaic virus	Berrie *et al.* (1998)	NC_003803 NC_003804	Southern Africa
East African cassava mosaic Cameroon virus	Fondong *et al.* (1998)	NC_004625 NC_004630	East and West Africa
East African cassava mosaic Malawi virus	Zhou *et al.* (1998)	NC_022645 NC_022644	East and southern Africa
East African cassava mosaic Zanzibar virus	Maruthi *et al.* (2004)	NC_004655 NC_004656	East Africa
East **African cassava mosaic Kenya virus**	Bull *et al.* (2006)	NC_011583 NC_001584	East Africa
Cassava mosaic Madagascar virus	Harimalala *et al.* (2012)	NC_017004 NC_017005	Madagascar
African cassava mosaic Burkina Faso virus	Tiendrebeogo *et al.* (2012)	HE616777 HE616778	West Africa

History

Cassava (*Manihot esculenta* Crantz) is a root crop that is grown widely throughout the tropics, primarily for its value as a starchy staple food. From its origins in Latin America, cultivated cassava was introduced to Africa in the sixteenth century, and subsequently spread through much of Africa south of the Sahara. The first cultivation of cassava in Asia occurred in south India in the 19th century, before it spread more widely throughout South-East Asia and eventually as far as Southern China. Although initially used as a food crop, most cassava in Asia is now cultivated for industrial starch production.

None of the viruses infecting cassava in Latin America is known to have been carried to either Africa or Asia, but over time, the crop became infected by indigenous viruses. The first report of a virus-like disease in African cassava was made in 1894 from what is now northeastern Tanzania. The original German descriptor, 'Krauselkrankheit', made reference to the characteristic mosaic symptoms elicited in affected plants. It was not until just over a decade later, however, that the first firm indication was given that the disease had a viral etiology. In spite of these early reports, there seems to have been little concern about the impact of cassava mosaic disease (CMD) until the 1920s. Between 1929 and 1937, however, numerous reports were made of the spread and damaging effect on cassava crops of CMD from diverse locations throughout the continent, from the island of Madagascar off the southeastern shores of the African mainland, to Sierra Leone in West Africa. These developments provided the stimulus for the earliest concerted efforts to develop approaches to controlling the viruses that caused this damaging crop disease. Although substantial progress was made in the development of cassava varieties that were resistant to cassava mosaic-causing viruses in the 1930s and 1940s, the viruses themselves remained poorly understood, and it was not until 1983 that the first definitive study confirming the viral etiology of CMD was published. Geminate virions were shown to encapsidate a bipartite genome of single-stranded circular DNA, leading to the designation of these viruses as geminiviruses in the genus *Begomovirus*. In Asia, symptoms of cassava mosaic disease were first observed from southern India in the 1950s, and subsequently from Sri Lanka in the 1980s. Since 2015, the disease has spread more widely, affecting Cambodia and Vietnam in South-East Asia.

Virion Structure

The two single-stranded circular DNA molecules (DNA-A and DNA-B) of CMGs are encapsidated within a virion having a unique architecture composed of two incomplete icosahedral particles (hemicapsids) fused to form a twinned, geminate particle from a single type of capsid protein. For each icosahedral half, 55 CP chains assemble into 11 capsomers. The near-atomic resolution of the virion structure of ACMV has been resolved by cryo-electron microscopy to 4.2 Å revealing that alternating conformations of

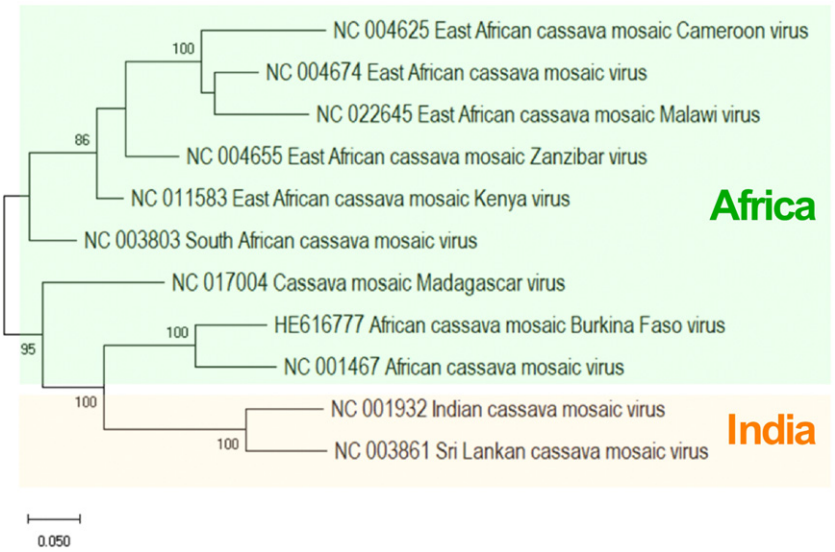

Fig. 1 Estimated phylogeny of DNA-A genome sequences of cassava mosaic geminivirus species within the genus *Begomovirus*, family *Geminiviridae*. The evolutionary history was inferred using the Maximum Likelihood method with the percentage of trees in which the associated taxa clustered together shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The evolutionary analyses were conducted in MEGA X.

the N-terminus of the CP confer stability of the twinned particle and most probably mediate the interaction with the encapsidated DNA (Fig. 2). This was further confirmed by similar studies with Ageratum yellow vein virus, and resolution at an even higher level (3.3 Å) showed that three distinct conformations of CP form the capsid and arrange the hemicapsid interface. The proposed model for geminivirus capsid assembly and DNA binding suggests that genomic DNA is crucial for the formation of the capsids and for directing the assembly of the capsomers and the virus particle. The high resolution model of the ACMV virion structure has also shown the position of amino acid side chains implicated in *B. tabaci* transmission of begomoviruses. The most critical, Q130 and N131, are located on the particle surface and are accessible to interact with vector insect receptors while other residues may influence particle stability and thus have indirect effects on virus transmission.

