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Cassava mosaic geminiviruses in Africa

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Abstract

Cassava mosaic disease (CMD) caused by cassava mosaic geminiviruses (CMGs) (*Geminiviridae*: *Begomovirus*) is undoubtedly the most important constraint to the production of cassava in Africa at the outset of the 21st century. Although the disease was recorded for the first time in the latter part of the 19th century, for much of the intervening period it has been relatively benign in most of the areas where it occurs and has generally been considered to be of minor economic significance. Towards the end of the 20th century, however, the inherent dynamism of the causal viruses was demonstrated, as a recombinant hybrid of the two principal species was identified, initially from Uganda, and shown to be associated with an unusually severe and rapidly spreading epidemic of CMD. Subsequent spread throughout East and Central Africa, the consequent devastation of production of the cassava crop, a key staple in much of this region, and the observation of similar recombination events elsewhere, has once again demonstrated the inherent danger posed to man by the capacity of these viruses to adapt to their environment and optimally exploit their relationships with the whitefly vector, plant host and human cultivator. In this review of cassava mosaic geminiviruses in Africa, we examine each of these relationships, and highlight the ways in which the CMGs have exploited them to their own advantage.

Historical perspectives

After the earliest report in 1894 of CMD from what is now Tanzania, the first suggestion that it might be caused by a viral pathogen was made by Zimmerman (1906) some years later. All the information accumulated about the causal viruses of the disease point towards an African weed origin, cassava becoming the primary host over the years (Fauquet and Fargette, 1990), but there is no direct proof to support this hypothesis. During the late 1920s and early 1930s, there was widespread interest across sub-Saharan Africa in the spread of CMD, and records which were most likely associated with 'first encounter' epidemics were made from Sierra Leone, Ivory Coast, Ghana, Nigeria, Madagascar and Uganda. It seems apparent that by the end of the 1930s CMD had spread to virtually all cassava-growing environments of the African mainland and its islands. The first detailed studies of CMD and the viruses assumed to be causing the disease were conducted by Storey and colleagues at the Amani research station in the Usambara mountains in the north-eastern part of what is now Tanzania (Storey and Nichols, 1938). Further evidence was provided for the viral etiology of CMD, the whitefly *Bemisia tabaci* (Genn.) was shown to transmit the putative virus(es), seasonal effects on epidemiology were described and the first reference was made to the occurrence of mild and severe virus strains. As this research program matured, a greater emphasis was placed on developing resistant germplasm which provided the basis for the major breeding initiatives launched later in the century.

The viral etiology of CMD was not fully described, however, until the implementation of a major virology project in Kenya, which, working

with laboratories in the UK, was able to benefit from the new tool of electron microscopy coupled with techniques of virus purification and diagnostics. Virions associated with CMD were described as 30 nm × 18 nm geminate particles (Bock, 1975), a *ca*. 30 kDa protein was shown to be the subunit making up the paired icosahedral coat structure (Bock *et al*., 1977) and the genetic material was found to comprise two components of single-stranded circular DNA, both of which were *ca*. 2800 bp long (Harrison *et al*., 1977). The first virus isolated from CMD was initially named cassava latent virus (CLV) because in early experiments it was not possible to infect cassava and produce similar symptoms. The first sequence of DNA-A was published in 1983 (Stanley and Gay, 1983), and shortly after this, successful infection back to cassava was achieved from *Nicotiana benthamiana* Domin., fulfilling Koch's postulates, and leading to the naming of the causal virus as *African cassava mosaic virus* (ACMV) (Bock and Woods, 1983). These early characterization studies provided the basis for a comprehensive range of both field and laboratory-based studies, which extended the understanding of viruses that were increasingly coming to be recognized as some of the most important pathogens affecting agriculture in Africa.

Molecular characterization of the cassava mosaic geminiviruses

Etiology

Serological techniques initially developed to detect ACMV (*Geminiviridae*: *Begomovirus*) were subsequently used to demonstrate the occurrence and distribution of distinct serotypes (Swanson and Harrison, 1994) and three cassava mosaic geminivirus (CMG) species were described on the basis of DNA sequence comparisons (Hong *et al*., 1993), two occurring in Africa, namely ACMV and *East African cassava mosaic virus* (EACMV) and one restricted to India designated *Indian cassava mosaic virus* (ICMV). The earliest full-length DNA-A sequences published were both from ACMV, collected from Kenya (Stanley and Gay, 1983) and Nigeria (Morris *et al*., 1990). Analysis of these sequences revealed the presence of six open reading frames (ORFs), four on DNA-A and two on DNA-B (Stanley *et al*., 1986) and a conserved intergenic 'common region' (IR or CR) of *ca*. 200 bp shared by the two DNA components. Genes on DNA-A were shown to code for the coat protein (AV1, CP), the replication-associated protein (AC1, Rep) and proteins associated with movement (AV2), transactivation of AV1 and BV1 (AC2, TrAP) and replication enhancement (AC3, REn) (Hanley-Bowdoin *et al*., 1999). Genes on DNA-B were demonstrated to have important roles in nuclear transport (BV1, NSP) and cell-to-cell movement (BC1, MP) (Sanderfoot *et al*., 1996).

Coat protein structure appears to be important for vector specificity. Coat protein mutants are not transmitted by the whitefly vector, *B. tabaci*, and chimeric mutants combining the ACMV genome with the coat of the leafhopper-transmitted beet curly top virus (*Geminiviridae*: *Curtovirus*) were transmitted by the leafhopper vector (Briddon *et al*., 1990). However, different African CMGs appear to be transmitted with similar efficiency by African biotypes of *B. tabaci* from different geographical locations (Maruthi *et al*., 2002), whilst efficiency of transmission of Asian CMGs by African *B. tabaci* biotypes is poor, and *vice versa* (Maruthi *et al*., 2002). These results reflect co-adaptation between CMGs and their whitefly vector and also highlight the fact that there is substantial uniformity in coat protein structure arising from homogeneity within AV1 sequences.