Genome

The eight genes on the DNA-A and DNA-B genome components are located in sense and complementary sense orientations on the circular covalently-closed single-stranded DNA molecules (Fig. 3). A conserved intergenic region (IR) of ca. 200 bp is shared between both genome components. This unique IR identifies DNA-B to be transreplicated by its cognate DNA-A only. Embedded in the IR is a stem loop comprising the TAATATTAC sequence that is highly conserved among almost all geminiviruses and which represents the origin of replication. While DNA-A can infect and replicate autonomously in a host plant it requires DNA-B for movement and systemic infection of the host. CMG genes are multifunctional and proteins are responsible for encapsidation and for directing the host machinery to provide cellular functions favorable for replication. In virion sense orientation on DNA-A, the structural protein gene CP (AV1) codes for the capsid protein, which is involved in whitefly transmission and transport, and which interacts with host proteins and binds to ss and double-stranded (ds)DNA. A functional CP, however, is not required for infectivity and movement. Thus, DNA-A with a defective or missing CP is still infectious and capable of systemic movement within its host, however its capacity for vector transmission is compromised. Upstream of CP, the AV2 gene also provides movement function. On the complementary-sense strand, the replication initiation protein Rep (AC1) initiates specific steps in DNA replication; TrAP (transcriptional activator protein)(AC2) and REn (replication enhancer protein)(AC3) further downstream of Rep, function as suppressers of post-transcriptional gene silencing (PTGS) and support efficient DNA replication respectively. AC4 is nested within Rep and involved in symptom expression and possibly in counter-defense. Genes on DNA-B enable virus movement within the cell and nucleus (nuclear shuttle protein, NSP)(BV1) as well as sustaining long distance spread and movement within the plant (movement protein, MP)(BC1).

Replication

Geminiviruses replicate in the nuclei of infected cells and depend on host DNA and RNA polymerases for their replication and transcription. Geminiviruses predominantly replicate in the phloem companion and parenchyma cells and some geminiviruses

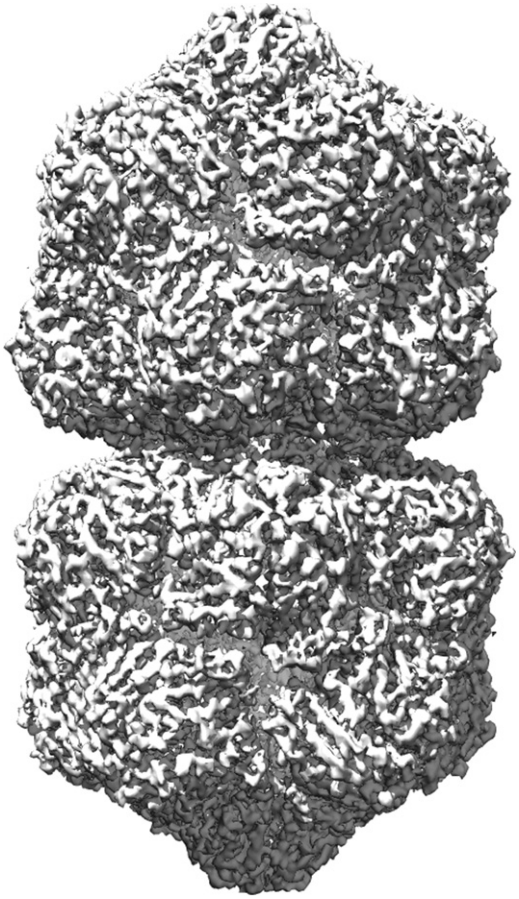

Fig. 2 Structure of African cassava mosaic virus determined by cryoelectron microscopy. Courtesy of Katharina Hipp.

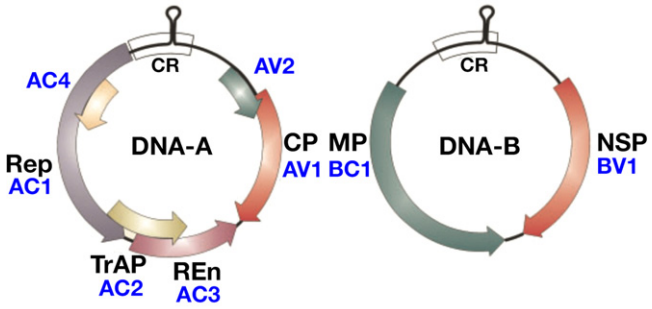

Fig. 3 Genome organization of bipartite cassava mosaic viruses. The proteins encoded by the components are indicated as follows: DNA-A component on the sense orientation encodes for the following proteins: CP or AV1; the coat protein, and the AV2 protein; and on the antisense orientation: Rep or AC1; replication associated protein, TrAP or AC2; the transcriptional activator protein, REn or AC3; the replication enhancer protein, and the AC4 protein. The DNA-B component encodes for the following proteins: NSP or BV1; the nuclear shuttle protein on the sense orientation and MP or BC1; the movement protein on the antisense orientation. The conserved region (CR) is the common region between the DNA-A and DNA-B components. For each component the conserved hairpin structure, containing the nonanucleotide sequence TAATATTAC within the loop structure, is shown at position zero.

can also invade *mesophyll* cells but are never found in meristems. Thus, geminiviruses replicate in differentiated cells. Using host proteins the ssDNA genome is replicated by rolling circle- and recombination-mediated mechanisms in which double-stranded DNA intermediates are formed that are chromatinized with host histone proteins to form viral minichromosomes inside the nucleus. Rep recruits host factors, binds specifically to iteron sequences within IR and creates a nick in the conserved nonanucleotide TAATATT↓AC to initiate DNA replication. Rep also binds to a plant homolog of retinoblastoma protein to catalyze a series of intracellular reactions that move the cell from G to S phase to mobilize host factors essential for DNA replication.

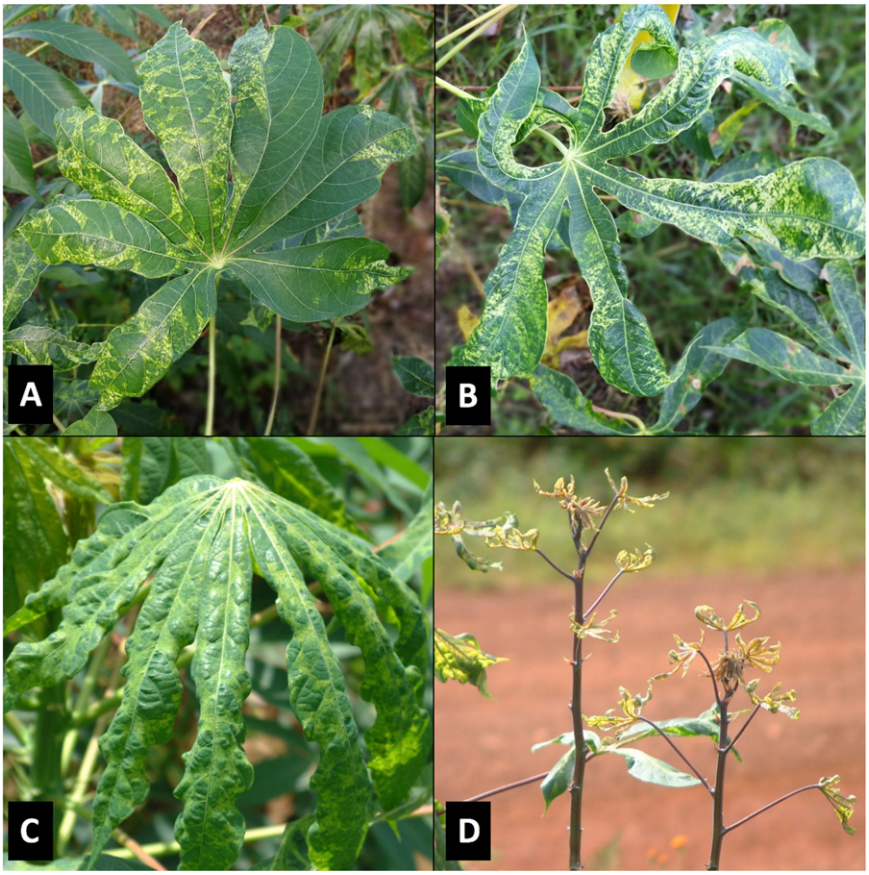

Fig. 4 Cassava mosaic disease (CMD) symptoms: (A) & (B) – Sri Lankan cassava mosaic virus in Vietnam; (C) – African cassava mosaic virus in Gabon; (D) – mixed infection of African cassava mosaic virus and East African cassava mosaic virus-Uganda in Burundi.

Double-stranded DNA (dsDNA) produced in the replication process then assembles under the control of TrAP and REn into transcriptionally active mini-chromosomes. The nuclear export is mediated by NSP, from nucleus to the cytoplasm, and movement out of the cell through plasmodesmata and long distance transport and systemic movement are facilitated by MP.

Transmission

CMGs, in common with all other members of the genus *Begomovirus*, are transmitted by the whitefly vector *B. tabaci*. Transmission can also be achieved through grafting, and relatively inefficiently through mechanical inoculation of indicator plants, but there is no seed-borne transmission. Cassava is normally propagated through the use of vegetative cuttings, and this is perhaps the most frequent source of infection in new crops under field conditions. *Bemisia tabaci* adults feed, mate, and reproduce preferentially on the upper newly-emerged leaves of cassava plants, and almost all transmission occurs here. Transmission is persistent, and ACMV has been shown to be retained by *B. tabaci* adults for up to 9 days. Transtadial, but not transovarial transmission, has been demonstrated, although the larval instars are unimportant in the epidemiology of CMGs in view of their sessile nature. Minimum periods for each of the stages of transmission for ACMV by *B. tabaci* adults are: acquisition (3 h), latent period (3 h), and inoculation (10 min). Inoculated plants begin to show symptoms of infection after 3–5 weeks. Varying levels of transmission efficiency have been reported, ranging from 0.3% to 10%, depending primarily on the nature of the CMG infection in the plants from which virus is acquired. There is evidence for a limited degree of co-evolutionary adaptation between the whitefly vector and CMGs, as Indian whiteflies are significantly better at vectoring Indian than African CMGs and vice versa. However, within Africa, there is currently no indication that whiteflies from a particular location are more efficient at vectoring locally-occurring CMGs than they are at vectoring CMGs from another part of the continent. There is, however, an important balance between the pattern of transmission and the nature of the virus infection. More severe infections, caused either by more virulent virus species/strains or by mixed virus infections, lead to a greater frequency of whitefly-borne infection but diminished propagation through cuttings by farmers. Conversely, more moderate or mild infections, caused by less virulent virus species/strains occurring in single infections, lead to less frequent whitefly-borne infection and increased propagation through cuttings by farmers. This balance dictates the epidemiological characteristics of CMG infection in any given location, area, or region.