Replication

Geminivirus replication mostly occurs in the nuclei of the infected cells, where virus particles and virus induced doughnut structures can be seen in electron microscopy. Rep initiates viral DNA replication by binding specifically to reiterated motifs (iterons) within the intergenic region (Fontes *et al*., 1994) and introducing a nick into the conserved TAATATT/AC sequence (Heyraud-Nitschke *et al.,* 1995). Rep also binds to the plant homologue of retinoblastoma protein (Rb) to regulate cell-cycle progression, altering the environment of terminally differentiated cells to provide host factors that support viral DNA replication (Kong *et al.,* 2000). TrAP transactivates expression of virion-sense gene expression from both DNA-A and DNA-B (Sunter and Bisaro, 1992) and also

functions in the suppression of post-transcriptional gene silencing (Voinnet *et al*., 1999). The REn protein, although not essential, will boost viral DNA replication several fold (Sunter *et al*., 1990). The NSP and MP proteins coded by the DNA-B component are essential to shuttle viral proteins and DNAs from the cytoplasm to the nuclei and from one cell to the next (Sanderfoot and Lazarowitz, 1995).

Variability

An important development in the understanding of the molecular characterization of CMGs came in 1997 after reports of the rapid spread of an unusually severe form of CMD in central Uganda (Gibson *et al*., 1996). Sequences determined for virus isolates obtained from severely diseased plants suggested the occurrence of a CMG for which the DNA-A had arisen by inter-species recombination (Deng *et al*., 1997; Zhou *et al*., 1997). Examination of the coat protein encoding sequence revealed that whilst the 5′-end 219 nucleotides (nt) and 3′-end 93 nt were almost identical to those of EACMV, together with the rest of its DNA-A, the central 459 nt portion of the coat protein was highly homologous with that of ACMV. The recombinant virus, initially referred to as the Uganda Variant (UgV) (Zhou *et al*., 1997), was finally considered to be a strain of EACMV (Deng *et al*., 1997), and has recently had its definitive designation as EACMV-UG confirmed after a comprehensive review of the taxonomy of the family *Geminiviridae* (Fauquet and Stanley, 2003).

Subsequent to this finding, characterization of viruses occurring in CMD-diseased plants from a wide range of locations has begun to reveal an increasingly complex picture of recombination, pseudorecombination and virus mixtures.

Recombination

Sequence comparisons of a large number of geminivirus species and strains have shown that recombination is a very common occurrence, and clearly has an important role to play in the evolution of these viruses (Padidam *et al*., 1999). For the CMGs, however, it appears that an important distinction can be drawn between ACMV, which shows a high degree of homology regardless of the location of collection, and EACMV-like viruses, for which variation is considerable and recombination frequent (Pita *et al*., 2001a). In addition to the EACMV-UG case mentioned above, further recombination events were identified in other CMGs. In the case of *East African cassava mosaic Cameroon virus* (EACMCV), recombination events were present in the AC2–AC3 region of DNA-A and the BC1 region of DNA-B (Fondong *et al*., 2000). Interestingly, the DNA-B of EACMCV is almost completely different from the DNA-B of EACMV with the exception of a 500 nts recombinant fragment in the BC1 ORF (Fondong *et al*., 2000). Contrastingly, for *South African cassava mosaic virus* (SACMV), the B component is identical to the B component of EACMV, with the exception of the CR which is similar to the A component, and the A component has a short 500 nt fragment in the AC2–AC3 ORFs identical to that of EACMV (Berrie *et al*., 2001). Recombined portions of AV2 in Malawian isolates of EACMV showed strong homology with a strain of *Tomato yellow leaf curl virus* (Zhou *et al*., 1998).

Taxonomy

Recent taxonomic guidelines have been developed to provide a framework on which to base definitions of species and strains (Fauquet and Stanley, 2003), but given the apparent propensity of the CMGs to exchange genetic material, this should be viewed as a dynamic tool to aid those working with these organisms rather than a fixed system. Based on this new approach, in which the sequence homology demarcation between species has been set at 89% for the DNA-A component of begomoviruses, six African and two Indian CMG species are recognized. These are: ACMV, EACMV, EACMCV, *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Zanzibar virus* (EACMZV), SACMV, ICMV and *Sri-Lankan cassava mosaic virus* (SLCMV) (Fauquet and Stanley, 2003) (Figure 1). Undoubtedly, many additional species remain to be identified, since comprehensive sampling and characterization work has only been done for material collected from a fraction of the geographical range affected by CMD. Clearly an important future aim in this area of study should be to expand on the