Pathogenesis and Symptoms

Following initial infection of a previously uninfected cassava plant by a CMG, viral DNA moves through the phloem to the newly developing phloem and parenchyma cells immediately behind the meristem where rapid multiplication of virus particles takes place. Plants have developed defense responses to virus multiplication through the process of PTGS, but in response, CMG species/strains have developed various effective mechanisms to overcome this, and the degree of this effectiveness seems to be the main factor in determining the severity of disease resulting from infection. Both AC2 and AC4 have been shown to act as suppressors of PTGS. Significantly, in mixed ACMV + EACMV-like virus infections, the responses of the two viruses to host plant PTGS are complementary, leading to a synergistic interaction between the two viruses, greatly increased titers of both, and a concomitant increase in severity of the disease symptoms expressed in the plant. Molecular studies of these interactions have shown that the abundance of short interfering RNA (siRNA) molecules associated with the host plant PTGS response increases over time in pure ACMV infections, yet remains low over similar time periods for ACMV + EACMV-UG co-infections. Synergism has been described for ACMV + EACMCV mixtures in Cameroon, but its significance is greatest in the widely occurring ACMV + EACMV-UG mixed infections that cause severe CMD in many parts of East and Central Africa.

As CMG virions replicate in infected cells, chloroplasts become distorted and reduced in number leading to the appearance of a patchy mosaic of chlorotic leaf portions interspersed with normal green leaf tissue. The chlorotic mosaic patches have discrete, non-diffuse borders, but these may vary greatly in size, ranging from small portions of individual leaflets to the entire leaf (**Fig. 4**). In moderate to severe infections, leaves are distorted and reduced in size and the growth of the plant is stunted. The severity of symptoms is affected by several factors, including the virulence of the CMG strain, the occurrence of mixed virus infection, temperature, host susceptibility and the stage of growth of the plant. Varieties display a full range of responses to CMG infection, ranging from near immunity to extreme sensitivity resulting in plant death. The most frequent cause of severe symptoms is where ACMV and EACMV (or related viruses) occur in mixed infection. This is a consequence of the synergistic interaction between these viruses which leads to increased virus titer of both. There are currently no anecdotal or published records of synergistic interactions between ICMV and SLCMV in South Asia.

Plants infected through the cutting typically express symptoms in the first-formed leaves immediately on sprouting. Plants that sprouted without virus infection, but which were subsequently infected by the whitefly vector express symptoms only in leaves above the point of infection, although there is a latent period of 3–5 weeks between inoculation and the appearance of symptoms. These contrasting patterns of symptom expression make it possible to distinguish plants infected through the cutting from those infected during crop growth by the whitefly vector. Separating these two infection types has become an important facet of field research into the epidemiology of CMGs. Symptoms of CMD caused by ICMV and/or SLCMV in Asia vary in severity, depending on the factors described above, but the characteristics of the symptoms are the same as those of African CMD, as is the distinction between cutting-borne and whitefly-borne infection.

There are currently no reported cases of cassava varieties with immunity to infection by CMGs. However, there are many varieties that are highly resistant, and when these are infected, symptoms expressed are often confined to small portions of a few leaves. A common feature of resistant varieties is symptom recovery, as lower symptomatic leaves drop naturally and new foliage is asymptomatic. This phenomenon is linked to reversion, which is the absence of virus in a proportion of cuttings taken from an infected parent plant. This is thought to be a consequence of the incomplete systemicity of CMGs in resistant varieties.

Epidemiology

Diversity and Distribution

Distribution of the African CMGs

The earliest studies of CMG diversity and distribution used serological techniques to characterize variability, and then utilized that variability to develop diagnostic tests based on the use of monoclonal antibodies in the enzyme-linked immunosorbent assay (ELISA). Using these methods, two principal groups of CMGs were recognized that were subsequently confirmed as distinct species following the sequencing of DNA-A molecules. These were ACMV and EACMV. The earliest distribution maps of the African CMGs showed EACMV to occur in the coastal east African areas of Kenya, Tanzania, and Madagascar, as well as Malawi and Zimbabwe. ACMV, by contrast, occurred throughout the remainder of the cassava-growing areas of Africa, from South Africa and Mozambique in the southeast, to Senegal in the northwest. Significantly, at this time there was no reported zone of co-occurrence of the two virus species. With the increased use of polymerase chain reaction (PCR)-based diagnostics from 1990s onward, it became possible to identify differences not solely associated with the coat protein. This led to two important developments in the understanding of CMGs in Africa. First, several new species were identified, most of which were more closely related to EACMV than to ACMV. Second, it was shown that virus mixtures belonging to different species occurred frequently. A notable consequence of this finding was the concomitant evidence for the more widespread distribution of the EACMV-like viruses than had hitherto been recognized. New virus identifications included: SACMV (1998), EACMMV (1998), EACMCV (2000), EACMZV (2004), EACMKV (2006), ACMBFV (2012), and CMMGV (2012). Significantly, all but one (ACMBFV) of the CMGs occur in different parts of East and Southern Africa, while ACMV predominates in West Africa (**Fig. 5**). EACMCV, the only EACMV-like virus occurring in West Africa, is less frequent and often occurs in mixed infections together with ACMV. ACMV is absent from coastal areas of Kenya and Tanzania. Since there has been very little CMG characterization in many of the cassava-growing countries of Africa, it seems likely

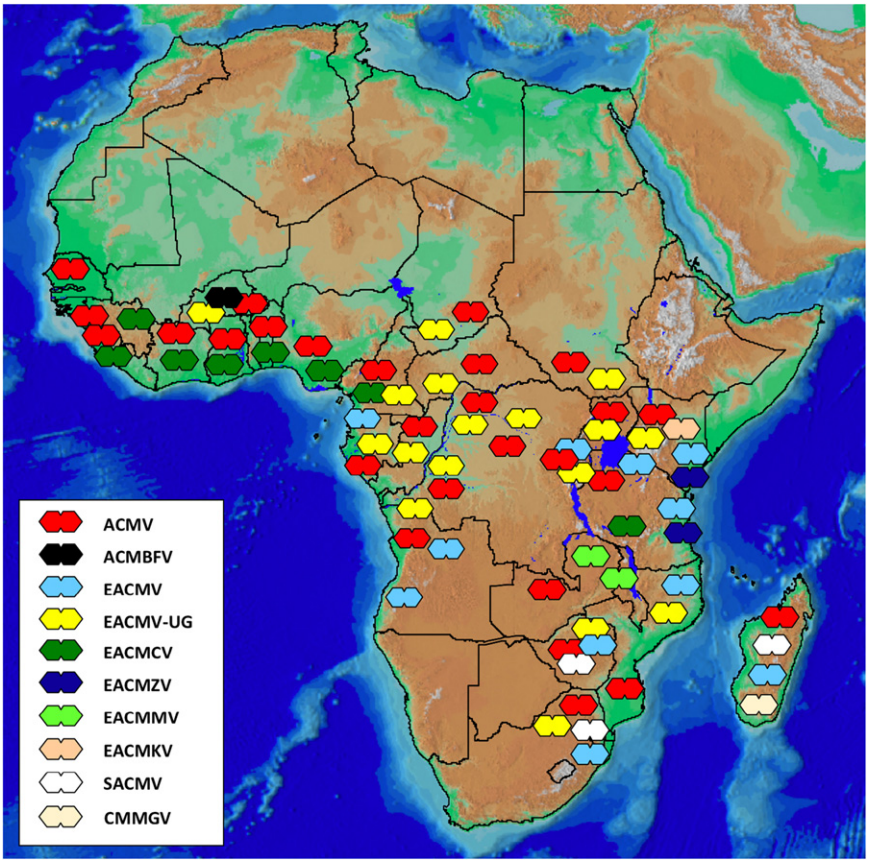

Fig. 5 Distribution of cassava mosaic geminiviruses in Africa: ACMV (African cassava mosaic virus); ACMBFV (African cassava mosaic Burkina Faso virus); EACMV (East African cassava mosaic virus); EACMV-UG (East African cassava mosaic virus-Uganda); EACMCV (East African cassava mosaic Cameroon virus); EACMZV (East African cassava mosaic Zanzibar virus); EACMMV (East African cassava mosaic Malawi virus); EACMKV (East African cassava mosaic Kenya virus); SACMV (South African cassava mosaic virus); CMMGV (Cassava mosaic Madagascar virus).

that further variability within this group of viruses still remains to be revealed. An assessment of the data currently available has led to the conclusion that East Africa is the center of diversity for the EACMV-like CMGs and is probably the home for yet-to-be-identified wild hosts of the proto-CMGs that were first introduced to cassava by *B. tabaci* sometime between the earliest introductions of cassava to this part of Africa in the eighteenth century, and the first report of CMD in 1894.

Distribution of CMGs in Asia

ICMV and SLCMV are considered to be native to South Asia and have been recorded from cassava-producing areas of India and Sri Lanka for several decades. Early serology-based work identified ICMV from southern India. However, following the characterization of SLCMV as the causal agent of CMD in Sri Lanka in 2002, subsequent surveys in India showed that SLCMV was more widely distributed there than ICMV. The CMGs in India have been identified from Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Madhya Pradesh, and West Bengal states, a zone covering all of the major cassava-producing parts of the country. Although it has been suggested that SLCMV is a more aggressive virus than ICMV, and has been expanding its geographical coverage, the absence of systematic surveys conducted over several years means that the evidence for this remains circumstantial.

CMD was reported for the first time from Southeast Asia (SE Asia) from a single location in Ratanakiri Province in Northeastern Cambodia in 2015. The causal virus was SLCMV, and it was assumed that this new outbreak had arisen through the introduction of infected planting material from South Asia. Since that time there has been rapid spread of CMD and associated SLCMV through most of the major cassava-producing areas of Cambodia, as well as southern Vietnam. An isolated report of the disease from Ha Tinh Province in northern Vietnam presents a risk for spread to neighboring Laos, and there are also several reports of CMD in Thailand that have yet to be officially confirmed. The most recent confirmed reports of the occurrence of CMD in Asia come from the southern Chinese regions of Hainan and Fujian, in both cases coupled with positive diagnoses of SLCMV. Rapid spread of SLCMV through SE Asia is thought to be driven by extensive cross-border and intra-regional commercial trade in cassava stems, although there is also clear evidence of local spread through whitefly transmission. The progress of the SE Asian pandemic has also been exacerbated by the extreme susceptibility to CMG infection of virtually all of the cassava varieties currently being cultivated. Given the rapid spread of CMD through this region from 2015 to 2019, it seems likely that new occurrences will be reported from

Laos, Thailand and Myanmar in the near future, and there is also a greatly increased threat to other major-cassava producing countries in the region – Philippines and Indonesia.