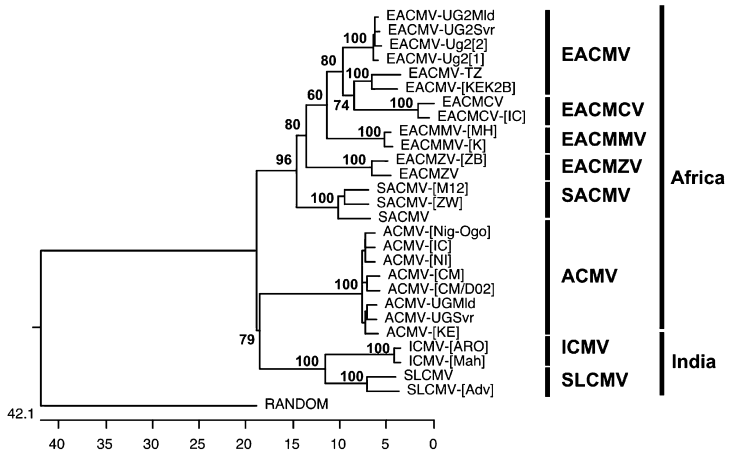

Figure 1. Dendrogram based on complete DNA-A component nucleotide sequences of 27 geminivirus isolates representing eight species of geminivirus infecting cassava. Accession numbers and nucleotide sequences were obtained from GenBank. Sequences were aligned using the Clustal algorithm (MegAlign 3.11, DNAstar) and the bootstrap analysis was done with PAUP 4.0 (values indicated at the branching). The vertical axis is arbitrary and the horizontal axis represents distance expressed in percentage of nucleotide substitution ×100. The scale at the bottom indicates the horizontal distance in percentage of differences.

coverage of material characterized, since different viruses have very different biological characteristics often with gross differences in the severity of disease caused (Harrison *et al*., 1997; Fondong *et al*., 2000; Pita *et al*., 2001b) and there is an obvious potential economic advantage to be gained from understanding which virus species, strains and mixtures occur and how they are distributed.

Pseudorecombination

Pseudorecombination in which the DNA-A of one virus co-occurs with and trans-replicates the DNA-B of another virus has also been reported for CMGs occurring in Uganda. The most frequent naturally occurring CMG infections in Uganda involve the co-occurrence of EACMV-UG2 DNA-A and EACMV-UG3 DNA-B (Pita *et al*., 2001a), and the other two molecules, i.e. EACMV-UG2 DNA-B and EACMV-UG3 DNA-A, are no longer prevalent in nature. The infections comprising ACMV DNA-A and EACMV-UG3 DNA-B have also been reported from PCR analysis, although it has not been ruled out that the corresponding DNA-A and DNA-B molecules were present at some stage during the infection, and results from tobacco protoplasts show that the EACMCV-CM DNA-A and ACMV-CM DNA-B combination behaves in a similar way.

Synergism between cassava mosaic geminiviruses

Mixed ACMV and EACMV infections were shown to be an important feature of the severe CMD first reported from Uganda and subsequently in neighboring countries (Harrison *et al*., 1997; Legg, 1999; Pita *et al*., 2001a). Plants infected with EACMV-UG expressed more severe symptoms than those infected with ACMV, but plants infected with the two viruses together were more severely diseased than both of the single infection conditions (Harrison *et al*., 1997; Pita *et al*., 2001a), and measurement of virus concentrations in all three infection conditions suggested the occurrence of a synergistic interaction between the two viruses. A similar synergistic interaction was reported for mixed ACMV/EACMCV infections in Cameroon (Fondong *et al*., 2000). This synergism is the only case known for geminiviruses and the only case known for plant viruses belonging to the same family. This biological phenomenon is of primary importance for the emergence of new geminivirus diseases and has been shown to be a key factor in the genesis and spread of the CMD pandemic in East and Central Africa (Harrison *et al*., 1997; Legg, 1999).

Transmission and epidemiology

Subsequent to the early studies of Storey and Nichols (1938) that provided clear-cut evidence for

B. tabaci as the vector of the CMGs, more detailed studies on the characteristics of transmission were conducted by Dubern (1994). Optimal (and minimum) times for different periods of the persistent transmission process were shown to be: acquisition 5 h (3.5 h), latent 6 h (3.5 h) and inoculation 30 min (5–10 min). Additionally, it was observed that *B. tabaci* adults could retain the virus for at least 9 days, although this was considered to be an underestimate (Dubern, 1994). Variable results have been obtained for studies of transmission efficiency. Data from the Ivory Coast with field-collected whitefly adults indicated that 0.15–1.7% of adults were infective (Fargette *et al.*, 1985), whilst for cage-based studies, Dubern (1994) reported 13% and Maruthi *et al.* (2002) a range from 4.4% to 7.5%.

Primary considerations of epidemiological studies have been patterns of spread of the virus disease within and between fields and the conditions which favor or inhibit such spread. Many of the fundamental epidemiological data were derived from the comprehensive set of experiments conducted within the framework of the Ivory Coast-based CMD research program of the 1980s. Environmental gradients were demonstrated (Fargette *et al.*, 1985) in which most new disease in initially CMD-free plantings was recorded from the upwind edges, and it was demonstrated that external sources of inoculum were more important sources of new infection than internal sources. Regional differences were demonstrated in rates of spread in Ivory Coast (Fauquet *et al.*, 1988) and Uganda (Legg *et al.*, 1997), with the general finding that spread was more rapid in humid environments with greater densities of cassava cultivation. Abundance and distribution of populations of the whitefly vector have been shown to be key determinants of patterns of spread (Fargette and Vie, 1994), although a study of factors determining patterns of CMD spread into initially CMD-free plots revealed that the level of inoculum in surrounding fields was a more important determinant of final CMD incidence in the test plot than whitefly abundance (Legg *et al.*, 1997). In Uganda, inconsistencies apparent in the association between the epidemiological characteristics of CMD spread and environmental conditions (Legg and Ogwal, 1998) were subsequently shown to be a result of the overriding importance of the epidemic of severe CMD associated with EACMV-UG. As a consequence, rapid spread occurred in areas in which EACMV-UG was present, regardless of the specific agro-ecological or other environmental conditions. A primary reason for this is the fact that in dual ACMV + EACMV-UG infections, synergism leads to 10–50-fold increases in viral DNA accumulation, substantially increasing the potential for a higher efficiency of vector transmission (Harrison *et al.*, 1997; Pita *et al.*, 2001a). These findings provided the first indication of the importance of considering the nature of the virus or virus mixtures causing CMD when making epidemiological assessments.