Recombination and the CMD pandemic

The CMGs represent a very dynamic group of viruses, and evidence has been presented for the occurrence of both pseudo-recombinants, in which the DNA-A of one virus species co-replicates with the DNA-B of another species, as well as true recombinants, in which portions of the DNA-A or -B of one species have been spliced into the DNA-A or -B of another. In fact, all of the EACMV-like viruses other than EACMV show evidence for recombination events either with known or as yet unknown begomoviruses. One of the most important developments in the study of CMGs in Africa was the recognition that an unusually damaging strain, referred to as EACMV-UG, had arisen through a recombination event between EACMV and ACMV in which a 340 nt portion of ACMV AV1, had replaced the equivalent portion of the DNA-A of EACMV. The consequence of this was that ELISA-based diagnostic tests erroneously identified this strain as ACMV. This is one of the reasons why PCR-based diagnostic methods are now used almost exclusively for CMG monitoring work. EACMV-UG was associated with the rapid epidemic-like spread of CMD in East and Central Africa through the latter part of the twentieth and early 21st century. Countries affected by the severe CMD pandemic included: Angola, Burundi, Cameroon, Central African Republic, Democratic Republic of Congo (DRC), Gabon, Kenya, Republic of Congo, Rwanda, South Sudan, Tanzania, and Uganda. Localized identifications of EACMV-UG have also been made from Burkina Faso, Chad, Equatorial Guinea, South Africa, Swaziland, and Zimbabwe. The dynamic character of CMG diversity and distribution cannot be over-emphasized, and the propensity that this group of viruses shows to produce both naturally occurring pseudo-recombinants, as well as novel true recombinants, ensures that the patterns of diversity and distribution will continue to evolve. It is significant to note, however, that most of the CMG variability occurs amongst the EACMV-like viruses, which show a high propensity for recombination. This contrasts strongly with ACMV, for which all 311 publicly-available accessions have $>94\%$ homology and can be considered to be a single strain of the same species.

Field-Level Epidemiology

Epidemiological studies of the CMGs can be broadly categorized into two groups: those that describe patterns of infection at the field level and those that relate to area or region-wide spread. In the case of the former, external sources of infection have been shown to be most important for determining the rate and quantity of infection in initially CMD-free plantings in the normal field environment. Gradients of infection occur in which new infections are most frequent on the windward sides of cassava crops and these gradients are matched by similar patterns of vector distribution. Multiple regression relationships have been used to describe the association between measures of inoculum pressure and final CMD incidence in test plots. Gompertz curves model patterns of infection increase in plots of CMG-susceptible cassava cultivars, and incidence increases rapidly to 100% over the first 3–6 months of growth. For resistant varieties, however, under similar conditions of inoculum pressure, rates of infection increase are much lower and final incidences typically range from 0% to 50%. Mathematical models have been generated that predict patterns of CMD spread under varied conditions of virus infection and vector abundance. The great majority of epidemiological research has focused on CMD in Africa, although several studies in India have confirmed that patterns of cutting-borne and vector-borne spread of CMGs are largely the same as those observed in Africa.

Regional Epidemiology

Following the earliest 'first colonization' descriptions of African CMD epidemics in the 1920s and 1930s, a few reports were made of rapid area-wide spread of severe CMD at other times during the twentieth century, some of the most notable of which were epidemics in Cape Verde and south-eastern Nigeria in the 1990s. Of much greater importance, however, has been the African CMD pandemic that was first reported from the northern-central part of Uganda in the late 1980s. CMD associated with the epidemic was unusually severe and was rapidly spread by super-abundant populations of *B. tabaci*. During the 1990s it became apparent that the zone affected by this severe CMD was expanding southwards at a rate of 20–30 km per year, and in 1997 molecular studies revealed that the severe disease phenotype was associated with the occurrence and spread of an 'invasive' recombinant CMG, EACMV-UG, commonly in mixed infection with the locally occurring, but now synergized ACMV. Regular monitoring surveys conducted throughout the East and Central African region through the 1990s and early part of the 21st century have used PCR-based diagnostics to map the spread of the EAMCV-UG associated with this 'pandemic' of severe CMD. This work has given rise to first reports of EACMV-UG and resulting severe CMD in ten additional countries in East and Central Africa: Kenya (1996), South Sudan (1997), Tanzania (1999), DRC (1999), Republic of Congo (1999), Rwanda (2001), Burundi (2003), Gabon (2003), Angola (2008), and Cameroon (2010). Significantly, the pandemic 'front' advanced through north-western Tanzania from the Uganda border a distance of c. 400 km between 1999 and 2004. Although the severe CMD pandemic threatened to spread further through southern Africa and westwards to Nigeria, the period post-2010 saw a decline in new reports of spread. Evidence from Uganda in 2019 indicates that EACMV-UG has now largely been replaced by ACMV, although the reasons for this pattern of change are currently unclear. An important contributing factor to this amelioration in the CMD pandemic in Africa is likely to be the increasingly widespread adoption by farmers of CMD-resistant varieties.

Diagnosis

CMD symptoms are very characteristic, although they cannot be used to discriminate between the CMG virus species causing the infection. Additionally, symptoms are almost always expressed in infected plants at some stage during their period of growth, which means that symptoms are widely used as an indication of infection during surveillance activities and by regulatory authorities during certification inspections. The characteristic mosaic pattern of CMD symptoms has also made it possible to use machine learning and artificial intelligence to develop a smartphone app – Nuru AI – which allows untrained users to recognize CMD with a high degree of accuracy. Laboratory-based methods are required, however, to increase the accuracy of detection as well as to identify the various CMG species.

ELISA was the first of these approaches to be widely applied to CMG detection. Monoclonal antibodies allowed a distinction to be made between ACMV, EACMV, and ICMV, at a time when only these three species were recognized. The emergence of the severe CMD epidemic in Uganda, however, highlighted the weakness with this serology-based method, as EACMV-UG was misdiagnosed as ACMV by ELISA, since EACMV-UG was a recombinant virus with the coat protein of ACMV. From the late 1990s onwards PCR was used almost exclusively for the detection and identification of CMG species. PCR presents an easy and robust detection system for CMGs and specific primer sets and a diverse array of PCR protocols are available to achieve reliable detection of these viruses in cassava. To verify specificity, sequencing of PCR amplicons confirms virus identity. However, because of recombination at various positions across DNA-A, amplification of genome fragments may not fully resolve the diversity of DNA-A and the association of sequence to a particular virus species. Hence amplification followed by sequencing of the entire DNA-A genome component is required for reliable virus identification. This is achieved by using inverse, abutting PCR primers located in a conserved region of DNA-A (CP) to amplify the entire circular DNA-A molecule. A more general approach is rolling-circle amplification (RCA) using phi29 (Φ29) DNA polymerase. Plant DNA including the circular virus DNA is amplified in an isothermal process using random oligonucleotide primers. Phi29 polymerase is highly processive and in this unique RCA process, small circular DNA molecules are replicated nearly infinitely. By enzymatic restriction of the RCA products using rarely-cutting restriction enzymes, CMG DNA fragments or complete virus genome components can be resolved and the entire begomovirus composition of a plant sample can be analyzed without prior knowledge of virus sequences. With this approach it is possible to detect unexpected and unknown begomoviruses, mixed infections and satellites. Sample preparation and DNA extraction are identical to other methods and commercial kits are available. RCA generates genome DNA sequences as concatemers. Virus identification can then be performed after restriction digestion, cloning and sequence analysis.

Economic Importance

CMD symptoms of leaf chlorosis, reduction of leaf size and plant stunting lead to a reduction in the quantity of photosynthetic assimilates channeled into the tuberous roots, and through this, a reduction in yield. The degree of yield loss varies greatly, depending primarily on the susceptibility of the cassava cultivar, the virulence of the CMG species/strain, and the stage of growth at which infection occurred (being most severe for cutting-infected plants). Typically, individual plants sustain yield losses ranging from 20% to 100%. The only study of the effects of specific viruses on yield showed average yield losses to be 42% for ACMV alone, 12% for EACMV-UG mild, 68% for EACMV-UG severe, and 82% for mixed ACMV + EACMV-UG. The relatively moderate losses attributable to ACMV infection, coupled with moderate to low incidences, have been the reasons for the apparent lack of concern through large parts of cassava-growing Africa about the impact of CMGs and CMD. This outlook changed markedly, however, following the emergence and regional spread of the CMD pandemic. Extensive surveys of CMD incidence, CMD severity, and the occurrence of the causal viruses have made it possible to make continent-wide yield loss estimates for CMD. The last of these (2006) provided estimates for losses in pandemic-recovered (16% loss), pandemic-affected (47%), and as yet unaffected (18%) countries in sub-Saharan Africa, leading to an overall loss figure of 34 million tons per year, which was equivalent to roughly a third of total African production of fresh cassava roots. Following the region-wide spread of the pandemic, major control programs were implemented, and anecdotal evidence suggests that this has resulted in reductions in CMD incidence and reduced losses. This declining impact of the disease in Africa is likely to be the reason why there have been no comprehensive assessments of losses since 2006.

For CMD in India, losses of up to 84% have been recorded at field level, although these are said to vary, as in Africa, depending on the varietal response, type of infection and virus species/species mixture causing the infection. There are currently no published records of overall losses due to CMD in South Asia or for the more recently affected countries of Southeast Asia.

Control

The most widely practiced approaches to controlling the CMGs that cause CMD include the deployment of host plant resistance and the use of cultural methods, particularly phytosanitation. More recently, considerable attention has been directed toward the use of genetic engineering techniques to produce transgenic virus-resistant cassava plants.

Host Plant Resistance

The potential value of introgressing virus resistance genes from wild relatives of cassava was recognized from the earliest days of CMD research in the 1920s/1930s, and inter-specific crosses combining cultivated cassava with Ceara rubber (*Manihot glaziovii*) were developed independently through breeding programs in modern-day Tanzania and Madagascar. F1 progeny were triple backcrossed with cultivated cassava to produce plants that combined acceptable food quality with significantly enhanced resistance to CMGs. Germplasm developed in this way formed the basis for the later continental breeding program run from the Nigeria-based International Institute of Tropical Agriculture (IITA), which from its establishment in 1967, to the present day, has developed thousands of CMD-resistant cassava clones. Many of these have been sent to cassava-producing countries in Africa for use either specifically for CMD management programs, or more generally for cassava development. Germplasm derived from the initial interspecific crosses uses the name prefix 'TMS' for 'Tropical Manihot species'. This resistance source is multigenic and has provided high levels of resistance which have been very durable when used against all CMGs and CMG combinations. Four distinct mechanisms of resistance are recognized: resistance to infection, resistance to virus multiplication, resistance to virus movement (leading to incomplete systemicity), and resistance of normal plant function to the effects of virus infection. During the 1990s, resistant landraces from West Africa, given the name prefix 'TME' for 'Tropical Manihot esculenta', were incorporated into the breeding program. These have been shown to possess alternative sources of resistance, one of the most important of which has been characterized through genetic analyses as a single dominant gene designated CMD2. Molecular marker approaches have been used subsequently to combine the multigenic *M. glaziovii*-derived resistance with CMD2. In recent years, improvements in sequencing technologies have enabled researchers to undertake genome-wide association studies. These have been used to identify major QTLs associated with CMD resistance and they have provided new tools to improve the efficiency of the breeding process.

Hundreds of CMG-resistant varieties have been released and disseminated throughout the cassava-growing areas of sub-Saharan Africa. However, their adoption by farming communities has been most widespread in CMD pandemic-affected countries, where they have provided the only effective means of restoring cassava production to pre-pandemic levels. In South Asia, sources of resistance have been drawn from the breeding work undertaken in Africa. Evaluations of seedling populations obtained from introduced germplasm have led to the development of varieties such as CMR1, CMR129, CR21–10, and CR43–11 which combine high starch content with strong resistance to ICMV and SLCMV. Some of this germplasm, together with other CMD-resistant varieties from IITA in Nigeria are currently being introduced to Southeast Asia as part of regional efforts to control the CMD outbreak there.