Molecular epidemiological studies

The development of PCR-based diagnostic techniques enabling the separation of virus species and strains and the detection of mixed infections, has allowed more detailed virological assessments to be made of CMD epidemiology both at the regional and field levels. Molecular diagnostics have been used to investigate patterns of CMG infection in initially CMG-free cassava plantings of a susceptible variety at locations in Uganda where both ACMV and EACMV-UG occur (J. Legg, unpublished data). Preliminary results suggest that EACMV-UG infection is greatest during early stages of growth. However, as incidence increases, mixed ACMV + EACMV-UG infections become more frequent resulting in increased virus concentrations through synergism and rapid spread of both viruses to all plants. Comparisons of mildly CMD-diseased with initially healthy plants of a local CMD-susceptible cultivar have also shown that mild strains of EACMV-UG appear to provide some form of cross-protection against more severe strains of the same virus (Owor, 2002). By contrast, initially ACMV-infected plants are super-infected with EACMV-UG at the same frequency as initially CMG-free plants are infected with EACMV-UG (J. Legg, unpublished data). Although such studies are complex and expensive, the biological importance of different single and mixed virus infections means that they will be an increasingly important component of future epidemiological studies.

CMD epidemics

A brief history

After the 'first encounter' epidemics reported from many parts of Africa in the 1920s, further epidemics were described from Uganda in the 1930s/1940s and Madagascar also in the 1930s. In the latter case, the detailed description of symptoms provided by Cours (1951) highlighted the intense chlorosis, reduction in leaf size and candlestick-like architecture of plants infected by the severe CMD associated with the epidemic. Such symptom descriptions do not feature in the literature again until the first reports were made of the epidemic of unusually severe CMD in Uganda (Gibson *et al*., 1996; Otim-Nape *et al*., 1997) (Figure 2B–E). Brief descriptions have been made of epidemics in Cameroon (Fondong *et al*., 2000), Ghana (Fauquet, pers. commun.), Ivory Coast (Pita *et al*., 2001a), Akwa Ibom State in Nigeria and the Cape Verde Islands (Calvert and Thresh, 2002), but none of these situations seems to have developed beyond the local level.

The African CMD pandemic

The epidemic of severe CMD that spread to affect most of Uganda in the 1990s devastated the country's cassava production, causing losses valued at in excess of USD 60 million annually between 1992 and 1997 (Otim-Nape *et al*., 1997). Farmers literally abandoned the crop in large parts of the country, and in eastern districts widespread food shortages led to some famine-related deaths (Thresh and Otim-Nape, 1994). During the second half of the 1990s, the epidemic spread to the neighboring countries of Sudan, Kenya, Tanzania and eastern Democratic Republic of Congo (DRC), with a similar impact on cassava cultivation (Legg, 1999). Key characteristics of what was by this stage known as the CMD pandemic, were high incidences of severe CMD (Gibson *et al*., 1996), rapid vector-borne spread (Otim-Nape *et al*., 1997) and super-abundant *B. tabaci* populations (Legg and Ogwal, 1998). The pandemic was also described as advancing along a 'front' that within Uganda was estimated to be moving at 20–50 km per year (Legg and Ogwal, 1998). Studies of the relationship between CMGs and the pandemic, with the specific primer PCR diagnostics developed by Zhou *et al*. (1997), revealed a consistent association of the recombinant EACMV-UG with the pandemic, commonly in mixed infection with ACMV, and single ACMV infections alone in unaffected areas (Harrison *et al*., 1997; Pita *et al*., 2001a). Subsequent to this finding, EACMV-UG has been identified as the dominant virus in pandemic affected areas of western Kenya and north-western Tanzania (Legg, 1999), Rwanda (Legg *et al*., 2001), and in 2003 also from eastern DRC (P. Phemba, unpublished data) and Burundi (Bigirimana *et al*., 2003). Similar associations have been made between severe CMD and the occurrence of EACMV-UG from the western part of DRC, the central and northern areas of the Republic of Congo (ROC) (Neuenschwander *et al*., 2002) and most recently from eastern Gabon (Legg *et al*., 2003). This has led to the assertion that this part of west-central Africa represents the western extremity of the African CMD pandemic. However, there is currently no clear evidence for a link between EACMV-UG and rapid CMD spread, as has been reported for East Africa, although this may in part be due to the limited amount of research attention that has been directed towards CMD in DRC, ROC and Gabon, and the lack of regular monitoring in patterns of CMD infection in initially CMD-free crops. EACMV-UG is also reported to occur in four countries of southern Africa, completely outside the reported area of coverage of the African CMD pandemic (Berry and Rey, 2001). These occurrences, described for Mozambique, South Africa, Swaziland and Zimbabwe, were most commonly in mixed infection with ACMV, EACMV or SACMV, but in no case was there an association with unusually severe disease or rapid epidemic-like spread, and there are currently no reports of such disease situations from any of the southern African countries. The best assessment of the coverage of the pandemic in 2003, therefore, is that it extends from western Kenya, to western DRC, and from southern Sudan and northern DRC to central DRC, central Burundi and the Lake Zone of Tanzania in the south (Figure 2A). The occurrence of EACMV-UG in southern Africa, in the absence of the epidemic-like behavior that characterizes its presence in East Africa, raises an important question about the causal link between the two. EACMV-UG does not seem to be unique in being able to elicit severe symptoms in cassava, as simi-

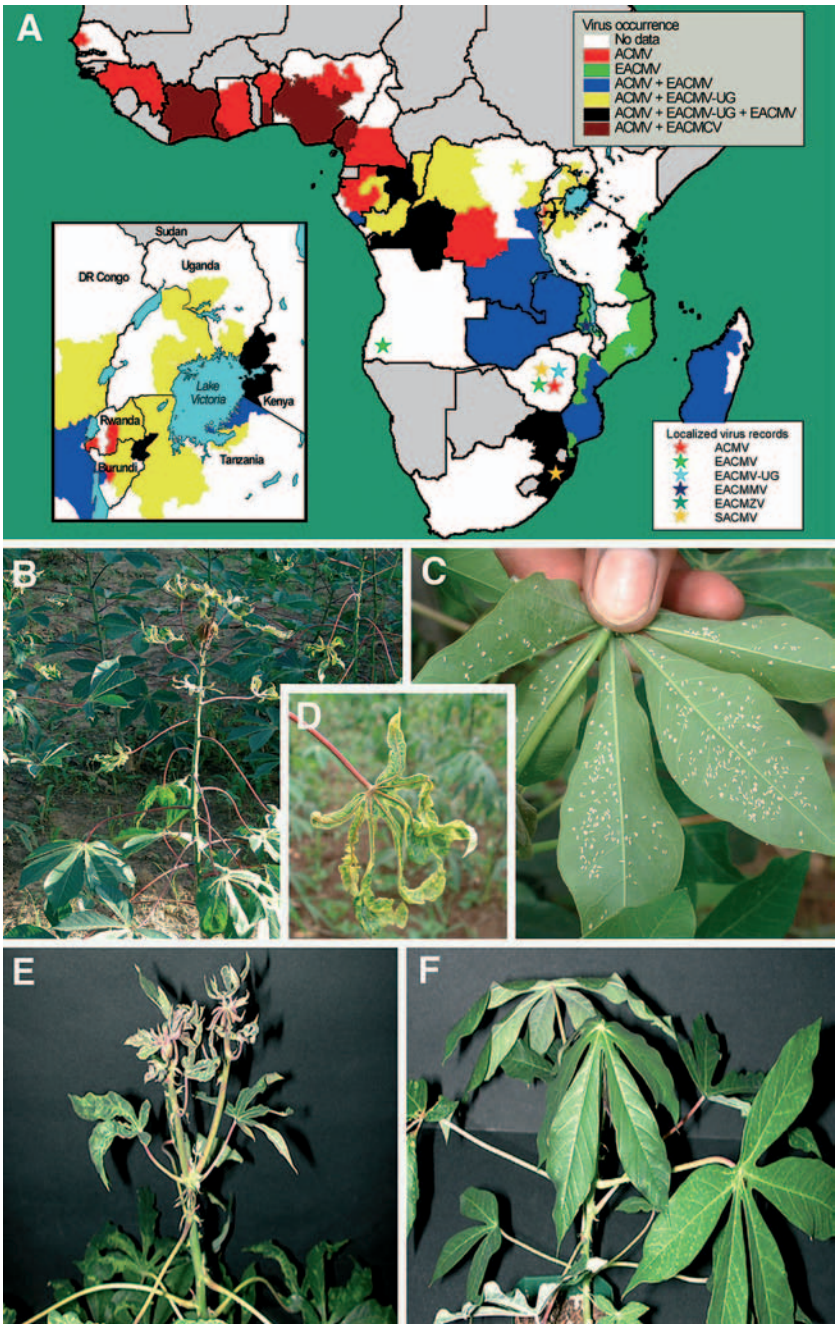

Figure 2. (A) Distribution of cassava mosaic geminiviruses in Africa obtained from CMD surveys in Africa, 1998–2003. See legend in map for the significance of the colors. Source of the information: Fondong *et al*. (1998, 2000), Zhou *et al*. (1998), Offei *et al*. (1999), Legg and Thresh (2000), Berry and Rey (2001), Legg *et al*. (2001), Pita *et al*. (2001a), Neuenschwander *et al*. (2002), Bigirimana *et al*. (2003), Bull *et al*. (2003), Briddon *et al*. (2003), Legg *et al*. (2003), Ogbe *et al*. (2003), Okao-Okuja *et al*. (2004), ■. Tata-Hangy (unpublished data, Democratic Republic of Congo), ■. Okao-Okuja (unpublished data, Mozambique) and P. Markham (unpublished data, Benin and Madagascar). (B) typical symptoms of cassava infected with ACMV and EACMV-UG in Tanzania. (C) Unusually large whitefly populations on CMD-resistant MM 96/0245 clone in Uganda. (D) Close-up of a typical symptom of EACMV-UG on a young cassava leaf. (E) TMS 60444 infected with ACMV and EACMV-UG infectious clones showing typical synergistic symptoms. (F) Transgenic TMS 60444 challenged with ACMV and EACMV-UG infectious clones and not showing symptoms 2 months after inoculation.

lar characteristics have been described both for ACMV (Pita *et al*., 2001b) and other EACMVs (Pita *et al*., 2001a). Similarly, mixed ACMV/EACMV, or EACMV-like virus, infections in which synergism occurs, leading to high virus titer and very severe symptoms, have been reported from many locations outside of the pandemic-affected zone. These include: southern Africa (Berry and Rey, 2001), Cameroon (Fondong *et al*., 2000), Nigeria (Ogbe *et al*., 2003), Ghana (Offei *et al*., 1999) and Ivory Coast (Pita *et al*., 2001a). In all locations in Central and West Africa, however, EACMVs or EACMV-like viruses rarely occur in single infections, even where CMD incidence is relatively low. This contrasts strongly to the situation in the pandemic zone in which EACMV-UG is the predominant virus, occurring either in single or mixed infection. This would seem to suggest that the DNA-As of many isolates of EACMVs may be dependent on the presence of ACMV to facilitate their vector transmission. An alternative hypothesis for the apparent absence of single infections of EACMV-like viruses is that their concentrations are very low except when synergized through co-infection by ACMV, as has been reported for EACMCV (Fondong *et al*., 2000).