Cultural Methods

A range of cultural methods has been proposed for the control of CMGs. The methods most widely recommended have been the removal of infected plants (roguing) or the selection of disease-free planting material for the establishment of a new crop (selection). Crop isolation, adjusting crop disposition in relation to the prevailing wind, varying planting date, varietal mixtures, and intercropping cassava with other 'putative' protective crops have all been suggested at various times as potentially useful control options for CMGs. No convincing experimental evidence has yet been presented to confirm the value of any of these methods, however, and current field practice is restricted to selection and occasional roguing. Roguing is considered to be of value within the framework of institutional programs for the multiplication of CMD-resistant germplasm, in view of the requirement for the production of high-quality planting material. Experiments conducted in 'post-epidemic' areas of East Africa, first affected by the CMD pandemic five or more years previously, have provided clear evidence for the value of selection of CMD-free stems when choosing planting material. Local cultivars treated in this way provided equivalent yields to those of CMD-resistant varieties after two cropping cycles. A key drawback to the wider adoption of this approach, however, is the variability in effectiveness of the approach in relation both to the virus inoculum pressure of the location, as well as the relative susceptibility of the cultivar.

Systems for the phytosanitary control of CMGs have been greatly strengthened in several parts of Africa in recent years. Most notably, certification guidelines for cassava have been developed and appended to Seed Acts in countries such as Nigeria, Rwanda and Tanzania. These guidelines include maximum tolerance levels for CMD incidence. This has facilitated the certification of early generation cassava 'seed' (= planting material) and promoted the production of near virus-free planting material. The approach is helping to ensure that newly developed virus-resistant varieties are multiplied and disseminated in a healthy condition and it is reducing overall incidences of CMD.

Virus Resistance by Engineering

Engineering virus resistance by expression of virus sequences in transgenic plants is a strategy that is also used to achieve resistance against gemininiviruses. The most common approach is to engineer RNAi (RNA interference) constructs as inverted-repeats of virus sequence which are spaced by an intron. Splicing of the intron upon transcription generates a "hairpin" dsRNA that is targeted by the plants RNA silencing machinery. RNAi triggered antiviral defense using constructs comprising Rep sequences is effective against Tomato yellow leaf curl virus (TYLCV) and against Bean golden mosaic virus (BGMV). The stability of this resistance was verified in field trials showing virus resistance under field conditions for several seasons and the feasibility of the

approach to provide protection in both crops. In Brazil, transgenic common beans resistant to BGMV were released in 2011 as a commercial variety. Thus RNAi strategies may protect plants against DNA viruses as shown also for other crops including cotton as well as cassava, by boosting the antiviral defense. The effectiveness of the RNAi protection depends on the choice of the viral sequence and the abundance of small interfering RNAs generated from the hairpin RNA transgene. In addition, temperature and the intrinsic interaction of virus and plant host influence the effectiveness of silencing and the level of resistance achieved. RNAi strategies have been used to induce gene silencing against cassava infecting geminiviruses. In all approaches, symptoms were attenuated and virus DNA accumulation was drastically reduced. Broad spectrum resistance against several CMGs e.g., ACMV, EACMCV, and SLCMV can be achieved when there is sufficient sequence homology between the siRNAs and transcripts of the viruses.

Several studies have shown the effectiveness of CRISPR-Cas9 to engineer resistance against the geminiviruses Tomato yellow leaf curl virus (TYLCV) and Beet curly top virus (BCTV). A single-guide RNA (sgRNA) carrying the TAATATTAC nonanucleotide origin of replication of geminiviruses was used to induce a broad spectrum virus resistance. In cassava, CRISPR-Cas9 was used to engineer resistance to ACMV targeting AC2 and AC3 with a single sgRNA. However, CRISPR-Cas9 did not show any difference to the wild-type susceptible plants because of the emergence of a cleavage mutant of ACMV in transgenic plants. The selection of cleavage resistant mutants, as shown for ACMV, or new virus mutants that evade the sequence-specific action of transgene derived RNAi in TYLCV transgenic plants or ACMV transgenic cassava demonstrate the challenges of any of the engineering strategies employing targeting sequences of a replicating virus. However, this can be addressed by using multiplexed CRISPR/Cas9 with multiple sgRNAs to target virus complexes, as shown for the control of Cotton leaf curl virus (CLCuV). With more information on functional networks and genes involved in vital virus functions, CRISPR-Cas9, RNAi or similar approaches will be increasingly used for the editing or interfering with expression of host genes to achieve virus resistance in cassava.

Biosafety considerations

Cassava plants transformed for resistance to CMGs have been tested under field conditions in several African countries (Kenya, Nigeria, Uganda) through confined field trial arrangements, with promising results confirming the effectiveness of the strategy. However, uncertainty in most African countries about the consequences of the widespread cultivation of GMOs has slowed the adoption of biosafety guidelines and meant that at the time of writing (2019) there are no transgenic cassava plants that have been evaluated under 'real-world' conditions in farmers' fields. In January 2019, however, the National Biosafety Management Agency of Nigeria made its first approval of a GM crop – podborer-resistant cowpea. Since Nigeria is the world's largest producer of cassava, and since its cassava crop is widely affected by CMD, this development offers significant promise for the future release of GM CMD-resistant cassava varieties. Furthermore, this is a move that could provide a signal for other countries similarly affected by cassava viruses to follow suit. Given the huge impact that CMD has on the production of one of the world's most important staple food crops, the widespread adoption of science-based strategies to control may be essential if the food security of hundreds of millions of cassava-dependent households in Africa and elsewhere is to be assured.

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