Molecular characterization of *B. tabaci*

Bemisia tabaci has a pan-tropical distribution, has been recorded from more than 500 crops and weed host plants, and occupies a great diversity of niches with contrasting ecological conditions. In spite of this almost unique breadth of adaptation, genetic relationships remain poorly understood, and although there are important biological contrasts between populations, they cannot be readily distinguished on the basis of morphology (Mound, 1963). Biochemical and genetic markers have therefore been developed to separate populations (Brown, 2001). In Ivory Coast, two *B. tabaci* biotypes were distinguished using isozyme analyses (Burban *et al*., 1992), one of which was restricted to cassava, whilst the other colonized a range of crop and weed hosts. Similarly, in Uganda, esterase isozymes were used to distinguish a polyphagous biotype occurring on cotton and sweet potato from a cassava biotype (Legg *et al*., 1994). More recently, genetic markers, including portions of the mitochondrial DNA cytochrome oxidase 1 gene (MtCO1) (Legg *et al*., 2002), the internally transcribed spacer of ribosomal subunit 1 (ITS) (Abdullahi *et al*., 2003), and random-amplified polymorphic DNA (RAPD) (Maruthi *et al*., 2001) have been used to assess genetic variability amongst African *B. tabaci* populations. Both Maruthi *et al*. (2001) and Legg *et al*. (2002) examined the relationship between *B. tabaci* genotypes and the CMD pandemic in East Africa. Maruthi *et al*. (2001) demonstrated mating compatibility between populations obtained from both ahead of and behind the pandemic 'front' and the absence of genetic differences based on RAPD analyses. Similarly, it was shown that there were no differences in either the biological or virus transmission characteristics of these two populations. Legg *et al*. (2002), by contrast, found evidence for the occurrence of an invasive pandemic-associated *B. tabaci* genotype based on MtCO1 sequence analyses of *B. tabaci* adults collected along three transects running perpendicular to the pandemic 'front'. Mismatch analyses further suggested that the variability of the 'invader' haplotype was substantially less than that of the 'local' haplotype, indicating that the 'invader' group could be a rapidly expanding population that has been recently introduced. Given the apparent contradiction between these two data sets, and the continuing need to explain the super-abundance of *B. tabaci* populations in areas affected by the pandemic (Legg and Ogwal, 1998), further investigation of the biology, genetic relationships and host/virus interactions of these populations is required. Preliminary studies in Uganda have suggested that severely CMD-diseased cassava plants interact synergistically with *B. tabaci* populations (Omongo, 2003). Components of this interaction include increased colonization of CMD-diseased plants, concentration of egg-laying on symptom-free portions of diseased plants and enhanced rates of fecundity and nymphal development on CMD-diseased plants. However, further data are required from a wider range of cassava cultivar/*B. tabaci* population/virus and virus mixture combinations before more definitive conclusions can be drawn about the key underlying reasons for the increases in *B. tabaci* abundance observed on cassava in pandemic-affected areas (Figure 2D).

The economic impact of CMD

Studies investigating yield loss have been conducted in many locations under diverse conditions of cultivar susceptibility and inoculum pressure conditions and these have as a consequence provided a wide range of loss estimates from 20% to 95% (Fauquet and Fargette, 1990). Molecular evidence demonstrates clearly that different viruses and virus mixtures have strongly contrasting effects on the symptom expression and growth of cassava plants (Harrison *et al*., 1997; Fondong *et al*., 2000; Pita *et al*., 2001b), but there is little quantitative information available on effects of specific viruses and virus combinations on yield. In Uganda, however, it has been demonstrated that whilst plants infected with mild strains of EACMV-UG yielded only 12% less than CMG-free plants, yields of plants infected by ACMV were reduced by 42%, those infected by severe strains of EACMV-UG by 68% and those with mixed ACMV + EACMV-UG infections by 82% (Owor, 2002), highlighting the impact of the synergistic interaction between these two viruses. Further studies are required to provide comparable information for other cultivars and viruses and virus mixtures. Efforts have also been made to estimate yield losses associated with plants infected through the cutting and those infected by the whitefly vector at different stages of crop development. Studies in Ivory Coast using the moderately resistant cultivar 'CB' showed that losses were greatest (>75%) for cutting-infected plants, were less for whitefly-infected plants – decreasing as time prior to inoculation increased – and were negligible when infection occurred beyond 70 days after planting (Fargette *et al*., 1988).

Various attempts have been made to assess the continent-wide impact of CMD on African cassava production. Fargette *et al*. (1988) assumed 37% yield losses for infected plants and 100% incidence to arrive at an overall loss estimate of 30 million tons for Africa. Thresh *et al*. (1997) used more conservative assumptions of a 30–40% yield loss and an overall incidence of 50–60% to conclude that losses ranged from 15% to 24%. Most recently, Legg and Thresh (2003) used country-level incidence figures obtained from recent surveys carried out in all of the major producer countries, together with the 30–40% yield loss assumption, to estimate that continental losses in 2003 ranged from 19 to 27 million tons based on a total production of 97 million tons (FAO, 2003). Assuming a conservative financial value of USD 100 per ton, this amounts to an annual economic loss of USD 1.9–2.7 billion. Clearly, such losses mean that CMD is one of the most globally damaging if not *the* most globally damaging plant virus disease. It is of concern that although awareness is growing of the significance of CMD, and control programs are being implemented in many affected countries, the continued expansion of the CMD pandemic is further eroding the tentative production gains achieved in recent decades. Urgent measures are therefore required to tackle both the chronic losses sustained throughout the areas of Africa where cassava is produced, but also, and even more importantly, the severe and rapidly spreading CMD that characterizes the pandemic.

CMD management

In common with most plant virus diseases, CMD has been managed primarily by phytosanitation and conventional resistance breeding. Significant efforts, however, have been made to supplement these two basic approaches with alternatives, including: vector management, cross-protection, marker assisted selection and genetic transformation.

Phytosanitation

Phytosanitation comprises all those techniques that aim to keep plants of the crop or variety being grown in a virus-free condition. The most commonly advocated approaches are the removal of CMD-diseased plants from within a crop stand (roguing) and the selection of symptom-free cassava stems for planting the subsequent crop (selection). Both have inherent disadvantages, however. Roguing is unpopular with producers, very often since the loss of the plants removed is considered to outweigh the future and rather intangible gain that may result from reduced virus spread. Selection may be difficult to practice where symptoms are not clearly distinguished at the time of harvest and where there is a lag between harvest and replanting. In situations where symptoms are very obvious as a result of severe CMD, commonly

there may be no CMD-free plants available for selection. Roguing and selection are practiced informally by cassava producers in many situations, particular where there has been a history of strong extension support messages, but more commonly these measures are confined to official schemes for the multiplication of planting material.

More sophisticated approaches to the provision of virus-free germplasm involving the 'clean-up' of tissue culture material through meristem tip culture and thermotherapy have been proposed. To date, however, these practices remain largely confined to quarantine support facilities, such as the Ibadan, Nigeria, based tissue culture unit of the International Institute of Tropical Agriculture (IITA), in which tissue culture material is maintained virus-free to allow germplasm to be exported throughout the continent. Furthermore, it is unknown how long virus-free plants would remain virus-free and thereby benefit subsequent multiplication. Many wrongly believe that there is an 'enrichment' in virus load over serial cultures and that cuttings get smaller and less productive, when in actual fact an equilibrium is established at each generation between the resistance of the genotype and the virus load. An ambitious program using virus cleaning of mother plants would therefore be effective only with genotypes having a certain level of resistance or having a high level of reversion. Reversion is the genetic capacity of a particular genotype to grow virus-free from cuttings obtained from virus-infected plants. Computer models have been used to show that the use of varieties that revert at each generation could significantly lower the percentage of infected plants at an early stage and consequently the losses due to the virus (Fargette and Vie, 1994).

Additional cultural practices which have been advocated for CMD management at various times, including: crop isolation, planting strategies in space and time designed to minimize the risk of infection and inter-cropping, are all either only marginally beneficial or too impractical for producers to implement, and have not as a result been widely adopted.

Conventional resistance breeding

From the early years of research into CMD it was apparent to workers that cultivars varied in their response to the disease, and more importantly, that wild relatives to cassava displayed significantly higher levels of resistance to virus infection. The earliest resistance breeding programs, initiated almost simultaneously in the 1930s in Madagascar and at the Amani station in north-east Tanzania therefore used both intra-specific and inter-specific crosses with *Manihot glaziovii* Muell.-Arg. to produce progeny with increased levels of CMD resistance. Most success was achieved with the inter-specific *M. glaziovii* crosses, and the Amani group then used a series of backcrosses to restore root quality whilst retaining resistance. High levels of resistance were obtained, but the program was terminated in the late 1950s, although seed from one of the most resistant clones, 5318/34, was used to reinitiate the work at IITA from 1970. One of the clones derived from this seed, designated 58308, had a good combination of CMD resistance and root quality and formed the basis for much of the resistance breeding work that followed at IITA (Hahn *et al.*, 1980). Some of the most important clones from the Tropical Manihot Species series that resulted from this work included: TMS 4(2)1425, TMS 30337, TMS 91934, TMS 30001, TMS 60142 and TMS 30572, all of which have been widely distributed across the continent and are now grown by producers in many of Africa's main cassava-producing countries. Resistance derived from the *M. glaziovii* inter-crosses was found to be multigenic and was characterized by a number of mechanisms. These included: resistance to initial virus infection by the vectors, reduction in the rate of virus replication, restriction in the movement of virus particles within the plant and decreases in the effects of a given virus titer on growth and development of the plant (Fargette *et al.*, 1996). Since the 1990s, however, IITA has been exploiting a newly identified source of resistance conferred by a single dominant gene/locus (CMD2) which is derived from Nigerian landraces (Akano *et al.*, 2002). Crosses combining the multigenic *M. glaziovii* resistance with CMD2 have given rise to progeny which are both near immune to CMD and offer hitherto unrealized yields, exceeding what was previously considered to be a yield plateau of *ca.* 30 t/ha (A. Dixon, unpublished data). These materials now make up the bulk of germplasm being exchanged by IITA with national research programs and being promoted directly in emergency situations. The discovery of a single domi-

nant gene/locus conferring CMD resistance has opened up new opportunities for marker assisted selection (MAS) (Akano *et al*., 2002), but the routine implementation of this approach for CMD alone may be constrained by its relative expense in comparison with tried, tested and widely practiced field-based approaches to breeding and selection. Although the use of a single dominant gene/locus-based resistance strategy might lead to a vulnerability to resistance breakdown, the recent identification by the IITA breeding team of four additional sources of resistance to CMD opens up possibilities for pyramiding these genes thereby assuring durability (A.G.O. Dixon *et al*., submitted for publication).

Novel approaches to CMD control

Cross-protection

Field observations of the 'post-pandemic' situation in Uganda, 5 years or more after the initial passage of the 'front', have revealed increases in the frequency of occurrence of mildly CMD-diseased plants of local CMD-susceptible varieties. Diagnoses of virus infections have revealed the majority of these plants to be infected by mild strains of EACMV-UG, as reported by Pita *et al*. (2001b). Moreover, experimental comparisons of these mildly diseased plants with other initially CMD-free plants of the same variety have shown that whilst the initially CMD-free plants rapidly become infected and express very severe symptoms, the plants that were initially infected by mild strains of EACMV-UG remain mildly diseased (Owor, 2002). Initially mildly diseased plants grew taller and more vigorously than those that started CMD-free, and final yields at harvest were up to 45% greater in the mild treatment. These results contradict the general principle, derived from experiments conducted under different virus infection circumstances, that plants infected from the cutting yield less than plants that are infected during subsequent growth (Fauquet and Fargette, 1990). Furthermore, the results suggest that infection of plants with a mild strain of EACMV-UG confers some form of cross-protection, inhibiting subsequent super-infection by related severe strains. Further work is required, however, to improve the understanding of the molecular mechanisms underlying these observations, and if appropriate, to exploit and enhance the phenomenon as an additional CMD control strategy.

Transformation for CMD resistance

A novel approach is to genetically transform cassava with genes conferring resistance to the virus. This has been attempted by several groups and led to the production of transgenic plants that are resistant to several CMGs. The advantage of the method is the possibility to keep traits that are considered of primary importance to cassava producers and consumers such as processing and taste qualities of the roots, or to combine the virus resistance phenotype with high yielding qualities of some of the inbred lines. Currently the source of resistance is coming from the viruses themselves by expressing in the transgenic plants the full length or part of a viral gene. The highest level of resistance so far recorded in containment facilities using artificial methods of inoculation has been obtained using the so-called Rep gene of ACMV (P. Chellappan, unpublished data). A very susceptible genotype, TMS 60444, has been used for transformation, and near immune plants have been regenerated. In addition, these plants are resistant to other CMGs like EACMCV and SLCMV indicating a wide range of protection, which is a key requirement in view of the molecular variability of known and unknown CMGs. Furthermore, these plants have shown a very high level of resistance to the synergistic mixture of ACMV and EACMV-UG, indicating that the employed strategy is very effective against the natural mixture causing the pandemic. It appears that the most resistant plants are using the post-transcriptional gene silencing mechanism and that the broad spectrum of protection to other virus species is due to the presence of common short sequences between their respective Rep protein genes (P. Chellappan, unpublished data). An alternative approach has made use of anti-sense RNA technology (Zhang *et al*., this issue), in which targets for the anti-sense interference were the mRNAs of AC1, AC2 and AC3 of ACMV. Virus accumulation assays in transgenic plants revealed reduced or inhibited replication of ACMV. In a third approach, a hypersensitive response upon infection is elicited through the transformation of TMS 60444 with the bacterial *barnase* and *barstar* genes from *Bacillus amyloliquefaciens*, controlled by the ACMV A bi-directional promoter (Zhang *et al*.,

2003). Reductions of viral replication of between 86% and 99% have been demonstrated when comparing leaves of untransformed and transgenic plants. Whilst this strategy remains at the greenhouse testing stage, both the Rep and anti-sense RNA strategies have yielded plants ready for field testing, and the first results are likely to be available by 2005.

Conclusions

Cassava mosaic disease has been a major factor limiting the production of one of Africa's most important staple food crops for more than 75 years. However, far from ameliorating with time, CMD has actually increased in importance, and is now responsible for financial losses of several USD billion per annum, and could be considered now as the single most globally important plant virus disease. Changes in all the principal elements of the CMD pathosystem have had an influence on this development. Human population movements, both intentional and as a result of civil disturbance, have encouraged the movement of virus-infected cassava cuttings within the continent. The CMGs have been shown to possess a remarkable capacity for rapid evolutionary change and adaptation through recombination and pseudorecombination. The recent discovery that CMGs can synergize and cause unusually severe symptoms leading to almost total yield loss in infected plants is undoubtedly of major importance in explaining and understanding the recent pandemic of CMD in Africa. New research on the whitefly vector, *B. tabaci*, has also revealed adaptations both for host specialization and synergistic interaction with the virus-diseased condition encouraging population increase and further virus spread. The complex cocktail of factors has had a profound impact on the development of CMD in Africa, and helps to explain why, despite significant efforts to develop control measures, the disease continues to exert a heavy influence on the already fragile livelihoods of millions of African people. The CMD pandemic will undoubtedly continue to spread to affect yet more of the cassava-growing belt of sub-Saharan Africa in the immediate future. There are also fears that the CMGs could be inadvertently carried to other parts of the world where cassava is an important crop, such as south America and South-East Asia where they do not exist yet but where *B. tabaci* has already adapted to cassava. Determined efforts will be required from plant quarantine authorities and the scientific community to ensure that the risk of such an occurrence is minimized. There are important signs of hope, however. High levels of resistance have already been incorporated into germplasm with the qualities demanded by some producers and consumers. Vital breakthroughs have been made in recent years in understanding the nature, biology and interactions of the CMGs, *B. tabaci* and the cassava host. The first critical steps have also been made in the development of cassava transformation and regeneration systems, which in the mid-term offer the possibility of a whole new range of control options. The challenge for the future to be addressed by the research and development communities, with support from African governments and development investors, will be to bring adequate resources to bear in strengthening efforts to tackle CMD in Africa. Only a determined, well co-ordinated and comprehensive approach, addressing both research and development needs, will allow the true potential of cassava in Africa to be unlocked, enabling this most versatile of crops to provide food security, income and new commercial opportunities for a growing population.

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